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CONTENTS

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REMOTE SELLING OF MEDICINES: LIMITATIONS AND PROSPECTS

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The article presents the results of a study of consumer opinions regarding remote selling of medicines on the Russian pharmaceutical market. The segment of the electronic commercial pharmaceutical market today occupies more than 6.5% of the total pharmaceutical retail and is an attractive field of activity. In October 2015, the Ministry of Health of the Russian Federation made a proposal to regulate the market for trade in over-the-counter medicines remotely. Since the sale of medicines through offline pharmacies and online pharmacies has obvious differences, a survey was carried out on consumers' attitudes to online purchases, including medicines. It was found that the majority of respondents make purchases in online stores. A third part of respondents plan to purchase medicines remotely in online pharmacies, and about the same number do not deny this possibility, but in the absence of a medicine in a classic pharmacy. Respondents believe that the main reasons for preferring offline pharmacies to their online competitors are the importance of advice and assistance from pharmacists and the risk of purchasing a low-quality medicine in an online pharmacy. The conclusion is that consumers are ready to legalize the remote sale of over-the-counter medicines with the possibility of goods delivery to their homes.

Keywords: medicines, internet pharmacy, online trade, pharmaceutical retail trade, survey

Despite the fact that the sphere of production, delivery, sale and consumption of medicines is considered as one of the most stable in the economy, evolutionary changes in socio-economic conditions determine a new format for pharmaceutical business, which cannot be done without the use of digital technologies and various Internet services. For several years now, the regulator and representatives of the pharmaceutical community have been discussing the legalization of remote sales of over-the-counter medicines with the possibility of home delivery. In October 2015, the Ministry of Health of the Russian Federation initiated and prepared a draft Federal law "On amendments to certain legislative acts of the Russian Federation regarding the remote retail sale of medicines". It was proposed to regulate the market for remote sales of medicinal products and allow remote sales of medicinal products for medical and veterinary use, except for narcotic, psychotropic drugs, medicinal products subject to strict record keeping and storage, and medicines with a volume fraction of ethyl alcohol over 25% [1]. The State Duma Members in the autumn session considered the bill on distance selling of medicines, and in April 2020, against the background of coronavirus pandemic, when purchasing commodities through the Internet gained a special actuality, the Federal Government amended the legislation. The introduction of remote sales of medicines

required the formation of a sufficient regulatory and legal framework for this mechanism. On the basis of the Decree of the President of Russia dated 17.03.2020 No. 187 "On the retail trade of medicines for medical use", the Federal law dated 12.04.2010 No. 61-FZ "On circulation of medicines" was amended and the Resolution of the RF Government dated 16.05.2020 No. 697 "On approval of Rules of issuing permits for remote retail trade in medicinal products for medical use, implementation of such trade and delivery of the specified medicines to citizens and amendments of some acts of the Government of the Russian Federation regarding the remote retail trade in medicines for medical use" was approved.

The remote sales method includes receiving, forming, storing, and delivering the orders (within the territorial entity of the RF and the localities closest to its border in the neighboring region) and selling the medicines. In accordance with the Civil Code of the Russian Federation, the sale of goods remotely, in particular through an online store, is a sale under a purchase and sale agreement concluded on the basis of the buyer's familiarization with the seller's description of the goods contained in catalogs, brochures, booklets, or other methods that exclude the possibility of direct buyer's familiarization with the goods, as well as using the Internet information and telecommunications network. This practice is attractive both for pharmaceutical retail, also referred to as pharmretail, and for consumers, but its legal aspects are still not regulated. To date, there is no such kind of a pharmacy, as online pharmacy (types of pharmacies were approved by the Order of the Ministry of Public Health and Social Development of Russia dated 27.07.2010 No. 553н), at the same time on the websites of many pharmacy organizations, you can find offers of the seller relating to pre-order of medicines, their reservations and later purchase directly at the pharmacy which is territorially located in the most convenient for the customer place [2,3].

According to experts, the segment of the electronic commercial (e-commerce) pharmaceutical market today occupies more than 6.5% of the total pharmaceutical retail. Since 2018, 25 million online orders of pharmacy products at the amount of more than 40 billion rubles have been made. According to Data Insight Agency estimates, the volume of orders of medicines on Internet platforms is growing rapidly and online service Apteka.ru is the leader with 30% of the market volume. Currently, such marketplaces as Yandex, Mail.ru, Ozon are developing the pharmaceutical direction. On the one hand, the paradigm of the pharmaceutical market is changing because the marketing strategies require transformations in the digital format and digitalization of trade. Permission for online sales of medicinal products provides additional opportunities for manufacturers of medicinal products to promote their brands, as it allows market participants to interact with each other with minimal costs, regardless of distance and in a convenient mode for them. Information about the medicine assortment is placed on the virtual storefronts of the company's website, which allows the seller to significantly reduce the cost of maintaining retail space and increase the consumer audience. On the other hand, the main task of pharmaceutical retail is to meet the needs of the population for high – quality, effective and safe medicines. In this regard, classic pharmacies have a number of advantages over their online counterparts, including a clear and effective assortment matrix, the possibility of pharmaceutical consultation and selection of a substitute medicine, as well as the absence of delivery costs. The above determines the relevance of the study of consumer opinions regarding the remote sale of medicines [4,5].

The purpose of this work was to study the desire and willingness of consumers to make purchases of medicines in online pharmacies.

STUDY METHODS

The study was conducted using survey, ranking, and grouping methods, as well as comparative and logical analysis. For the first stage of the experiment, a questionnaire was compiled that included, in addition to the socio-demographic characteristics of the respondents, a set of questions that allow assessing the attitude of the respondents to online purchases, including medicines. The representative sample included 103 persons at age of 18 and over (Fig. 1), mainly women (72%).

RESULTS AND DISCUSSION

According to the survey results, 73% of respondents make purchases in online stores, 15% of respondents are committed to offline retail and do not plan to make online purchases, 12% do not deny the possibility of purchasing goods through Internet services (Fig. 2). At the same time, more than half (56%) of online store users prefer to pay for goods when receiving an order: 31% – by Bank card, 25% – in cash.

The provision of pharmaceutical services in the modern pharmacy business should be combined not only with commercial price incentives. Non-monetary aspects of work, i.e. methods of interaction with consumers, are important when a client is making a decision about choosing a pharmacy for making a purchase [6]. Despite the rapid growth of sales in online stores, about a third (34%) of respondents plan to make remote purchases of medicines in online pharmacies, and another 35% do not deny this possibility if the necessary medicine is not available in a classic pharmacy.

The most popular reasons why respondents preferred offline pharmacies to their online counterparts: importance of advice and assistance from a pharmacist (14%), risk of purchasing the low-quality medicines in an online pharmacy (13%), lack of experience in making purchases in online stores (4%) (Fig. 3).

At the next stage of the study, respondents had to compare a classic offline pharmacy with an online pharmacy according to the proposed criteria such as assortment, price level, product quality, service time, quality of service and pharmaceutical advice. Since different pharmacy indicators have different degrees of importance

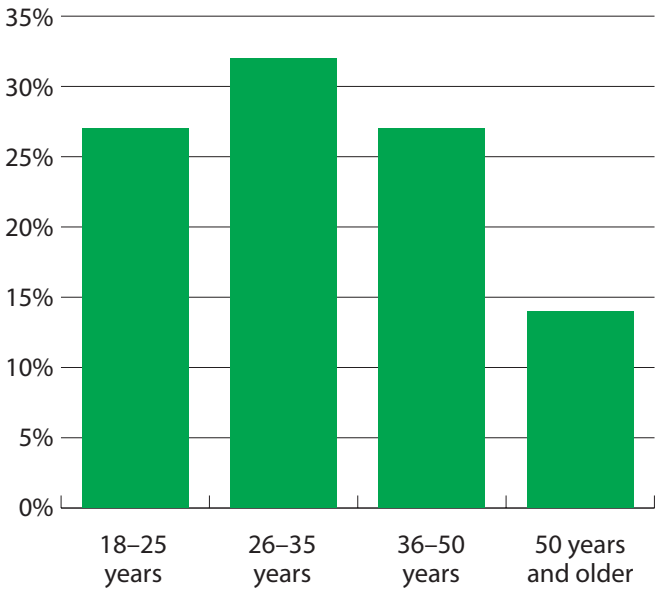

FIG. 1. *Age categories of respondents*

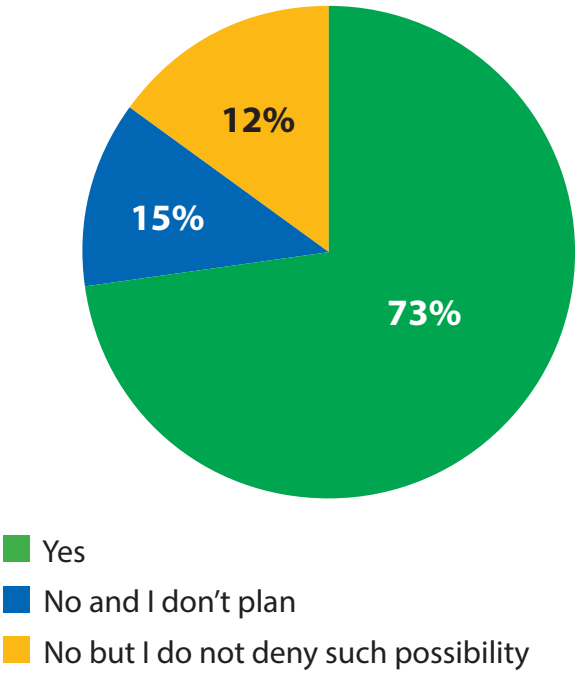

FIG. 2. *Respondents' opinion on purchasing the products in the online segment*

for consumers, a criterion weight was assigned to each of the listed parameters so that the total weight was 1.0. The rating was given by consumers based on a one-to-five scale, where 1 point is unsatisfactory, 2 points is bad, 3 points is satisfactory, 4 points is good, 5 points is excellent. After filling out and information acquisition, the questionnaires were subjected to statistical processing.

The rating (Ci) for each i^{th} criterion of pharmacies was calculated by the following formula:

$$Ci = \sum_{j=1}^{n} Aij \times Dj \times Ki / m, \quad (1)$$

where Aij – the rating of the i^{th} criterion by the j^{th} respondent; Dj – number of respondents, who assessed the i^{th} criterion; Ki – weight of the i^{th} criterion; m – total number of respondents.

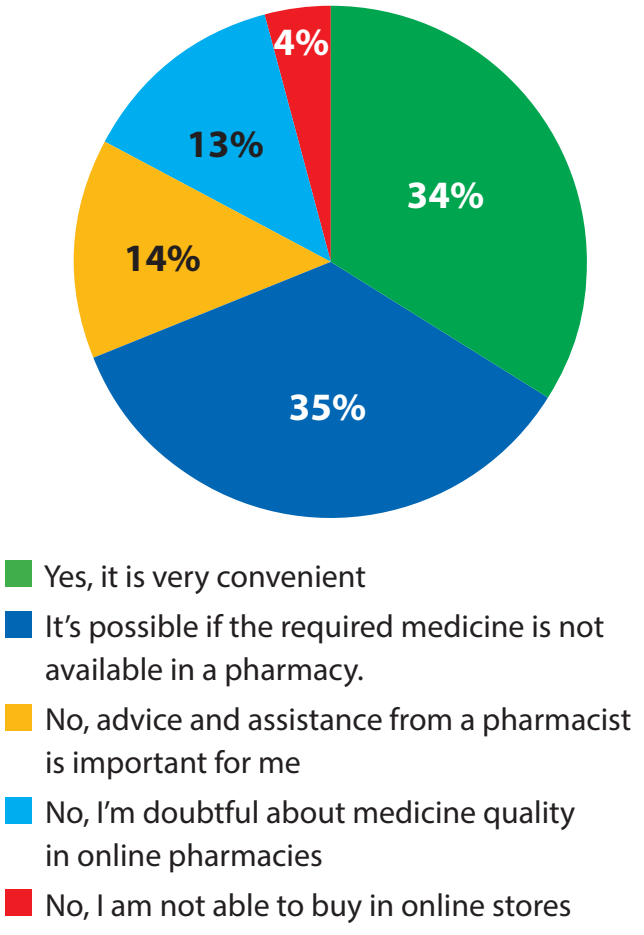

FIG. 3. *Respondents' opinions regarding the purchase of medicines in an online pharmacy*

Further, for clarity and further comparative analysis of the studied types of pharmacies, the integral indicator for the pharmacy was determined as a total score of all criteria. The results of calculating the integral score for a classic offline pharmacy based on a survey of 103 respondents are shown in Table 1.

Data in Table 1 show that in a classic stationary pharmacy, consumers rather highly evaluate the quality of the product, the product range and pharmaceutical advices. The resulting total scores are indicators for further comparative analysis of the studied types of pharmacies. The results of calculating the integral score for an online pharmacy based on the survey of 87 respondents are shown in Table 2.

Data in Table 2 show that for all respondents who purchase medicines in the e-commerce segment, of course, the quality and assortment of goods are of great importance. The price level index in the online pharmacy exceeds the same in the classical one by 0.1 point. Thus, according to the price criterion, consumers are more loyal to online retail. At the same time, there is a risk of encountering low-quality, counterfeit medicines in an online pharmacy, which is confirmed by a lower consumer rating of the "product quality" criterion in the Internet sector. The analysis on the "pharmaceutical advices" indicator translates buyers 'doubts about complete and timely pharmaceutical assistance when making online purchases of medicines. However, a slight difference in the integral scores of the analyzed types of pharmacies shows that consumers are ready to legalize the remote sale of over-the-counter medicines with the possibility of delivering them to their homes

One of the main principles of public health protection is the availability and quality of medical care, including medicinal care. The development of the segment of online trade in medicines will allow customers not only to get detailed information about the necessary medicines,

Table 1

RESULTS OF ESTIMATION OF PHARMACY INDICATORS BY RESPONDENTS

Criterion	Criterion weight	Number of respondents, who assessed					Criterion score
		1	2	3	4	5	
Assortment	0.2	2	3	19	59	20	0.8
Level of prices	0.1	1	7	42	47	6	0.3
Service time	0.1	2	6	26	42	27	0.4
Quality of goods	0.3	0	5	10	47	41	1.3
Quality of service	0.1	0	4	21	53	25	0.4
Pharmaceutical advice	0.2	0	1	27	41	34	0.8
Integral score	4.0						

their analogues and prices, but also to order the selected medicine with home delivery.

Analysis of the readiness of the market to the legalisation of distance selling of medicines shows that the format of the over-the-counter purchase of medicines will be enough demand in the consumer segment, but for the online selling of medicines the pharmacy is advisable to improve their business processes and have:

- an own online platform (website or mobile application) that meets the requirements of the information security and having certificate of conformity;
- a permission to carry out pharmaceutical activities in terms of retail sale of medicines remotely;
- an order acceptance and delivery service;
- a mobile acquiring system for making electronic payments directly at the place where the service is provided;
- equipment, ensuring the safety of medicines in accordance with the specified rules of good

Table 2

RESULTS OF RESPONDENTS' ASSESSMENT OF ONLINE PHARMACY INDICATORS

Criterion	Criterion weight	Number of respondents, who assessed					Criterion score
		1	2	3	4	5	
Assortment	0.2	2	1	6	44	34	0.8
Level of prices	0.1	1	5	23	42	16	0.4
Service time	0.1	3	2	19	41	22	0.4
Quality of goods	0.3	1	3	18	38	27	1.2
Quality of service	0.1	1	2	15	51	18	0.4
Pharmaceutical advice	0.2	9	9	44	19	6	0.6
Integral score	3.8						

practice of storage and transport (cold boxes, containers protecting fragile objects from breaking, etc.);
- local regulations (standard operating procedures) governing the delivery of medicines;
- personnel with a high level of professional skills and a desire for development and training.

It is assumed that access to Internet sites offering to purchase prescription medicines will be blocked by the Supervisory authorities that control the sphere of circulation of medicines. The potential risk of purchasing counterfeit products in the online segment, as well as a lack of awareness about the necessary actions to be taken if such products are suspected of being purchased, are the main reasons for refusing to buy medicines in an online pharmacy. In this regard, the main task of the regulatory authority is to monitor the compliance with the legal regulations relating to remote trade of medicinal products with a guaranteed high level of quality that meets the expectations of consumers.

Remote sale of medicines is a consequence of mass digitalization, the transition to a new level of information technology development. It is important that changes in legislation have a global character, and taking into account the fact that the issue under consideration is of international significance, it is advisable to adopt a regulatory act in the EEU space, as well as to develop cooperation at the CIS level and with the EU countries.

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PROBLEMS OF PHARMACEUTICAL CONSULTING AND WAYS TO SOLVE THEM: STANDARDIZATION, TRAINING, ADAPTATION OF INFORMATION

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Since 2017, quite a lot of studies have been devoted to the current legal status of pharmaceutical consulting and its informational content in the Russian Federation. Nevertheless, common approaches to this process are only being formed, and the problems of providing services directly in the pharmacy organization are not sufficiently developed. The study purpose was to compare the opinions of specialists and patients about the difficulties of pharmaceutical consulting in order to determine the vectors of process improvement. A survey of pharmaceutical specialists – retail pharmacists (n=93) and patients – visitors of pharmaceutical organizations (n=576) was conducted. Statistical analysis revealed that the main organizational complexity is specialist's limited time. The main communication problems are the fear of dissatisfaction of waiting visitors in the queue and the difficulty of interpreting the specialized information for the patient. The indicated information difficulties are the lack of own knowledge and the need for regular professional development. The main disadvantages for patients were the narrow range of medicines, the availability of a pharmacy employee, and the difficulty of understanding the terminology used. To improve the consulting process, first of all, it is necessary to optimize the assortment of pharmacy organizations, conduct staff training and improve standard operating procedures and algorithms for providing services with information adapted for the employee and patient.

Keywords: pharmaceutical consulting, pharmacy (drugstore), problems, organization, improvement

Data of sociological studies show high prevalence of self-medication among the population of the Russian Federation. The main reasons given by respondents are insufficient availability and quality of medical care, distrust of medical specialists and their qualifications, etc. However, the process of self-treatment is always associated with risks to the health of patients. Pharmaceutical specialists, in particular retail pharmacists, can significantly influence on the

use of medicines by patients to improve the safety of therapy first of all [1].

The pharmaceutical community, in turn, does not always properly implement the functions assigned to it by the health care system to ensure the continuity of pharmaceutical care in the chain of "medical specialist – patient – pharmacy employee" [2]. One of the ways to preserve and maintain the health of the population is pharmaceutical counsulting [3]. This pharmaceutical service is provided directly in the process of selling the products of the pharmacy assortment, which is determined by the Rules of Good Pharmacy Practice[1]. A large number of studies are devoted to pharmaceutical consulting, and there is a legal conflict in the current regulatory field, which consists in the lack of common approaches to the terminology and format of service provision [4]. On the one hand, in accordance with Federal Law No. 323 of 21.11.2011[2], the patient is the central figure in the provision of medical and pharmaceutical care. On the other hand, Federal Law No. 61 of 12.04.2010[3], and order No. 403н[4] of the Ministry of Health of the Russian Federation of 11.07.2017 refer to pharmacy visitors only as "a person purchasing a medicinal product", while order №647н[5] of the Ministry of Health of the Russian Federation of 31.08.2016 uses the term "buyer" [5]. This discrepancy in the status of a pharmacy visitor, in turn, can lead to a lack of a common understanding of the object of the pharmaceutical consulting process and the formation of a conflict of interest among its participants.

For professional consulting in a pharmacy organization, a specialist must have "medical and pharmaceutical knowledge, communication and commercial skills, methods of selling goods, analytical methods for evaluating the consumer behavior, etc." [6]. However, in the conditions of the modern pharmaceutical market, there is often a bias towards solving the marketing problems, which naturally leads to a shift in emphasis when recommending the medicines in favour of the medicines which are most promoted by manufacturers or distributors [7].

Another problem with the provision of qualified medical care is violation of communication. In the study conducted at Yaroslavl State Medical University (2018), it was found that a pharmacy employee must be "conflict-resistant, open, contactable, attentive, flexible, competent, informed, and able to ask correct questions" [8], which is formed not only due to the personal qualities, but also due to appropriate education and training. The situation is complicated by the limited time and resources of the pharmacy organization.

Nevertheless, the active introduction of digital technologies allows you to speed up and simplify the process, which leads to optimization of service delivery [9]. Consulting is relevant not only for self-medication, but also for the purchase of prescription drugs prescribed by a doctor [10], educational work to reduce the spread of antibiotic resistance, limit the unjustified and irrational use of antibacterial drugs [11], as well as to promote health and medical literacy,

[1] Order of the Ministry of Health of the Russian Federation No. 647н of August 31, 2016 "On approval of the Rules of Good Pharmacy Practice of medicines for medical use.

[2] Federal Law dated 21.11.2011 №323-FZ "On the basics of public health protection in the Russian Federation".

[3] Federal Law dated 12.04.2010 №61-FZ "About circulation of medicines".

[4] Order of the Ministry of Health of the Russian Federation No.403н of July 11 2017 "On approval of the Rules of sale of medicines for medical use, including immunobiological medicines, by the pharmacy organizations, individual entrepreneurs having the license for pharmaceutical activity".

[5] Order of the Ministry of Health of the Russian Federation No. 647н of August 31 2016 "About approval of Rules of good pharmacy practice for medicines for medical use".

prevent chronic diseases, improve compliance and prevent potential risks of side effects [12,13].

Despite the increasing number of studies devoted to external factors of consulting, the analysis of problems of providing pharmaceutical services directly in the practice of a pharmacy organization, confirmed by statistically reliable studies, is not yet sufficiently covered in the literature, and often the views of a specialist and a patient may differ significantly.

The purpose of the study is to compare the organizational, communication and information problems of specialists and visitors in pharmaceutical consulting in pharmacy organizations.

MATERIALS AND METHODS

100 pharmaceutical specialists (senior pharmacists and pharmacists) working directly with the population (retail pharmacists) were surveyed according to the developed questionnaire. 93 questionnaires were found to be suitable for further processing of the received information (the response was 93%, the sampling error was 8.6%) [14]. A survey of 600 pharmacy visitors in Khabarovsk and Moscow (432 and 144 respondents, respectively) was conducted using a specially developed questionnaire (response was 96%, sampling error was 4.1%) [14]. The respondents' responses were processed using Microsoft Office Excel 365 programs with the use of the "Subtotals" data package. The average value of the age of employees and patients, and the length of employment of specialists is presented as the average value ± standard error. Statistical data processing was performed using the IBM SPSS Statistics 25.0 software package. To determine the correlation with the gender of respondents and work experience in the specialty, a nonparametric criterion was used such as a Spearman's rank correlation coefficient. The correlation was considered as reliable with a two-sided significance of $p0<0.05$.

RESULTS AND DISCUSSION

This study examined three main factors in the pharmaceutical consulting process: organizational, communication, and informational. The problems identified during the actual implementation of the process in the pharmacy for both specialists and visitors were also analyzed in the context of these three aspects.

The analysis of the typological characteristics of respondents being the pharmaceutical specialists revealed that the majority of respondents were female – 95.7%; the average age was 38.69±1.31 years; the average work experience in the specialty was 14.01±1.34 years. Average profile of a pharmacy visitor: average age – 37.69±0.74 years; female – 67.4%; social status –working – 65.74%.

The main organizational problems of pharmaceutical consulting, according to pharmacy employees, are shown in Fig. 1.

The existence of organizational difficulties was confirmed in 96% of the questionnaires. The main difficulty in providing consulting services, most experts call the lack of time. This problem can be eliminated by hiring the additional employees to the staff of pharmacy organizations and / or their hourly employment directly during the maximum load on the main staff. One of the ways to reduce the service time may be to standardize the service by the introduction of an algorithm. However, only 3.7% of pharmacies indicated its absence, which proves the need to optimize the existing standard operating procedures for practical activities.

Another common response was the lack of a special area for pharmaceutical consulting, which is allowed by the Rules of good pharmacy practice. Establishment of a special department

is considered rational by 40% of visitors. Eliminating this disadvantage requires serious financial costs for equipment, and in addition, the availability of the appropriate space, which is not always available for a specific organization.

According to 7.5% of the surveyed specialists, pharmaceutical consulting is not in demand and there is no motivation for it. Since it is an integral part of the retail sale of pharmacy products, it is necessary to educate and train staff in pharmacy organizations. There was no correlation with the gender and work experience of employees.

At the next stage of the survey, the respondents were asked to clarify communication difficulties when working directly with visitors. The distribution of responses is shown in Fig. 2. The total number of affirmative responses was 93.5%.

The most common response from respondents was the risk of dissatisfaction among users waiting in line. Options for eliminating this disadvantage can be installing monitors directly in the sales room with demonstration of social or specialized advertising, redistributing the electronic queue based on the needs of visitors, using coupons, etc.

The main professional problem in this case is the complexity of interpreting the specialized medical and pharmaceutical information for both the patient and the specialist. This skill requires training and professional development of staff and/or availability of accessible and adapted sources of information/software products / algorithms, as well as systematic clarification of the patient's understanding of information and mitigation of possible negative reactions on his part. There was also no correlation between respondents' responses and their gender or professional experience

An equally important component of the pharmaceutical consulting process is information. The distribution of respondents' responses by difficulties in implementing of this component is shown in Fig. 3. The total number of positive responses was 89%.

The main problems of pharmaceutical consulting were identified by the majority of respondents as the lack of their own knowledge, the need for regular professional development, and the complexity of specialized information. Based on the survey data obtained, we can conclude that it is rational to train specialists, including directly at the workplace, as well as to create adaptive information materials with simplifying and combining the data. It is the need to systematize data in the implementation of pharmaceutical consulting that was most often indicated by respondents with the longer work experience in the specialty (rs=0.130,3220,49; p=0.002).

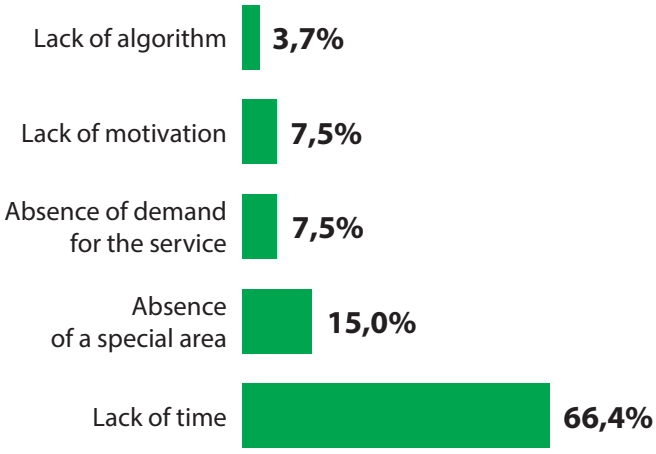

FIG. 1. *Organizational problems of specialists during pharmaceutical consulting*

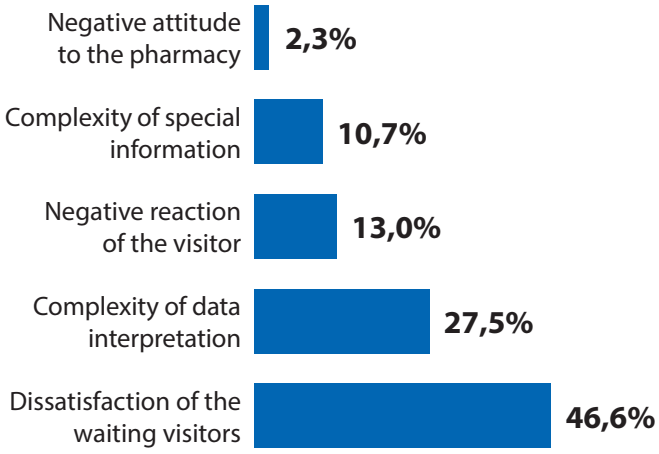

FIG. 2. *Communication problems of specialists during pharmaceutical consulting*

At the next stage of the survey, the opinions of specialists and patients were compared. The distribution of visitirs' responses is shown in Fig. 4.

70% of respondents did not mention any difficulties in obtaining pharmaceutical advice. 123 patients indicated the existence of disadvantages (hereinafter they were taken as 100%). The surveyed pharmacy visitors, as well as specialists, identify organizational problems as the main ones. There was no significant correlation with the gender and age of the respondents. The main difficulty was the lack of an assortment of medicines in the pharmacy organization. This disadvantage is eliminated by improving the marketing activities (demand analysis), logistics (product and transport), the system of inventory shortage accounting and order.

The opinions of visitors and specialists coincided in relation to the main organizational, communication, and information problems: employee unavailability, and the difficulty of adapting and perceiving the information by the user. At the same time, 15.4% of visitors indicated indifference of pharmacy employees, and only 7.6% of specialists indicated lack of motivation, which may be due to the presence of a conflict of interests between both participants in the process: "the effectiveness of treatment – the desire to save money", on the one hand, and "the effectiveness of treatment – commercial tasks", on the other.

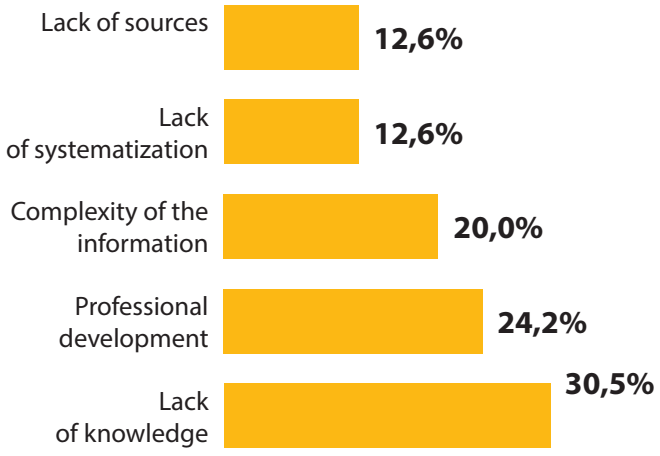

FIG. 3. *Information problems of specialists during pharmaceutical consulting*

Thus, when providing pharmaceutical consulting services, it is necessary to take into account the potential interests of all participants in the process, provided that the standards of customer service are constantly improved and updated and their practical implementation is monitored.

CONCLUSIONS

The majority of pharmacy visitors surveyed are satisfied with the quality of pharmaceutical consulting, with 70% of respondents not reporting any problems. Among these disadvantages, organizational ones prevail such as the lack of time for a specialist and the unavailability of a pharmacy employee for a patient. Among the most important communication and information disadvantages, the complexity of medical and pharmaceutical information, its interpretation for patients and difficulties in perception prevail. One of the most rational ways to eliminate these disadvantages is to optimize the algorithms for providing pharmaceutical consulting services with the adaptation of the information for the patient and the possibility of its use by the retail pharmacist directly at the workplace.

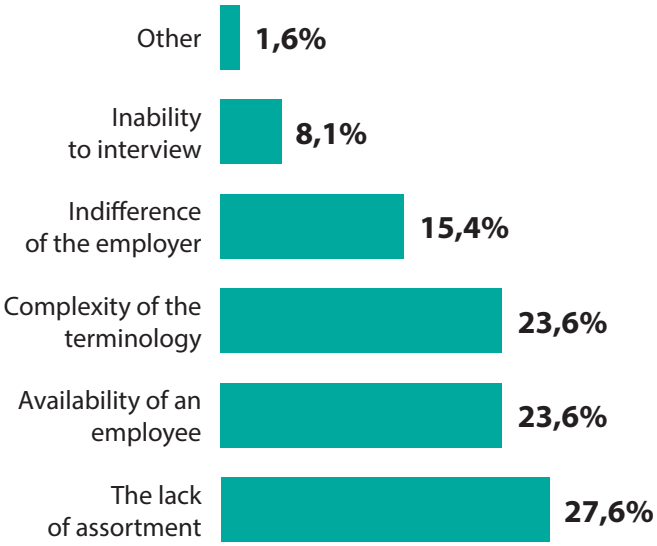

FIG. 4. *Disadvantages of pharmaceutical consulting in pharmacy organizations, according to patients*

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DETERMINATION OF THE PURITY OF THE NEW PHARMACEUTICAL SUBSTANCE 4.4'-(PROPANDIAMIDO) SODIUM DIBENZOATE BY CHROMATOGRAPHIC METHODS

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Methods for determining related impurities and residual organic solvents to determine the purity of a novel active pharmaceutical substance 4,4'-(propanediamido) sodium dibenzoate have been developed and validated using high performance liquid chromatography and gas chromatography methods

Keywords: active pharmaceutical substance, purity, related impurities, residual organic solvents, high performance liquid chromatography, gas chromatography

When standardizing a new pharmaceutical substance (APS), one of the most important characteristics is its purity, which is determined by such quality indicators as "Melting point", "pH", "Weight Loss on Drying", "Sulfate Ash", "Heavy Metals" and "Microbiological Purity", which are standard parameters. Procedures and criteria for assessing the quality of a substance are defined by the General Monograph (GM) of the State Pharmacopoeia of the Russian Federation (RF SP). Determination of impurities by the indicators "Related impurities" and "Residual organic solvents" (ROS) requires the development of analytical methods suitable for assessing the quality of the substance, taking into account the synthesis schemes (the initial compounds and solvents used).

The purpose of this study was to develop and validate methods for determining related impurities and ROS in the new APS "4,4'-(propanediamido) sodium dibenzoate" with the use of chromatographic methods of analysis.

MATERIALS AND METHODS

Substance 4,4'-(propandiamine) sodium dibenzoate (conditional name – Malaben), synthesized at the Department of Organic Chemistry of Saint-Petersburg State Chemical-Pharmaceutical University (SPCPU) is a derivative of Malonic ether (ME) and p-aminobenzoic acid (PABA) and has anti-atherosclerotic, anti-alcoholic and antioxidant effects [1,2]. The study was performed using microcolonies high performance liquid chromatograph "Milichrom A-02" with a UV detector and a column filled with sorbent ProntoSIL 120-5-C18 AQ, geometry 75×2 mm (manufacturer – "EcoNova", Novosibirsk, Russia) as well as gas chromatograph "Crystal-5000" with a flame ionization detector (FID) and a column filled with

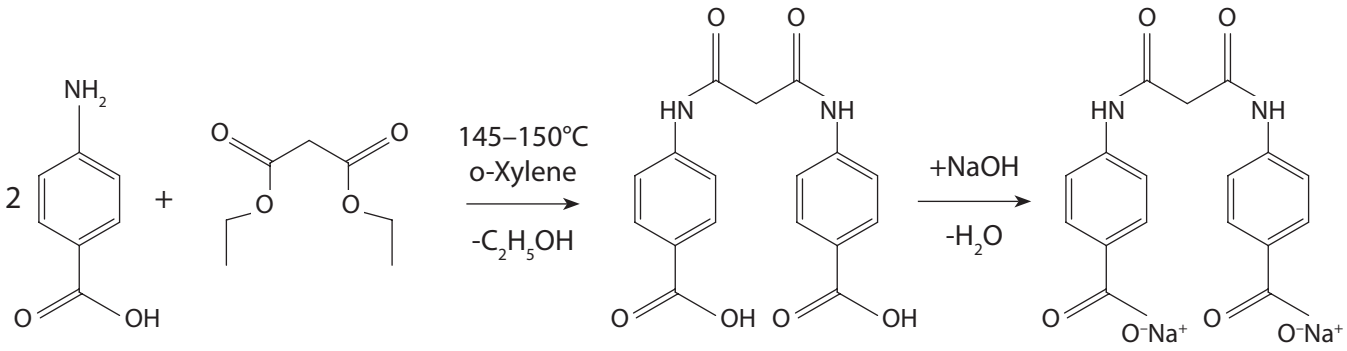

FIG. 1. *Scheme of synthesis 4,4'-(propandiamine) sodium dibenzoate*

polydimethylsiloxane liquid stationary phase Solgel MS-1 (manufacturer – SKB "Chromatek", Russia, Yoshkar-Ola).

RESULTS AND DISCUSSION

Malaben synthesis is carried out according to the scheme shown in Fig. 1, followed by recrystallization from alcohol. Malaben is a disodium salt which is easily soluble in water; practically insoluble in acetonitrile, ethanol, ethyl acetate, chloroform. Protonated form 4,4'-(propandiamine) sodium dibenzoate is insoluble in water, alcohol, acetonitrile, ethyl acetate, soluble in DMSO/DMFA.

As related impurities, based on the synthesis scheme, the target product may contain the initial PABA and ME compounds, as well as the by-product 4-((3-oxo-3-ethoxypropanoyl)amino) benzoic acid, which is a monosubstituted ME amide (conditional name – Etmaben) (Fig. 2).

When developing methods for the analysis of impurities, we were guided by the general standards for the maximum content of identified impurities imposed by the RF SP, which should not exceed 0.1%.

ROS of o-xylene belongs to toxicity class II, and its content in the substance is permissible within 0.217%. The weight loss on drying (WLOD) of the substance was less than 0.5%, so the ethyl alcohol used at the last stage of synthesis was not controlled [3].

Determination of non-volatile related impurities was performed by reverse-phase HPLC with UV detection.

The choice of the composition of the mobile phase was provided by the chemical properties of the determined impurities and APS. The studied objects of analysis are polar compounds, so to increase their affinity to the reverse-phase sorbent and, consequently, to increase the efficiency and selectivity of chromatographic separation, the pH of the mobile phase should be low. The value of pKa for the PABA is 4,85 [4], for malaben is 5.6 and 7.2 [5]. Based on these values, the optimal pH value of 2.8 was selected. It should be noted that a lower pH value leads to sedimentation of the protonated form of malaben.

At the selected pH value, all analyzed compounds have a maximum absorption of 270 nm. The selected conditions for chromatographic analysis [6] are specified below (Table 1).

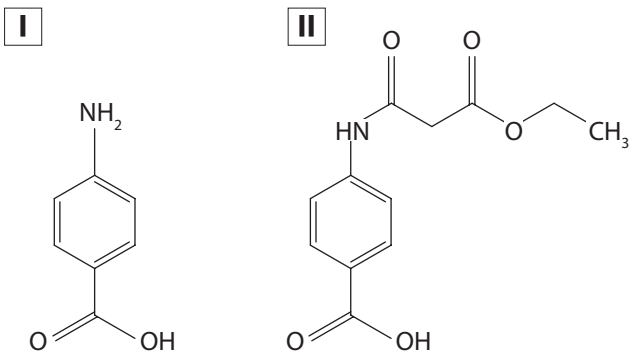

FIG. 2. *The structural formulas of the related impurities (I – PABA, II – Etmaben)*

Table 1

ANALYSIS CONDITIONS

Column	ProntoSIL 120–5-C18 AQ 75 mm × 2 mm × 5 µm, an alternative column that meets the requirements of the chromatographic system is allowed to be used.
Mobile phase	Phosphoric acid solution (pH=2.8) – methanol Linear gradient from 30 to 80% methanol. Chromatogram recording time – 20 minutes.
Flow rate	0,1 ml/min
Column temperature	40°C
Detector wave length	270 nm
Volume of sample	2 µl

The test solution was prepared at concentration of 1 mg / ml. Standard solutions of impurities were prepared by successive dilution of the stock solutions to concentration of 1 µg /ml. A 40% water solution of methanol was used as the solvent. To assess the resolution of the peaks, a standardized test solution was prepared (a test solution with additives of identified impurities). Typical chromatograms of the test and standardized test solutions are shown in Fig. 3

The results of the analysis of the tested solutions and spike solutions with additives of known impurities showed the presence of an impurity of PABA (less than 0.1%) and peaks of single unidentified impurities, as well as the absence of impurity of etmabene.

Determination of volatile related substance and residual organic solvents was performed by gas chromatography using a flame ionization detector. To determine the content of ME as a stationary phase (SP), it is advisable to use a chromatographic column with a moderately polar SP (for example, cyano-propylphenylmet hylpolysiloxane), for example, DB-624, ZB-624, DB-1701, DB-210. Since the number of analytes in the sample is insignificant, it is possible to use polyphenylmethylsiloxane SP, for example,

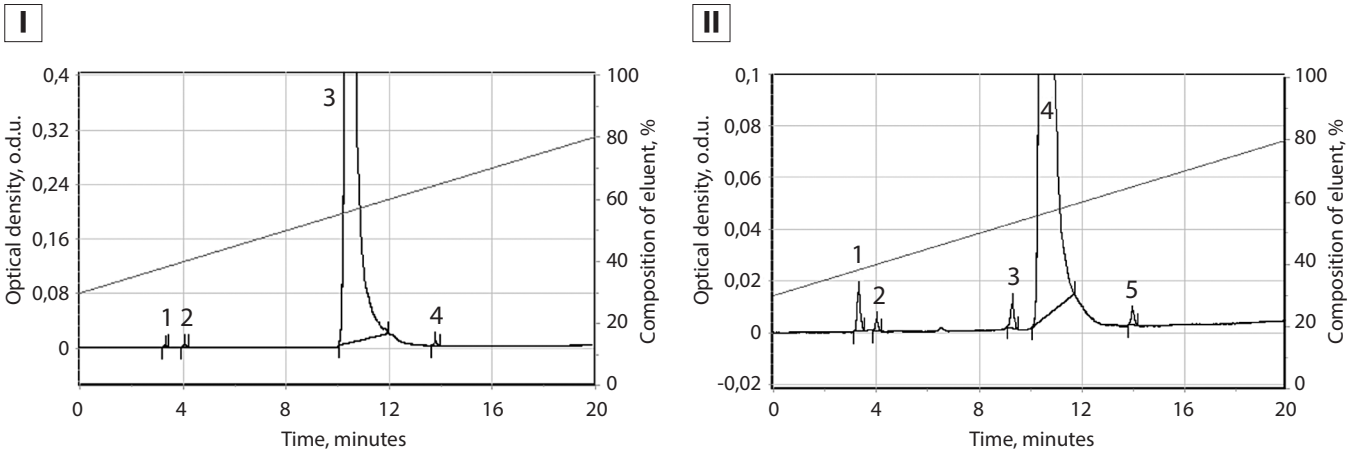

FIG. 3. *Typical HPLC chromatograms (I – test solution: peak 1 – PABA, peak 2 – i.u.i. (individual unspecified impurity), peak 3-malaben; II – test solution with the addition of specific impurities: peak 1 – PABA, 2 – i.u.i.., 3 – etmaben, 4 – malaben, 5 – i.u.i.)*

HP-5, ZB-5, DB-5, SE-54, OV-5, PPMS, or dimethylpolysiloxane, for example, HP-1, ZB-1, DB-1, SE-30, OV-1, PMS [7]. PS tests to determine the content of volatile related substances were performed under the following chromatographic conditions (Table 2).

Figure 4 shows typical chromatograms for determining the Malonic ester impurity under selected chromatographic conditions.

The residual content of o-xylene was determined under the same chromatographic conditions (Fig. 5).

To increase the reproducibility of the results, the analysis was performed using an internal standard (spike) (naphthalene).

For all the above methods of analysis, a validation assessment was performed for the following parameters: specificity, limit of quantification (LOQ), linearity, precision, correctness, analytical area, stability, and suitability of the chromatographic system. The validation results presented in table 3 show that the analytical methods are suitable for solving the tasks set.

Determination of the purity of the substance of 4,4'-(propanediamido) sodium dibenzoate was performed on three batches of samples [8]. The results of the analysis are presented in Table 4.

CONCLUSIONS

In course of experiments:

- the methods of chromatographic analysis (HPLC and GC) of related substances and ROS

Table 2

CHROMATOGRAPHIC CONDITIONS FOR THE CONTENT OF VOLATILE RELATED SUBSTANCES

Column	Chromatographic capillary column Solgel MS-1 with dimethylpolysiloxane liquid stationary phase (LSP), LSP film thickness is 0.25 micrometer, inside diameter of the column is 0.25 mm, column length is 30 meters, or similar, satisfying the criteria of suitability of the chromatographic system
Carrier gas	Helium, the pressure at the inlet of the column – 75 kPa, split ratio – 1/50
Column conditioning	At the temperature of the column oven 250°C for 30 minutes
Temperature of the column oven	Temperature programming mode: the initial temperature of the column oven is 100°C for 4 minutes, then increase at a rate of 5°C / min up to 155°C, then increase the temperature of the column oven up to 250°C for subsequent conditioning (purging)
Injector temperature	240°C
Detector temperature	250°C
Volume of a sample	2 µl
Detector	A flame ionization detector. Conditions of gas supply in accordance with its operational documentation or: air flow rate – 300 ml /min, hydrogen flow rate – 30 ml /min, blowing flow rate – 10 ml/min
Run Time:	The approximate run time is 15 minutes or 50% longer than the retention time of Malonic ester

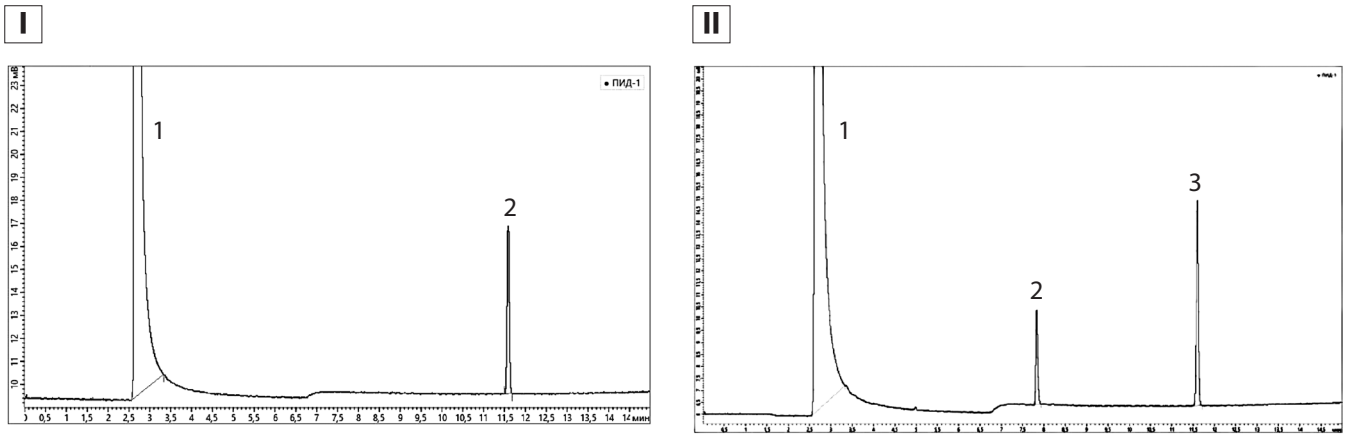

FIG. 4. *Typical GC chromatograms (I – test solution: peak 1 – ethyl alcohol, peak 2 – naphthalene; II-spike test solution with an addition of Malonic ester: peak 1 – ethyl alcohol, peak 2 – Malonic ester, peak 3 – naphthalene)*

in substance 4,4'-(propandiamine) sodium dibenzoate, showing pharmacological activity were developed;

- validation of the developed methods was carried out;
- the results of the analysis of three batches for determining the purity of substance 4,4'-(propandiamine) sodium dibenzoate in terms of "Related substances" and "Residual organic solvents" meet the stated requirements.

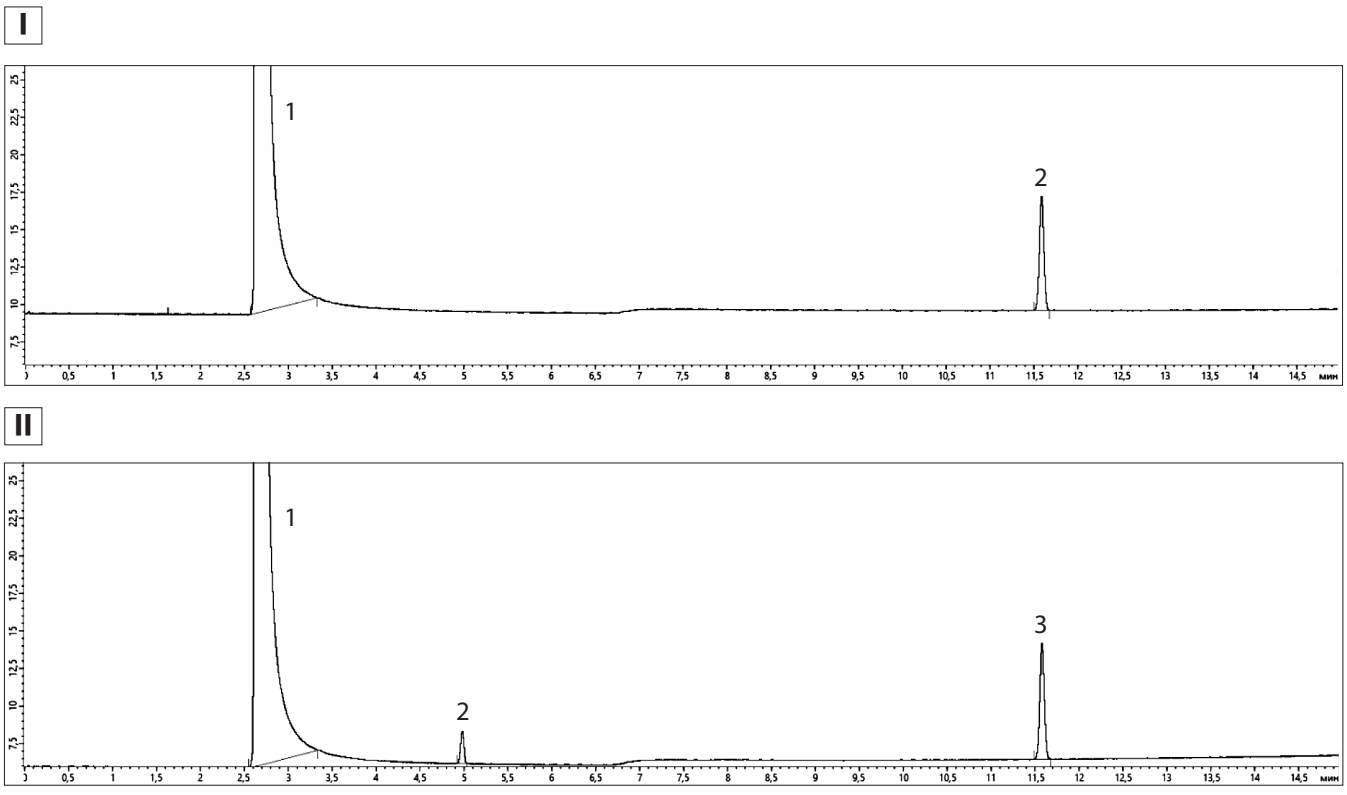

FIG. 5. *Typical GC chromatograms (I – test solution: peak 1- ethanol, peak 2 – naphthalene; II – spike test solution with addition of o-xylene: peak 1 – ethyl alcohol, peak 2 – o-xylene, peak 3 – naphthalene)*

Table 3

THE RESULTS OF THE VALIDATION OF ANALYTICAL METHODS

Validation factors	Result of checking the validation factors of the analytical method		
	Related substances by HPLC method	**Related substances by GC method**	**Residual organic solvents**
Specificity	• the solvent does not have system peaks and does not prevent the determination of related substances; • the resolution between the peaks is greater than 2.5	Chromatograms contain only those peaks that meet the requirements of the chromatographic system specificity, and there are no coeluating system peaks	Chromatograms contain only those peaks that meet the requirements of the chromatographic system specificity, and there are no coeluating system peaks
Linearity	• r = 0,9995; • Y-intercept, – 4,0%	• r = 0,9994; • the absolute value of the free term in the linear regression equation (0.0002) does not exceed its confidence interval (0.00745)	• r = 0,9970; • the absolute value of the free term in the linear regression equation (0.0024) does not exceed its confidence interval (0.045)
Limit of quantification (LOQ)	With concentration of 0.1 µg /ml: • signal-to-noise ratio 14.8; • RSD = 3,4%	With concentration of 3 µg /ml: • signal-to-noise ratio 30	With concentration of 1.6 µg /ml: • signal-to-noise ratio 30
Repeatability	RSD = 1,3%	RSD = 3%	RSD = 4%
Intermediate precision	• $F_{exp.} = 2,14 < F_{table} = 5,05$ • $t_{exp.} = 2,02 < t_{table} = 2,20$	• $F_{exp.} = 1,42 < F_{table} = 5,05$ • $t_{exp.} = 2,05 < t_{table} = 2,57$	• $F_{exp.} = 1,91 < F_{table} = 4,28$ • $t_{exp.} = 0,86 < t_{table} = 2,45$
Accuracy	The absolute value of the free term in the linear regression equation (0.0070) does not exceed its confidence interval (0.0075)	The difference between the obtained and specified ME contents in the solution is statistically insignificant with a confidence probability of 95%	The difference between the obtained and specified ME contents in the solution is statistically insignificant with a confidence probability of 95%
Range	from 0,1 to 1,2 µg / ml (from LOQ to 120% of impurity content)	from 3 to 80 µg /ml (from LOQ to 150% of impurity content)	from 1,6 to 40 µg / ml (from LOQ to 150% of impurity content)

Table 4

ANALYSIS OF SAMPLES OF 4,4'-(PROPANEDIAMIDO) SODIUM DIBENZOATE BY PURITY VALUES

Quality attributes	Normal values	Test results		
		batch 010317	batch 020417	batch 030517
Related substances, %	PABA impurity – not more than 0,1; i.u.i.* – not more than 0,1; total impurities – not more than 0,5	Not identified Less than 0,1 Less than 0,5	Less than 0,1 Less than 0,1 Less than 0,5	Less than 0,1 Less than e 0,1 Less than 0,5
	Malonic ester – not more than 0,1	Not identified	Not identified	Not identified
Loss of drying (LOD), %	not more than 0,5	0,31	0,35	0,4
Residual organic solvents (ROS), %	o-Xylene – not more than 0,217	Not identified	Not identified	Not identified

** i.u.i. – individual unspecified impurity*

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DISTRIBUTION OF PECTIN-CONTAINING SUBSTANCES OBTAINED FROM COSMOS BIPINNATUS BETWEEN TWO LIQUID PHASES

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In this work, we studied the distribution of pectin substances obtained from the Cosmos bipinnatus herb, of different varieties ("Purity", "Rosea", "Dazzler"). The concentration range of aqueous solutions is 0.1–1.0%, extraction systems are "ethyl acetate-water", "n-octanol-water". The efficiency of extraction is shown depending on the variety of Cosmos bipinnatus herb and the nature of the extractant.

Keywords: liquid extraction, pectin substances, ethyl acetate, n-octanol, Cosmos bipinnatus Cav., distribution coefficient.

Cosmos bipinnatus Cav. is an annual plant with height from 50 to 150 cm, strong, erect and unbranched stems. Leaves are opposite, double divided. Inflorescences are represented by baskets. The baskets are large, with a diameter of 3 to 7 cm. There are two types of flowers: medium are tubular, small, five-dimensional, actinomorphic, bright yellow; marginal are lingual, large, three-dimensional, zygomorphic, of various colors (depending on the variety). The fruit is represented by a gray-brown achene. Cosmos bipinnatus is found in the wild in the forests of South America and Mexico. The plant is cultivated in the climatic conditions of various territorial entities of the Russian Federation (Stavropol territory, Krasnodar territory, Rostov region, Moscow region, Volgograd region, Astrakhan region, the Urals, various regions of Siberia, the Republic of Buryatia, Transbaikalia, South Yakutia, etc.), blooms from early summer to late autumn. Cosmos bipinnatus forms dense high thicket; it is often used to create green hedges, as well as for the design of flowerbeds, ridges and decorative borders. In culture, the

plant is unpretentious, resistant to the plant, bacterial and fungal infections. It is propagated by seeds and vegetatively [1,2].

Cosmos bipinnatus is represented by a range of varieties, the most common of which are the following:

- Dazzler – fresh-blown inflorescences of this variety, they are red, but their color eventually changes to crimson or purple, the stems are high (up to 1.5 m), strong, erect, often pigmented in pink or purple;
- Purity – reed flowers of snow-white color with tubular bright yellow inflorescences which are baskets on flexible peduncles up to 1 meter high, stems are strong, erect, not pigmented in pink or purple;
- Rosea – inflorescences with pink tongue flowers, up to 1.5 m high, stems are strong, erect, pigmented in pink [3,4].

There is little data on the use of Cosmos in medicine, but it is known that its roots are used in Tibetan medicine as a hemostatic, antipyretic, diuretic, antitoxic, tonic.

Pectin substances are important bio-organic compounds that are involved in the production and storage of energy, water retention in cells, activation of the immune system, and detoxification of the body.

The use of pectin in pharmacy and medicine is very promising. The sources of its production are different: leaves, grass, fruits, vegetables, berries, etc.

Therefore, it was interested to study the physical and chemical properties and determine the possibility of using pectin substances isolated from the herb of numerous varieties of Cosmos.

The chemical composition of Cosmos bipinnatus Cav. is currently little studied. In the international literature, there is information about the presence of flavonoids and essential oils that have an antimicrobial effect, in plants of this type. The carbohydrate composition can be considered as practically unexplored, despite the relatively high yield of these compounds when they are obtained (4–9% of the initial weight of raw materials), as well as the large biomass of the plant, the unpretentiousness of cultivation and good resistance to plant infections [5–7].

Purpose of work is to determine the distribution coefficient of pectin substances in the two-phase system "organic solvent-water" and establish their sorption capacity. The high ability of pectin substances to pass from the aqueous phase to the organic phase during liquid extraction allows us to assume the ability of these pectin substances to pass through semipermeable membranes, including biological ones. Pectin substances are widely used in dosage forms as natural sorbents that can bind heavy metals. The penetration of pectin substances through biological membranes into cells increases their activity and prevents denaturation of cellular enzymes by binding to heavy metals. Therefore, it is very important to know the physical and chemical properties of pectin, in particular their ability to penetrate in model systems that reproduce the conditions of the internal environment of the body.

The studied samples of pectin substances were transparent and very light plates of light brown color with sour smell and taste, and were soluble in water. After acid hydrolysis, the monosaccharide composition of the resulting hydrolysate was determined in a water bath for 48 hours. It was found that the composition of all varieties of pectin substances includes monosaccharides: glucose, arabinose, galacturonic acid [8,9].

MATERIALS AND METHODS

Isolation of pectin substances from the herb of three varieties of Cosmos bipinnatus was carried using the method developed by N.K. Kochetkov and M. Sinner (Fig.1). The yield was 4.23% for the Purity variety, 6.85% for the Rosea variety, and 8.37% for the Dazzler variety, which in itself deserves attention from the point of view

of using this plant as a source of preparative production of these compounds [10]. In this regard, it was interested to study their physical and chemical characteristics, in particular the degree of extraction, distribution coefficient, and degree of association.

For the obtained pectin substances, their distribution between two phases of immiscible liquids was studied using conductometry to establish equilibrium concentrations during the extraction process. Conductometer of brand "Expert-002" was used in the work. Aqueous solutions were prepared in the concentration range from 0.1% to 1%, their electrical conductivity was measured, and a calibration graph was plotted as a function of C.

The method is based on different solubility of pectin substances in water and extractant. The better the substance dissolves in the extractant, the more chemically resistant it is to this

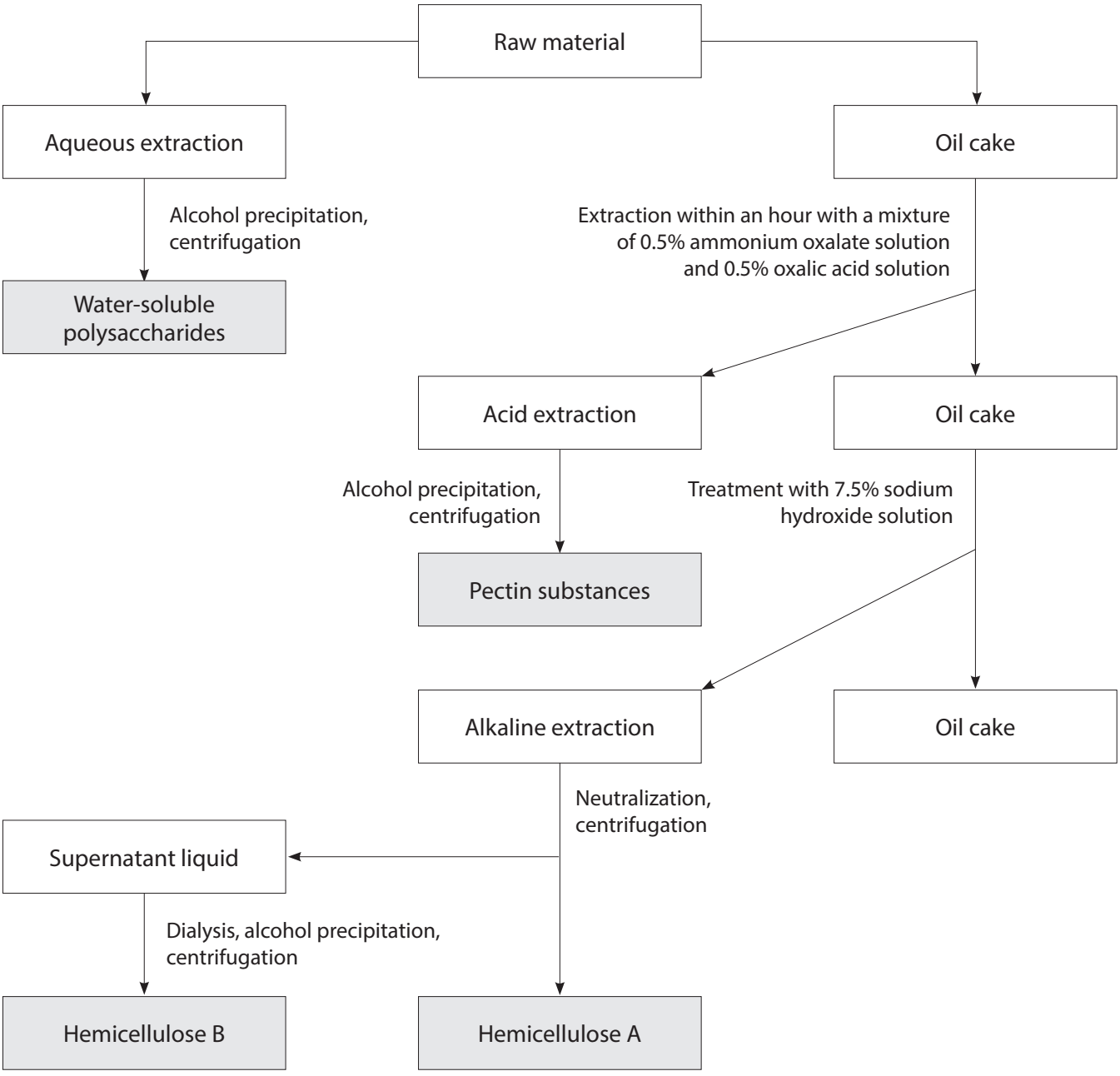

FIG. 1. *Flow-diagram for obtaining different fractions of polysaccharides from the herb of Cosmos bipinnatus*

extractant, the less the pectin substance dissolves in the extractant, the more effective the extraction process and the method itself are [12].

30 ml of an organic solvent was added to 30 ml of an aqueous solution of the pectin substance. As an organic solvent, ethyl acetate ester and alcohol – n – octanol of the "CP" brand were used. These solvents were selected taking into account the requirements for extractants such as low solubility in water, low vapor elasticity at room temperature, high extraction capacity, non-toxicity, density different from water. N-octanol was chosen because the membranes of the gastrointestinal tract are comparable to the "n-octanol – water"system.

The solutions were periodically shaken and after reaching concentration equilibrium (30 min.) the water phase was separated. The electrical conductivity of the aqueous solution was measured and the equilibrium concentrations of the pectin substance in both phases were determined. The distribution coefficient that is the concentration distribution constant was calculated using the formula (1):

$$K = \frac{C_1}{C_2^n}, \qquad [11]\ (1)$$

where n is the degree of association, and C_1 and C_2 are the equilibrium concentrations of the pectin substance in the raffinate and extractant.

The distribution coefficient characterizes the dynamic equilibrium in the extraction process. Its value depends on the nature of the pectin substance, the purity of the extractant, and the temperature.

The equilibrium concentration of the pectin substance in water was determined using linear dependences of 1/R0 on C, shown on the example of the pectin substance obtained from the Rosea and Dazzler Cosmos herb (Fig. 2).

The degree of association was determined using the bio-logarithmic distribution isotherm, shown by the example of the pectin substance obtained from the Cosmos bipinnatus herb of the Purity variety (Fig. 2).

The results of the experiment are presented in table 1.

The sorption capacity of the pectin substance with respect to lead ions was studied in vitro. Ostwald isolation method [8] was used which takes into account changes in the concentration of one participant in the complex formation process with minimal influence of the other. In this case, the reaction was carried out with an excess of the sorbent, then the sorption rate was proportional to the concentration of the reagent taken in the lack (lead ions).

After reacting lead acetate with the pectin substance aqueous solutions and separating the lead pectate precipitate, the determination of lead ions in the filtrate was performed by titration of 0.01 M EDTA (sodium ethylenediaminetetraacetate di-substituted) in the acetate buffer medium (pH=5). Xylenol orange was used as an indicator. Titration was performed before the transition of the crimson color to lemon-yellow [8].

The results of the experiment are presented in Table 2 using the example of the pectin

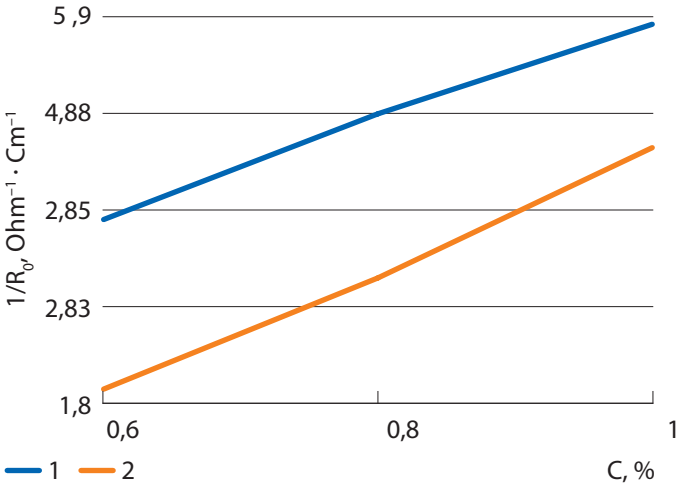

FIG. 2. *The dependence of electrical conductivity of the pectin substance aqueous solutions on concentration: 1 – the pectin substance, obtained from the Cosmos bipinnatus herb of the Rosea variety, 2 – the pectin substance, obtained from the Cosmos bipinnatus herb of the Dazzler variety*

Table 1

RESULTS OF DETERMINATION OF ELECTRICAL CONDUCTIVITY OF THE PECTIN SUBSTANCE WATER SOLUTIONS AND EQUILIBRIUM CONCENTRATIONS

C, %	$1/R_0 \cdot 10^3$, $Ohm^{-1} \cdot cm^{-1}$	$1/R_1 \cdot 10^3$, $Ohm^{-1} \cdot cm^{-1}$	C_1, %	C_2, %	lgC_1	lgC_2	K	n
Purity variety, extractant – ethyl acetate								
0.4	0.56	0.42	0.075	0.025	–1.12	–1.6	1.25	0.76
0.2	1.15	0.81	0.14	0.06	–0.85	–1.22	1.19	
0.1	2.30	1.6	0.28	0.12	–0.55	–0.92	1.4	
							$K_{av} = 1.28$	
Rosea variety, extractant – ethyl acetate								
0.6	3.76	2.67	0.48	0.12	–0.32	–0.92	0.76	0.22
0.8	4.87	3.38	0.52	0.28	–0.28	–0.55	0.68	
1	5.81	3.95	0.66	0.34	–0.18	–0.45	0.84	
							$K_{av} = 0.75$	
Rosea variety, extractant – n-octanol								
0.4	1.91	1.50	0.45	0.15	–0.35	–0.82	2.14	0.83
0.8	2.66	2.09	0.61	0.19	–0.21	–0.72	2.44	
1	3.20	2.50	0.80	0.20	–0.09	–0.69	3.07	
							$K_{av} = 2.55$	
Dazzler variety, extractant – ethyl acetate								
0.6	1.69	1.93	0.59	0.01	–0.23	–2.00	0.85	0.08
0.8	3.13	2.59	0.71	0.09	–0.15	–1.05	0.85	
1	4.50	3.06	0.96	0.04	–0.02	–1.39	1.0	
							$K_{av} = 0.9$	

substances obtained from the Cosmos bipinnatus of Rosea variety.

RESULTS AND DISCUSSION

Logarithm of the distribution isotherm equation helps to graphically determine the distribution constant and the degree of association:

$$lgK = lgC_1 - n lgC_2; \quad (2)$$

This equation of a straight line allows you to determine the degree of association as the tangent of the angle of inclination of the graph to the abscissa axis.

The intercept of the ordinate axis corresponds to lgK.

The studied pectin substances are polyelectrolytes, which, depending on the

Table 2

CHANGES IN THE CONCENTRATION OF PB2+ IONS DURING SORPTION ON THE PECTIN SUBSTANCES ISOLATED FROM THE COSMOS BIPINNATUS HERB OF ROSEA VARIETY

Variety	t, мин.	Number of ions Pb^{2+}, mg/g	Number of ions Pb^{2+}, mMol/L	Binding, %	Concentration, mg/g
Rosea	0	82.9	40.0	–	–
	5	53.9	26.0	65.0	359.2
	10	18.7	9.0	77.5	428.3
	20	16.6	8.0	80.0	442.1
	30	14.5	7.0	82.5	456.0
	60	14.5	7.0	82.5	456.0

pH, can be in both molecular and ionic form. The isoelectric point of the pectin substance determined in the acetate buffer solution was pH=4.7. This concentration of hydrogen ions was maintained during extraction. In the future, it is expected to study this process under conditions close to the acidity of the stomach, intestines and body temperature.

To evaluate the efficiency of extraction, we calculated the weight of the extracted pectin substances and the degree of extraction using the following formula:

$$m = m_0\left[1 - \left(\frac{KV_1}{KV_1 + V_2}\right)\right], \qquad \text{[12] (3)}$$

where m_0, m_1 are weights of initial and extracted pectin substances; V_1, V_2 – volumes of aqueous solution and ethyl acetate; K – distribution coefficient.

The degree of extraction was determined by the ratio of the weight of the extracted pectin substance in the extractant to the weight of the pectin substance contained in the initial aqueous solution. Calculations showed that the degree of the pectin substance extraction using ethyl acetate for Cosmos of Rosea, Purity, Dazzler varieties was 57.1%, 52.9%, 48.1%, respectively. The degree of the pectin substance extraction for Cosmos of Rosea variety using n-octanol was low and amounted to 28.3%.

Analysis of the extraction process shows that the transition of the pectin substance from the water phase to the hydrophobic phase is more free and effective for the pectin substance obtained from Cosmos of Rosea variety. Comparison of the results of the pectin substance extraction from Rosea herb using ethyl acetate and n-octanol (Table 1) showed that the extraction of this polysaccharide is more effective

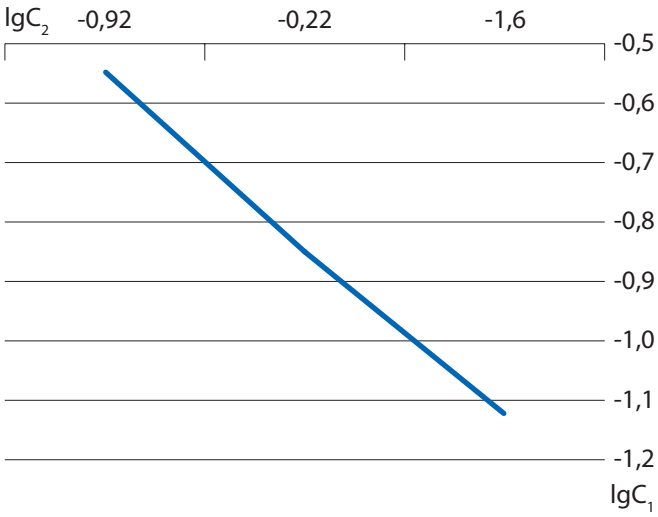

FIG. 3. *Logarithmic isotherm of distribution pectin substances obtained from Cosmos of Purity variety in the "ethyl acetate-water" solvent system*

Table 3

COMPARATIVE ANALYSIS OF SOME PHYSICAL-AND-CHEMICAL PROPERTIES OF THE PECTIN SUBSTANCE FROM COSMOS BIPINNATUS

Variety	M, g/mol	A, mmol/g	Sorption of Pb^{2+}, %	K	α*, %
Rosea	21612	26.4	85.2	0.75	57.1
Dazzler	13715	24.1	75.0	0.90	52.9
Purity	10071	22.4	70.0	1.08	48.1

** Extractant – ethyl acetate.*

in the presence of ethyl acetate. The value of the distribution coefficient in its presence was three times less. When extracted with ethyl acetate, the distribution coefficient was: 0.75 for the pectin substance obtained from Rosea Cosmos, 0.9 for the pectin substance obtained from Dazzler Cosmos and 1.28 for the pectin substance obtained from Purity Cosmos. However, when for the pectin substances obtained from Rosea Cosmos were extracted with n-octanol, the distribution coefficient increased up to 2.55, which indicates its low extraction capacity. This confirms the dependence of the distribution coefficient on the composition of both phases at the same temperature.

It is shown that in all cases the process of the pectin substance distribution into the organic layer proceeds spontaneously. The value of the Gibbs function was within the range of –0.26÷ –0.71 kJ/mol ($\Delta G<0$). The mass-transfer of the pectin substance from the aqueous phase to the organic phase was faster in the presence of ethyl acetate

This is not difficult for the pectin substance isolated from three different varieties of Cosmos herb under in vitro experimental conditions. Therefore, it will be currently important to study extraction in conditions close to the vital activity of the body. Regardless of the biological activity, the distribution coefficient is of great importance for the delivery of medicines to the site of its exposure.

The results obtained correlate with previous experiments for determination of the molecular weight (M), the value of experimental adsorption of Pb^{2+} (A) ions, and sorption activity (α) [13].

CONCLUSIONS

The comparative result shows that the pectin substances from Rosea Cosmos are characterized with the highest sorption activity in relation to Pb^{2+} ions and the lowest distribution coefficient. After studying the biological activity of the pectin substances, their practical application as natural heavy metal sorbents can be considered. With an increase in molecular weight, not only the adsorption, but also the absorption properties of the pectin substances obtained from of varieties of Cosmos bipinnatus increase, as evidenced by the data on the values of the distribution coefficient and the degree of extraction (α).

The conducted studies allowed us to determine the following scientific and practical significance of the experiment:

- Cosmos bipinnatus herb can be considered as a source of the pectin substances production;
- to assess the quantitative content of the pectin substances in aqueous solutions, the method of conductometry can be used;
- the use of liquid extraction and the calculation of the distribution coefficient allows us to estimate the spontaneous transition of the

pectin substances from the aqueous phase to the hydrophobic phase. It is shown that this is not difficult in an experiment in vitro, which will allow further to conduct research in vivo.

- the study of the complexing ability in relation to Pb^{2+} ions allowed us to determine a good sorption capacity (48–57%), which allows us to consider the pectin substances as a means used for intoxication of the body with heavy metals, without disturbing the biological balance of the body.

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MORPHOLOGICAL AND ANATOMICAL STUDY OF CANADIAN GOLDENROD HERB (*SOLIDAGO CANADENSIS* L.)

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The paper presents a morphological description of the Canadian goldenrod herb (Solidago canadensis L.), as well as a microscopic description of all its parts: leaves, stems, primordial leaves, flowers and achenes. Characteristic morphological and anatomical diagnostic features were revealed. The results obtained are proposed to be used to improve the regulatory documentation for Canadian goldenrod herb.

Keywords: Canadian goldenrod herb, *Solidago canadensis* L., morphological and anatomical features, medicinal plant raw material

The perennial herbaceous plant Canadian goldenrod (*Solidago canadensis* L.) is distributed throughout Russia as an ornamental and feral species. Competing as a weed with other representatives of the flora, Canadian goldenrod actively embraces new territories in China and other countries [1,2].

Medicinal products containing the total extract of the Canadian goldenrod herb (Marelin, Phytolysin, and Prostanorm) are used in medicine for kidney and bladder diseases [3]. Diuretic, anti – inflammatory, antibacterial, antioxidant, nephro-and antispasmodic activity were noted for the Canadian goldenrod herb [4]. Antitumor activity of chloroform and 50% alcohol extract of Canadian goldenrod against HeLa and MCF-7 cells [5] and fungicidal action against *Botrytis cinerea Growth* [6] were also studied.

Flavonoids and their aglycones, phenol-carboxylic acids, saponins, and essential oil were found in the chemical composition of the aboveground part of canadian goldenrod, and the presence of lignans, coumarins, carotenoids, and tannins was also detected [4,7].

The quality of the Canadian goldenrod herb is regulated by the Pharmacopoeial monograph FS-42-2777-91 [8]. This monograph describes in detail the morphology of the plant, and in section "Microscopy" the preparation of the leaf from the surface is considered and described. At the same time, there is no description of the stem and flowers. Taking into consideration the fact that in Russia the genus Goldenrod is represented by 26 species [9], of which only

the Canadian goldenrod herb is the official medicinal raw material, it is important to know the characteristic morphological and anatomical diagnostic features of the raw part of the *Solidago canadensis* plant when harvesting the plant raw materials.

Purpose of this work is study of the morphological and anatomical structure of the aboveground organs of Canadian goldenrod (leaves, stems, flowers, fruits) and identification of their characteristic diagnostic features.

MATERIALS AND METHODS

Morphological and anatomical study of raw materials was carried out on samples of the aboveground part of the Canadian goldenrod harvested in August 2018 on the territory of the medicinal plant nursery of Saint Petersburg State Chemical Pharmaceutical University during the flowering phase. Temporary preparations of plant organs (stem, leaf, flower) were prepared in accordance with the requirements of General Monograph (GM) 1.5.3.0003.15 "Technique of microscopic and microchemical study of plant raw materials and medicinal plant preparations" of the State Pharmacopoeia of the Russian Federation, XIV edition. Preparations clarified in a 5% alkali solution were examined from the surface. The leaf surface was studied using a scanning electron microscope (SEM). To do this, the objects were fixed with 3% solution of glutaric dialdehyde, dehydrated in a series of alcohols and passed through a mixture of alcohol and isoamyl acetate, as well as through pure isoamyl acetate, then dried at the critical point of liquid carbon dioxide. The dried objects were pasted on tables, sprayed with gold, and examined using a JSM 6390 (Jeol) microscope.

The anatomical structure of the leaf, stem, and flowers of Canadian goldenrod was studied on longitudinal and cross sections. To do this, the samples were fixed with a 3% solution of glutaric dialdehyde diluted with phosphate buffer with pH 7.4, and post-fixed with 2% solution of osmium tetraoxide for 24 hours. The material was dehydrated in a series of acetone solutions of increasing concentrations (from 30 to 100%), then enclosed in Epon-Araldite mixture. Semifine sections (2 and 4 microns) were obtained using the Ultracut E ultramicrotome (Reichert-Jung). Cutting was performed with a diamond knife. The preparations were stained with 1% toluidine blue solution and examined using Axio Lab. A1 (Zeiss) light microscope equipped with an AxioCam MRc5 digital video camera with Zen 2011 software.

A voucher sample of Canadian goldenrod is stored at the Department of Pharmacognosy of Saint Petersburg State Chemical Pharmaceutical University under number 794 (Fig. 1). the

Appearance of the herb was studied visually and using a binocular magnifying glass.

FIG. 1. *A voucher sample of Solidago canadensis L. (×4)*

Macroscopic analysis of raw materials was performed in accordance with the requirements of GM 1.5.1.0002.15 "Herbs".

RESULTS AND DISCUSSION

Morphological description of Canadian goldenrod herb

Raw materials are represented by dried leafy tops of shoots, individual leaves, inflorescences and their parts, pieces of stems. The leaves are simple, pubescent on both sides, ovate in the upper part of the stem, narrow-lanceolate in the lower part, with a pointed tip, a rounded-wedge-shaped base and a saw-like edge. Leaf venation is pinnate, with one pair of well-developed lateral veins extending from the middle vein in the lower part and then running almost parallel to it to the top of the leaf. The color of the leaves is green on the upper side and light green on the lower side. Leaf arrangement is alternate. The stem is from light green to brown, cylindrical, with a smooth or slightly ribbed surface and a weak pubescence in the upper part. Baskets are small (5–6 mm in diameter), numerous, collected in one-sided, arched panicled inflorescences. The floral receptacle is broad, concave. The involucre is cylindrical, multi-rowed; the primordial leaves are oblong, pale green. There are two types of flowers: median – tubular, golden-yellow; marginal – pseudo- ligulate, pale-yellow. The fruit is a small cylindrical achene 3–4 mm long, pubescent, with a brownish tuft of falling bristles.

Anatomical description of aboveground parts of Canadian goldenrod

Lamina. Leaves are amphistomatous. The adaxial epidermis consists of isodiametric, polygonal or oval-shaped cells with uniformly thickened cell walls (Fig. 2a). The cells of the epidermis along the veins are longitudinally elongated. Stomatal apparatus is of the anomocytic type with 2–3 near-stomatal cells and bud-shaped closing cells. The abaxial epidermis is represented by isodiametric cells with weakly branched walls and a folded cuticle (Fig. 2b). Stomata are located in the same plane relative to the surface of the epidermis. Stomatal apparatus is of the anomocytic type, with 4–8 near-stomatal cells and bud-shaped closing cells

The leaves of Canadian goldenrod are hairy and covered with glands. Using light and scanning electron microscopy, 4 types of hairs were detected: simple multicellular, whiplike, T-shaped (equilateral) and bristly (marginal).

Simple multicellular hairs consist of 4–5 cells and are localized along the conducting veins on the adaxial side of the leaf (Fig. 2c). The cell walls that are closer to the base of the hair are thickened. Cells of the leaf epidermis along the veins are longitudinally elongated. Whiplike hairs are located on the entire surface of the leaf. They consist of two parts: the base and the apical part. The base is represented by 2–3 cells, slightly tapering to the top, the apical part consists of 2–4 very narrowed and long cells, the final cell is pointed (Fig. 2d). T-shaped hairs which are characteristic for many members of the *Asteraceae* family [10] are localized on the adaxial side (Fig. 2e). When preparing the sample, the cross cell often breaks off and cannot be visible. The marginal bristly hairs are arranged as if in three rows. Two hairs are located on the sides of the leaf edge, and the third one is in the middle between them, but shifted slightly forward, towards the next pair. The marginal bristly hairs are mainly composed of three cells, the terminal cell is pointed. The main cells of the epidermis surrounding the bases of bristly hairs are slightly raised, and it seems that they are part of the hair, forming a multicellular base (Fig. 2f).

There are two types of glands: large (up to 30 microns in diameter) and small (up to 10 microns). Large glands have oval shape, are immersed in the recesses of the epidermis and are localized along the leaf veins (Fig. 2g). Small glands can occur both along the veins and on the adaxial side

(Fig. 2h). They often contain brownish substance, probably, of essential oil nature (Fig. 2i).

The cross-section of the leaf shows that the leaf is dorsoventral, with a two-row palisade (Fig. 2j). The cells of the epidermis are arranged in a single row. Directly above the veins with conducting bundles, the cells of the upper epidermis are smaller. On the lower side of the leaf, in the area of the veins, the bases of simple multicellular hairs are found.

In the central part of the vein there is a bundle of closed collateral type. The conductive system

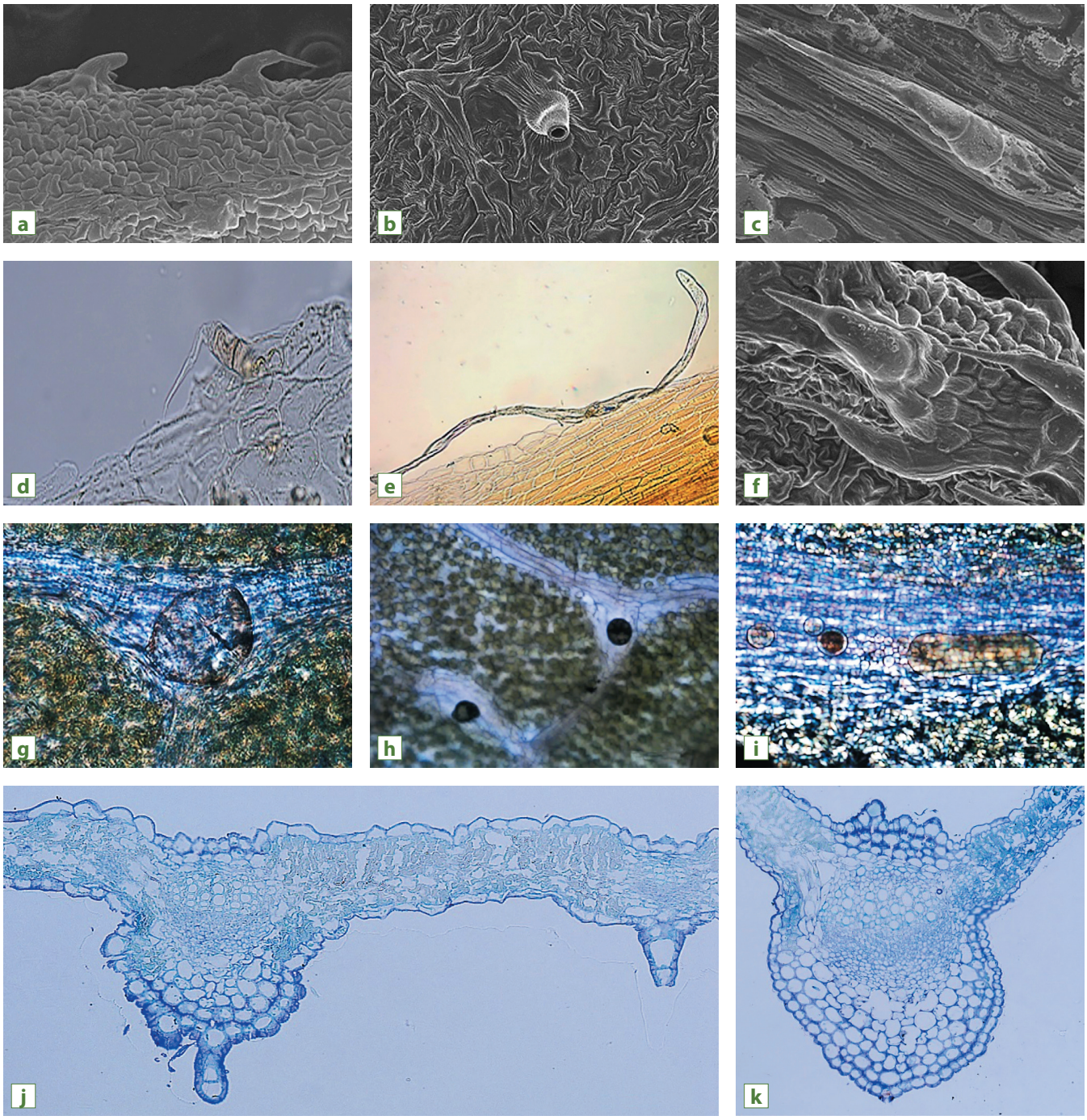

FIG. 2. *Anatomy of S. canadensis L. (a – ×250; b – ×430; c – ×700; d, h – ×400; e, j, k – ×200; f – x450; g, i – ×1000): a) fragment of the adaxial epidermis (SEM); b) fragment of the abaxial epidermis (SEM); c) simple multicellular hair (SEM); d) whiplike hair; e) T-shaped hair; f) bristly hairs (SEM); g) large gland; h) small glands; i) glands with brown essential oil content; j) cross-section of a leaf; k) central vein of a leaf (cross-section)*

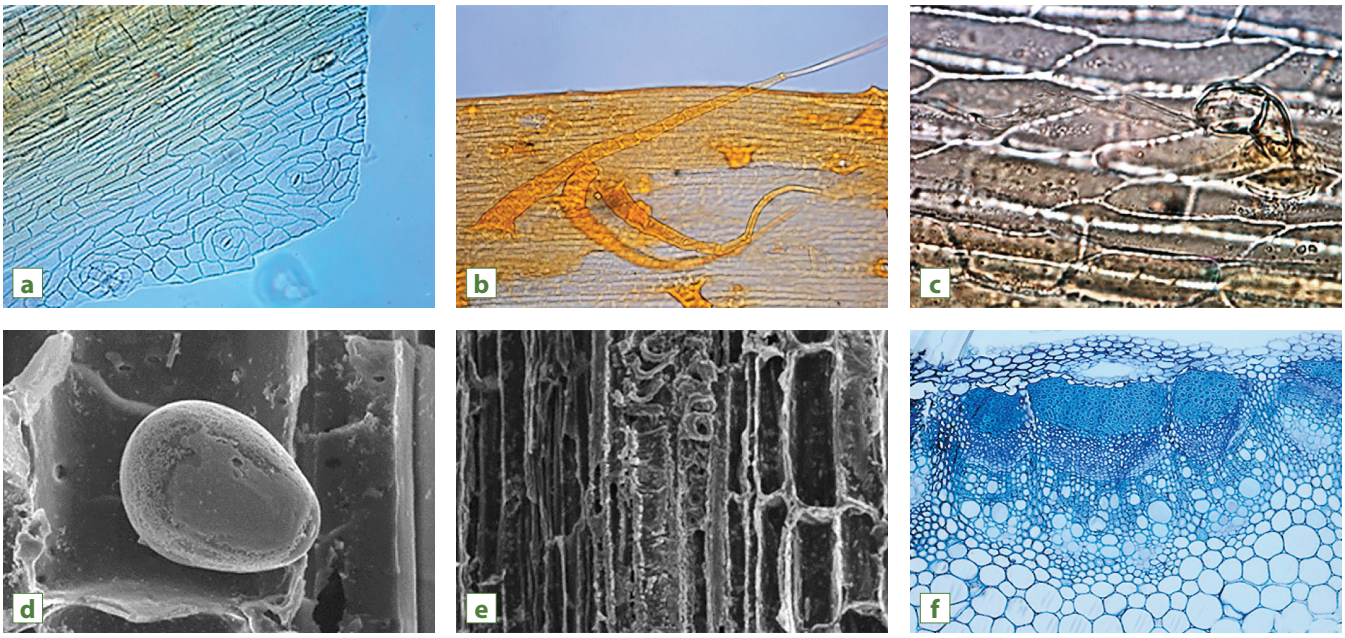

FIG.3. *Stem of S. canadensis L. (a, b, f – ×200; c – ×1000; d – ×2000; e – ×550): a) epidermis with stomata; b) multicellular cone-shaped hair; c) whiplike hair; d) gland (SEM); e) ladder-shaped vessels (SEM); f) cross-section*

of a bundle is represented by xylem and phloem. Between the epidermis and the bundle there are cells of the lamellar collenchyme. The rest of the vein consists of large thin-walled parenchymal cells (Fig. 2k).

Stem. The epidermal cells of the stem are oriented along its long axis. There are single stomata of the anomocytic type with 3–5 near-stomatal cells and closing bud-shaped cells (Fig. 3a). On the surface of the stem, there are 2 types of hairs: multicellular (10 or more cells) cone-shaped hairs (Fig. 3b) and whiplike hairs, similar in structure to the whiplike hairs of the leaf (Fig. 3c). There are also glands with a diameter of 20–25 microns (Fig. 3d), immersed in the epidermis. The conducting system is represented by ladder-shaped vessels (Fig. 3e).

On the cross-section of the stem (Fig. 3 f), made at a distance of 7–8 cm from the flower-bearing part, the cover tissue, bark and central axial cylinder are clearly distinguished.

The epidermis of the stem is single-rowed. The cortical part is represented by cells of the angular collenchyma, under which there is a single-row layer of underdeveloped endoderm. In the parenchymal layer of cortical cells above

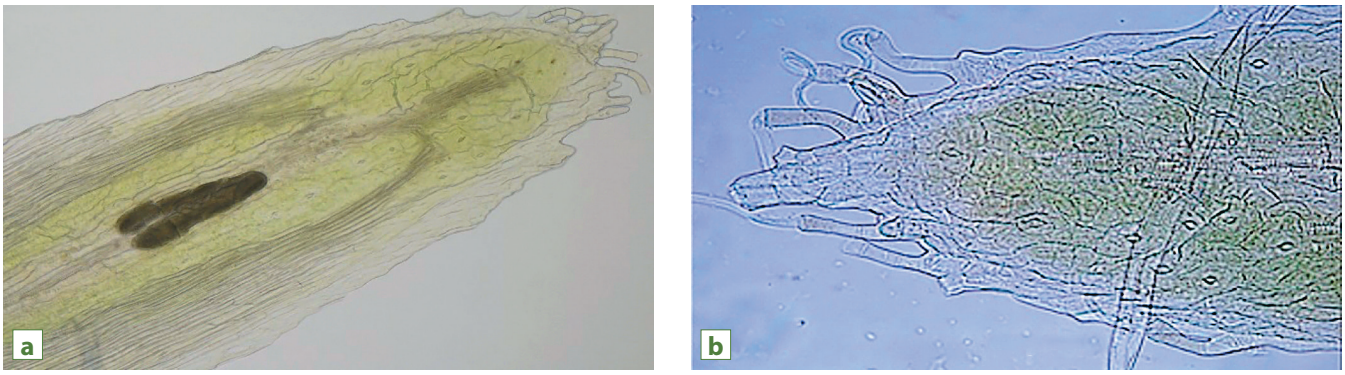

FIG. 4. *Primordial leaf of S. canadensis L. (a – ×200; b – ×400): a) primordial leaf with an intercellular canal; b) terminal part of the leaf with whiplike hairs and stomata*

the endoderm and the largest bundles, there are conceptacles of the schizogenous type. The lining cells of the conceptacles are isodiametric, arranged in a single row

The central cylinder contains conducting bundles of a collateral type arranged radially in a single row. Larger conducting bundles alternate with smaller ones. In the upper part of the bundles, as well as in the zone of the secondary xylem between the vessels, there are groups of cells of mechanical tissue. The central part of the stem consists of a thin-walled parenchyma.

Inflorescences-anthodes. The involucre is represented by leaves permeated with intercellular canals (Fig. 4a). The epidermis of the primordial leaf consists of thin-walled parenchymal cells. Stomata are of the anomocytic type with closing bud-shaped cells and 3–4 near-stomatal cells immersed in the epidermis. In the terminal part of the leaf, whiplike hairs are found, the base of which is most often represented by 4 cells, in contrast to similar hairs of the leaf and stem (Fig. 4b).

Tubular flowers (Fig. 5a) are in the central part of the inflorescence and have a pentamerous corolla and androecium. Corolla cells are polygonal in shape, without visible inclusions (Fig. 5b). The lower parts of the stamens are attached to the corolla. The stamen filaments are free, and the anthers coalesce to form a tube (Fig. c). The tube cells are isodiametric, and the

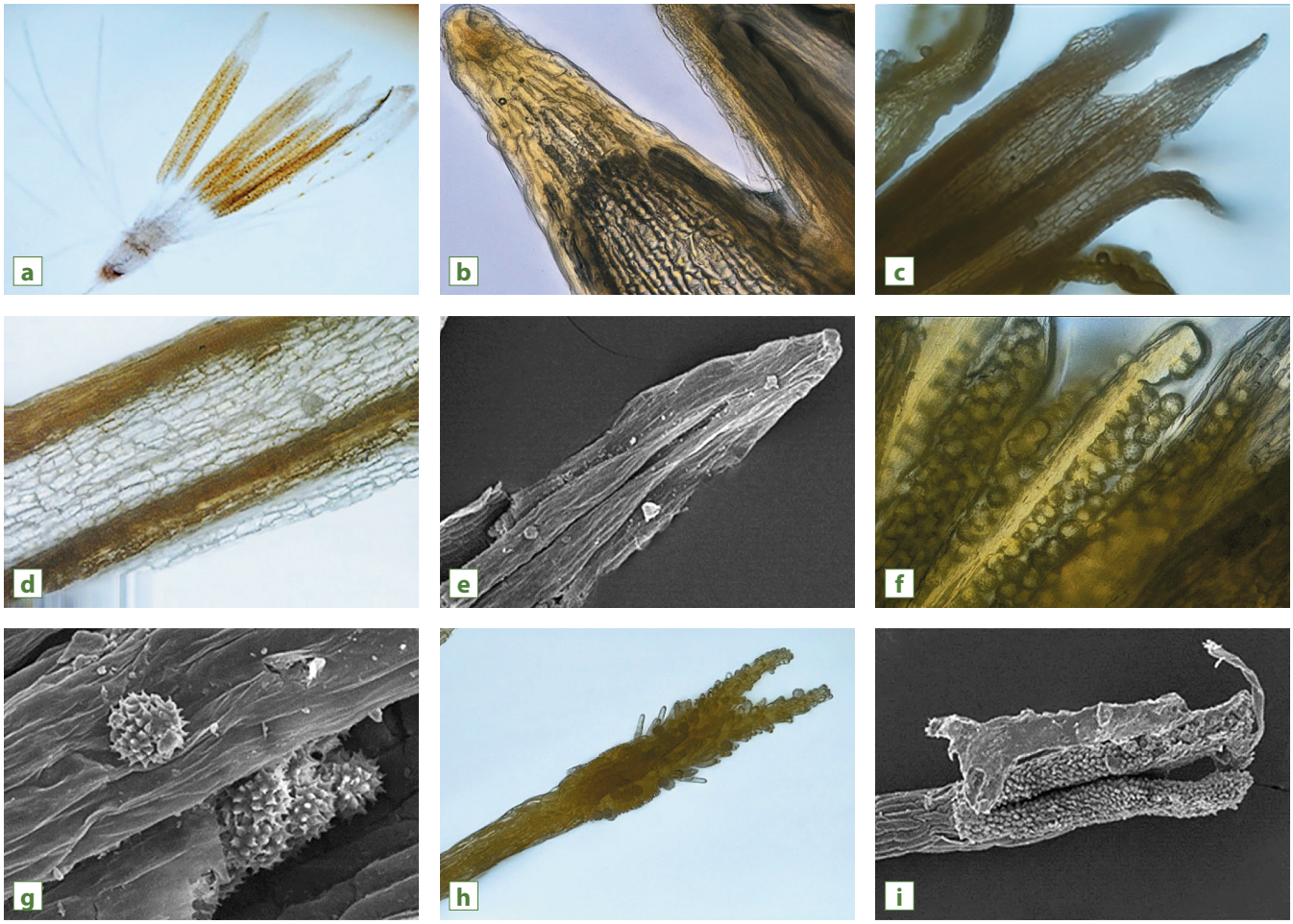

FIG. 5. *Tubular flower of S. canadensis L. (a – ×50; b, d, f, h – ×400; c – ×200; e – ×250; g – ×1400; i – ×330): a) general view; b) fragment of a corolla; c) fragment of a pollen tube; d) epidermis cells of a pollen tube; e) stamen (SEM); f) pollen in an anther; g) general view of pollen (SEM); h) fragment of gynaecium – stigma with baculum; i) stigma (SEM)*

cell walls are uniformly thickened (Fig. 5d, e). No stomata, hairs, or conceptacles were found. The pollen is numerous, spiny, and localized inside the pollen tube (Fig. 5f, g). The gynaecium consists of a single pistil (Fig. 5 h), whose elongated baculum consists of polygonal cells. Papillary cells of the epidermis are localized on the surface of the stigma (Fig. 5i).

Pseudo-ligulate flowers (Fig. 6a) occupy a marginal position in the inflorescence. The corolla is represented by three coalesced petals that diverge only in the terminal part. Corolla cells are oval in shape and arranged in 2–3 rows. In the terminal part of the petals, there are cells with numerous golden-yellow drops containing, presumably, essential oil (Fig. 6b).

The gynaecium is represented by a single pistil with closed stigma laminas and a short style attached at the base to the corolla (Fig. 6c). The surface of the cells of the epidermis of the stigma is folded. The terminal part of the stigma contains papillae (Fig. 6d). The glands protrude above the surface of the epidermis (Fig. 6e).

Achenes are cylindrical, obtuse-ribbed and medium-pubescent (Fig. 7a). The tuft consists of multicellular three-row hairs arranged in a single circle (Fig. 7b). Double hairs typical for *Asteraceae* were found on the surface of the achene [11]. The hair cells are tightly closed and characterized with pointed ends (Fig. c). No glandular trichomes were found.

CONCLUSIONS

During the study of herbarium samples of inflorescence shoots of *Solidago canadensis* L. using light and scanning microscopes, the morphological and anatomical features of the leaves, stems, primordial leaves, flowers and achenes of this species were expanded and supplemented. The following features of this species previously described in the literature [8,12] were confirmed: an anomocytic stomata in the epidermis of leaves, stems and primordial leaves, the presence of simple multicellular hairs and

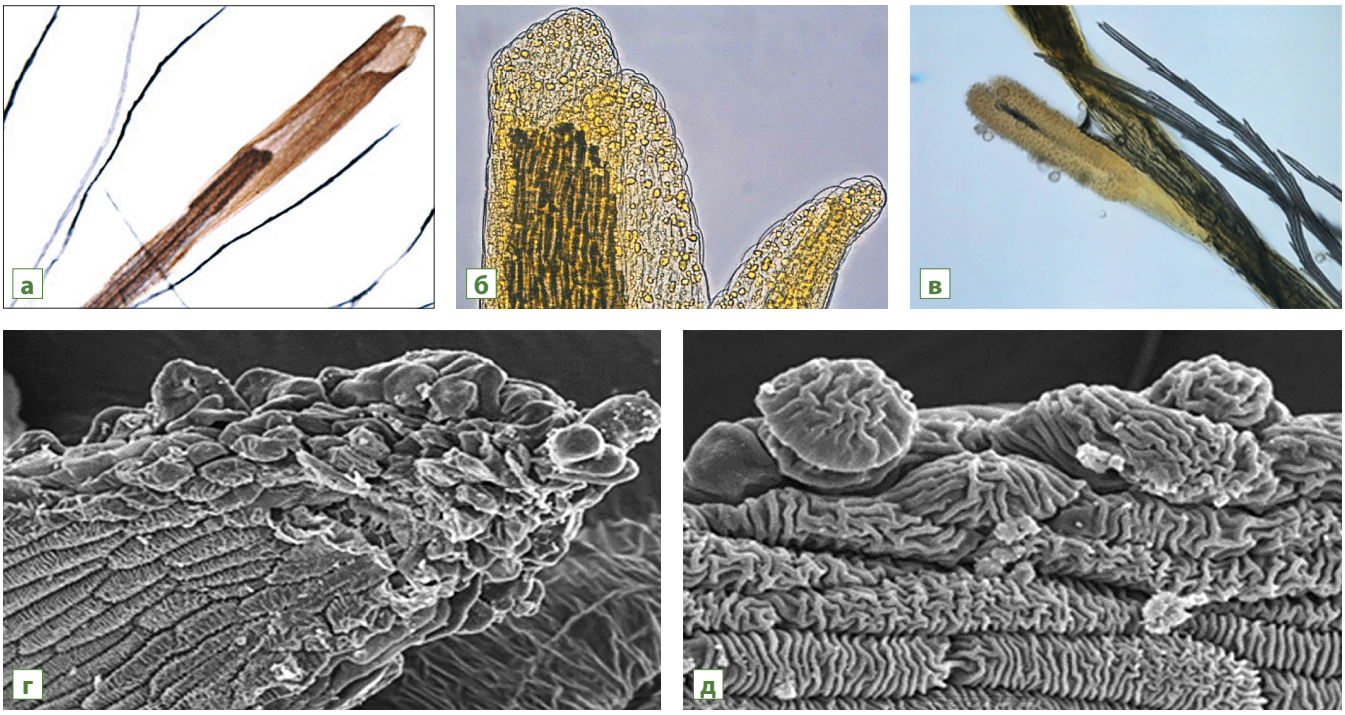

FIG. 6. *Pseudo-ligulate flower of S. canadensis L. (a – ×100; b – ×400; c – ×200; d – ×700; e – ×1900): a) general view; b) a terminal part of corolla; c) fragment of gynaecium – stigma with a style; d) a terminal part of a stigma (SEM); e) part of a stigma with glands (SEM)*

FIG. 7. *Achenes of S. canadensis L. (a – ×180; b – ×200; c – ×1000): a) general view (SEM); b) fragments of trichomes of a tuft; c) double hair*

essential oil structures such as glands, called conceptacles in previous works [12]. For the first time, the presence of T-shaped hairs in the leaves, whiplike hairs in the leaves, stems and primordial leaves, the presence of schizogenic conceptacles over large whorls, papillae and glands on the epidermis of the gynaecium of the pseudo-ligulate flower was identified. The nature of the marginal hairs of the leaf and the hairs of the achenes was specified.

The results of the studies allow us to recommend entering the additional morphological and anatomical data on the structure of leaves, stems, primordial leaves, flowers and achenes into the pharmacopoeial monograph on the raw material "Canadian goldenrod herb" for identification of raw materials.

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STUDY OF ANTI-INFLAMMATORY AND GASTROSPARING ACTIVITY OF JAPANESE PAGODA TREE (*SOPHORA JAPONICA* L.) FRUIT

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To study the anti-inflammatory and gastrosparing activity of fractions from the Japanese pagoda tree (Sophora japonica L.) fruits, screening studies were conducted in conditions of experiments in vitro using a specific enzyme biotest-system based on inducible NO-synthase. It was found that the glycoside fraction, represented by the glycosides of genistein, quercetin and kaempferol, shows anti-inflammatory activity. As a result of pharmacological studies conducted in conditions of experiments in vivo on laboratory animals, it was found that the glycoside fraction from the fruits of Sophora japonica is low-toxic, has a significantly pronounced anti-inflammatory and gastrosparing effect, and is promising for further study and development of new effective herbal medicines.

Keywords: Sophora japonica, specific enzyme biotest systems *in vitro*, acute toxicity, anti-inflammatory activity, gastrosparing action

Today, interest in herbal medicines on the Russian market is steadily growing, as the number of consumers using herbal medicine as a milder and more complex method of treatment is increasing [1]. Increase in the share of herbal medicines (HM) in the total number of medicines registered on the domestic market may be associated with increase in the share of chronic diseases that require long-term and mild therapy, a wide range of actions, safety and affordable price.

The fruits of Japanese pagoda tree (*Sophora japonica* L.) are a promising object for study. According to the literature, this plant material contains flavonoids (quercetin, quercetin-3-rutinoside, quercitrin, isoquercitrin, rutin, kaempferol-3-sophoroside, genistein-2-sophorabioside, sophoraflavonoloside, sophoricoside, sophorabioside, genistein, genistin, isorhamnetin, nicotiflorin, tamarixetin, isorhamnetin); triterpenoids (betulin, sophoradiol); phenol carbonic acids (chlorogenic, caffeic, gallic); nitrogen-containing

compounds; coumarone-chromones (cacsophorophenolon, medicagol, pseudobaptigenin, orobol); polysaccharides; monosaccharides; amino acids; macro-and microelements. Fruit seeds accumulate up to 10% of fat oil [2–6].

The predominance of secondary metabolites of phenolic nature in the fruits of Sophora japonica determines the prospect of using this raw material as a source of pharmaceutical substances with anti-inflammatory and gastrosparing effects.

The aim of our research was to study the pharmacological activity of fractions from Japanese pagoda tree (*Sophora japonica* L.) fruits using the specific enzyme biotest systems in experiments *in vitro* and using biological models on laboratory animals to create a new effective and safe herbal medicine.

MATERIALS AND METHODS

The object of the study was the fruits of Japanese pagoda tree (*Sophora japonica* L.) harvested in 2017 in the Krasnodar territory. The dried fruits were crushed to the size of particles passing through a sieve with holes 2 mm in diameter. The crushed fruits were triple extracted with 70% ethyl alcohol at a temperature of 60°. The combined extracts were concentrated on a rotary evaporator. The sedimentation released during concentration was separated and dried (glycoside fraction). The supernatant part of the concentrated extraction was treated with chloroform. The chloroform fraction was separated, evaporated, and dried until the solvent was removed (lipophilic fraction). The supernatant part of the extraction purified from lipophilic compounds was concentrated and fully dried (aqueous-alcoholic fraction). The obtained fractions were used for screening studies.

In experiments *in vitro* a commercial formulation of inducible NO-synthase (iNOS) prepared from mouse macrophages expressed by E. coli from Sigma-Aldrich (USA) was used. In the enzymatic reaction, the following Sigma-Aldrich reagents (USA) were used: NADPH, magnesium acetate, 6,7-dimethyl-5,6,7,8-tetrahydropterin (DMTP), dithiotreitol (DTT), DMSO, and GERBU reagents (Germany): N-(hydroxyethyl) piperazine-2-ethanesulfonic acid (HEPES), L-arginine, oxyhemoglobin. Changes in the absorption of solutions at 340 nm were monitored for 3 minutes.

To assess the anti-inflammatory activity of the presented samples, solutions of the samples were prepared in 70% ethyl alcohol at the initial concentrations of 2 and 4 mg/ml. The anti-inflammatory activity of the samples was calculated based on the inhibitory effect of BAS contained in the samples on the iNOS reaction rate, which was determined spectrophotometrically with a two-beam spectrophotometer of the Shimadzu UV1800 brand (Japan) at 340 nm using the kinetic research program. In experiments *in vitro*, the rate of the enzymatic reaction catalyzed by iNOS was determined before (control) and after adding 20 µl of the tested substances to a 3 ml sample (experiment). Based on the results of preliminary screening using the specific enzyme biotest systems, the glycoside fraction of Japanese pagoda tree (*Sophora japonica* L.) fruit was selected for further study.

Pharmacological studies in vivo were performed in accordance with the Rules of laboratory practice in the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 199н of 01.04.2016, National standard of the Russian Federation GOST 33044–2014 "Principles of good laboratory practice", "Guidelines for preclinical research of medicines", 2012) and in accordance with the Federal law of 12.04.2010 No. 61-FZ (ed. of 28.11.2018) "On the circulation of medicines". The studies were approved by the Bioethical Commission of the All-Russian Scientific Research Institute of Medicinal and Aromatic Plants.

In an experiment to study acute toxicity using the Kerber method, 36 non-linear male mice with

a body weight of 20–22 g were used. Laboratory animals were divided into 6 groups consisted of 6 individuals each: the first group – control animals; the second, third, fourth, fifth, sixth groups – experimental animals. The glycoside fraction of Sophora fruit was administered intragastrically to animals in doses of 100, 500, 1000, 1500, 2000 mg /kg. 1% starch paste in equivalent volume was injected to the control group of animals intragastrically. The duration of observation of laboratory animals was 14 days. During the experiment, the behavior of mice, their appearance, motor activity, and reaction to external stimuli were observed [7].

In an experiment to study the anti-inflammatory activity of the glycoside fraction of the study on a model of histamine edema of mouse paws, 40 non-linear male mice weighing 19–20 g were involved. The animals were divided into 4 groups with 10 individuals in each. The first group is a control group; the second, third and fourth groups are experimental ones. The second group received the glycoside fraction of Sophora fruits at a dose of 10 mg /kg, the third – the glycoside fraction of Sophora fruits at a dose of 100 mg /kg, the fourth – butadion as the comparison drug at a dose of 10 mg/kg for 3 days intragastrically. All medications were suspended in 1% starch paste. 1% starch paste in equivalent volume was injected to the control animals also for 3 days. Histamine edema was caused by a single subplant injection under the aponeurosis of the right hind paw of the mouse one hour after the last injection of 0.05 ml of 0.25% histamine. An hour after that, at the peak of inflammation, the animals were euthanized with carbon dioxide and the mass of amputated limbs of mice with the development of edema and control mice was recorded and the increase in exudate volume (mg) was calculated.

In the experiment to study the anti-inflammatory activity of the objects of study on the model of formalin edema of the paws of mice, 40 non-linear male mice weighing 19–20 g were also involved. Formalin edema was caused by a single subplant injection under the aponeurosis of the right hind leg of the mouse one hour after the last injection of 0.05 ml of 1% formalin. The animals were divided into groups and the test fraction and the comparison drug were prepared, just as in the previous experiment. All substances were administered within 3 days. On the third day, an hour after the introduction of the glycoside fraction and the comparison drug, formalin edema of the hind limbs was caused, and an hour after that, the animals again received the glycoside fraction and the comparison drug intragastrically. After 3 hours at the peak of inflammation, the animals were euthanized with carbon dioxide and the mass of amputated limbs of mice with the development of edema and control mice was recorded and the increase in exudate volume (mg) was calculated. The development of edema was judged by the difference in weight in control and experimental animals and the anti-exudative effect was calculated.

In experiments on models of histamine and formalin edema the anti-exudative effect was calculated according to the formula:

$$\% \text{ of inhibition of edema} = P_к - P_o/P_к \times 100,$$

where Pк is the difference of the masses of the paws with and without edema in animals of the control group; Ro is the difference of the masses of the paws with and without edema in the experimental animals [8].

The influence of samples on the state of the gastric mucosa of rats in conditions of acute experimental ulcers was studied using ethanol and indomethacin models. The comparison drug was omeprazole at a dose of 20 mg/kg.

Experimental work was performed on 64 nonlinear white male rats. For each model, 32 individuals were used, which were divided into 4 groups. The pharmacological properties of the glycoside fraction were studied by intragastric administration to rats in 1% starch paste for

3 days at doses of 10 mg /kg (Group 1) and 100 mg/kg (Group 2). Omeprazole at a dose of 20 mg /kg (Group 3) was also administered in 1% starch paste for three days. Control animals (Group 4) received only 1% starch paste.

To reproduce the pathological state of the gastric mucosa on an ethanol model, a single injection of 96% ethyl alcohol at a dose of 1 ml per animal was used, followed by euthanasia of rats in a CO2 chamber 1 hour after ethanol administration.

To reproduce the pathological state of the gastric mucosa on an indomethacin model, a single injection of indomethacin at a dose of 30 mg/kg was used, followed by euthanasia of rats in a CO2 chamber 24 hours after indomethacin administration.

All medications were administered to rats intragastrically using a probe in the morning, 1 hour before feeding. After euthanasia of rats, the stomach and duodenum were removed. The stomach (along large curvature) and duodenum were cut and washed in an isotonic solution of NaCl. Then, using a binocular stereoscopic microscope MBS-10 (magnification 1, millimeter scale), the area of ulcerative lesions of the gastric and duodenal mucosa was calculated, the Pauls index (PI) and the therapeutic effect (TE) were calculated [9].

Statistical processing of the results was performed using the statistical analysis software package Statistica 10.0 (StatSoft, USA). To assess the significance of differences between samples with a distribution approaching normal one, the Student's t-test was used. The critical significance level P was assumed to be 0.05 when testing the statistical hypotheses. The data is presented as M±m, where M is the arithmetic mean value and m is the error of the arithmetic mean value [10].

The work was performed in accordance with the research plan, code 0576–2019–0009 "Conducting preclinical studies of individual fractions, substances and medicinal products prepared from medicinal plant raw materials".

RESULTS AND DISCUSSION

For the screening of BAS prepared from medicinal plant raw materials, it is advisable to use specific enzyme biotest systems in experiments *in vitro*. Inducible NO-synthase (iNOS) is a key enzyme for nitric oxide synthesis in the development of the inflammatory process in tissues, which contributes to the suppression of infection. In inflammatory diseases, iNOS is known to play an important role in protecting the body from an infectious agent by catalyzing the synthesis of the active radical nitric oxide (NO), which has a wide range of biological effects [11]. The direct influence of the researchable objects on the enzymatic activity of the NO-synthase biotest system *in vitro* was studied. The enzyme activity was determined before (control) and after (experiment) adding the tested solutions. The enzyme biotest system used in this work is a part of the unique scientific facility of the VILAR's "Biological collections of specific enzyme biotest systems *in vitro* (BC-SEBTS) ".

The experimental data obtained are presented in Table 1.

As can be seen from the results presented in Table 1, at the initial concentration of 2 mg/ml, the BAS contained in the glycoside fraction inhibited the rate of the enzymatic iNOS reaction by 37%, and samples of the aqueous-alcoholic and lipophilic fractions reduced the reaction rate by only 13%.

Thus, it follows from the data obtained that at concentration of 2 mg/ml, the glycoside fraction exhibits more active anti-inflammatory properties compared to samples of aqueous-alcoholic and lipophilic fractions in experiments *in vitro*.

When increasing the concentration of samples in solution to 4 mg/ml it was observed the increasing of the inhibitory effect of BAS on the reaction rate. Thus, samples of the lipophilic and aqueous-alcoholic fractions reduced the iNOS reaction rate by about 2.6 times (up to 38–39%), and the sample of the glycoside fraction – be

Table 1

EFFECT ON THE RATE OF THE ENZYMATIC REACTION OF INDUCIBLE NO-SYNTHASE OF SAMPLES OF FRACTIONS PREPARED FROM JAPANESE PAGODA TREE FRUIT AT INITIAL CONCENTRATIONS OF 2 AND 4 MG/ML

Variants of the experiment		Rate of enzymatic reaction of inducible NO-synthase	
		Nmol NADPH / mg protein per minute (M±m)	Decrease in the iNOS reaction rate relative to the control (%)
Control		2.04±0.007	100
Glycoside fraction	2 mg/ml	1.29±0.005*	63
	4 mg/ml	0.465±0.006*	23
Aqueous-alcoholic fraction	2 mg/ml	1.77±0.006*	87
	4 mg/ml	0.81±0.008*	39
Lipophilic fraction	2 mg/ml	1.77±0.007*	87
	4 mg/ml	0.79±0.007*	38

*Note: here and hereinafter * – statistical significance of differences from the control at p≤0,05*

4.3 times (up to 23%). Consequently, the anti-inflammatory effect of the studied samples is enhanced at concentration of 4 mg/ml.

Thus, during the initial screening for BAS with anti-inflammatory activity, using a specific enzyme biotest system based on inducible NO-synthase, it was found that the glycoside fraction from the fruits of Japanese pagoda tree shows anti-inflammatory activity and is recommended for further in-depth pharmacological study.

To characterize the chemical composition of the glycoside fraction, its acid hydrolysis was provided. When studying the obtained hydrolysate by HPLC-UV method using the standard samples, genistein, quercetin and kaempferol were identified (Fig. 1) and, consequently, it was confirmed that the fraction

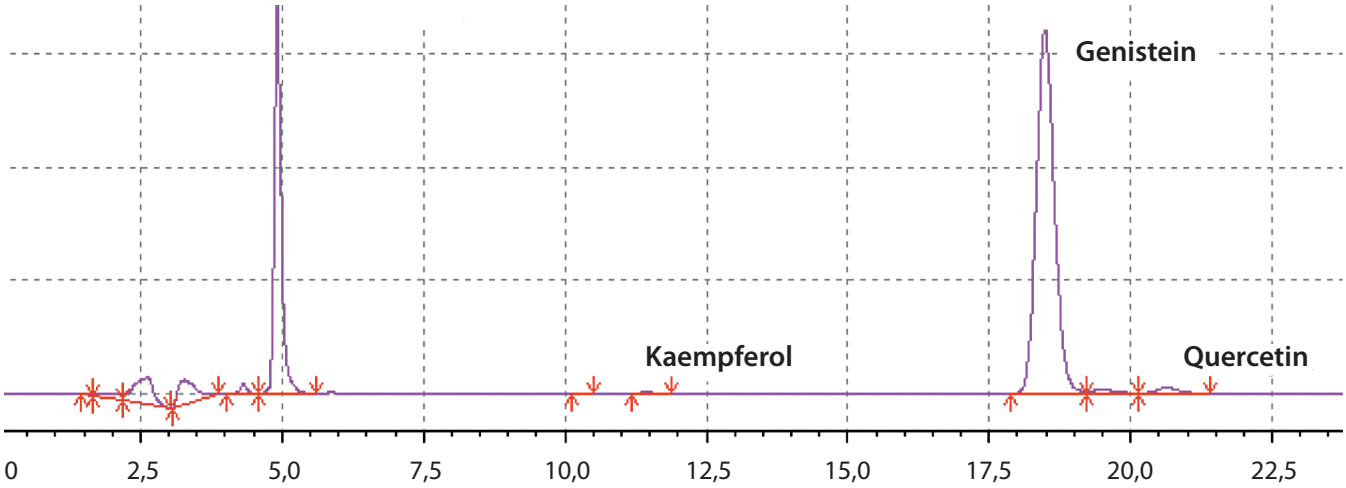

FIG. 1. *HPLC-UV chromatogram of hydrolysate of the glycoside fraction of Japanese pagoda tree fruit*

is represented by their glycosides. From the ratio of peak areas on the chromatogram, it can be assumed that the predominant content in the studied fraction is genistein glycosides.

Probably, due to the affinity for NO-synthase, one of the possible molecular mechanisms of action of BAS contained in the glycoside fraction from the fruits of Japanese pagoda tree is their ability to effectively interfere with the synthesis of the active radical of nitric oxide.

Based on the data obtained in experiments *in vitro*, the pharmacological activity of the glycoside fraction from Japanese pagoda tree fruits was further investigated in experiments in vivo.

At the first stage of research, we studied the acute toxicity of the sample. It was found that the glycoside fraction did not cause the death of animals in all groups, and there were no changes in the appearance and behavioral responses of mice. Since no animal deaths were observed during the entire observation period, it was not possible to determine the LD50 of the object under study. The maximum dose of administration to animals is 2000 mg/kg. Thus, the glycoside fraction from the fruits of Japanese pagoda tree belongs to low-toxic substances, according to the classification of chemical toxicity under GOST 12.1.007–76.

Further, in accordance with the tasks set, we studied the anti-inflammatory activity of the glycoside fraction from the fruits of Japanese pagoda tree in animal experiments on models of formalin and histamine edema.

The results of an experiment to study the effect of the glycoside fraction at doses of 10 and 100 mg /kg on the exudative stage of inflammation caused by 0.25% histamine, in comparison with butadione being the known anti-inflammatory drug at a dose of 10 mg/kg are presented in Table 2.

As can be seen from Table 2, the glycoside fraction had an anti-inflammatory effect when administered to animals for three days. It reduced histamine edema at a dose of 10 mg/kg by 19.46% and at a dose of 100 mg/kg by 19.28% compared to the control group of animals, but was inferior to the anti-inflammatory effect of butadione, which reduced edema by 41.6%.

The results of an experiment to study the effect of the glycoside fraction from Japanese pagoda tree (Sophora japonica) fruit at doses of 10 and 100 mg /kg on the exudative stage of inflammation caused by 1% formalin, in comparison with the known anti-inflammatory drug such as butadione at a dose of 10 mg/kg are presented in Table 3.

As can be seen from Table 3, the sample of the glycoside fraction had a dose-dependent anti-inflammatory effect after three days of administration. It reduced formalin edema at a dose of 10 mg /kg by 17.14% and at a dose

Table 2

ANTI-INFLAMMATORY EFFECT OF THE GLYCOSIDE FRACTION FROM THE FRUIT OF JAPANESE PAGODA TREE (SOPHORA JAPONICA) WHEN ADMINISTERED TO MICE FOR THREE DAYS ON A MODEL OF HISTAMINE EDEMA

Groups of animals, n=10	Dose, mg/kg, (oral)	Increase in exudate volume at the peak of inflammation, mg	Anti-inflammatory effect, %
Control	–	55.25±5.19	–
Glycoside fraction	10	44.5±1.87*	19.46
Glycoside fraction	100	44.6±1.45*	19.28
Butadione	10	32.25±1.33*	41.6

Table 3

ANTI-INFLAMMATORY EFFECT OF THE GLYCOSIDE FRACTION FROM THE FRUIT OF JAPANESE PAGODA TREE (SOPHORA JAPONICA) WHEN ADMINISTERED TO MICE ON A MODEL OF FORMALIN EDEMA

Groups of animals, n=10	Dose, mg/kg, (oral)	Increase in exudate volume at the peak of inflammation, mg	Anti-inflammatory effect, %
Control	–	93.9±2.22	–
Glycoside fraction	10	77.8±3.6*	17.14
Glycoside fraction	100	76.0±3.04*	19.06
Butadione	10	63.44±1.62*	32.43

of 100 mg /kg by 19.06% compared to the control group of animals, but was inferior to the anti-inflammatory effect of butadione, which reduced edema by 32.43%.

Thus, it was found that the glycoside fraction of Japanese pagoda tree (Sophora japonica) fruit at doses of 10 and 100 mg /kg has a significantly pronounced anti-inflammatory effect in the conditions of experimental histamine and formalin edema.

At the next stage of the research, to study the gastrosparing activity of the most active glycoside fraction according to the results of primary screening of this fraction, the effect of its administration at doses of 10 and 100 mg/kg on the healing of experimental stomach ulcers in rats caused by the introduction of ethanol and indomethacin was studied. The drug omeprazole at a dose of 20 mg/kg was used as a comparison drug.

The results of an experiment to study the effect of the glycoside fraction from Japanese pagoda tree (Sophora japonica) fruit at doses of 10 and 100 mg /kg on an ethanol experimental model of gastric ulcer are presented in Table 4.

As can be seen from Table 4, under the conditions of an ethanol experimental model of gastric ulcer, when the extract was administered at a dose of 10 mg / kg, a gastrosparing effect was revealed i.e. reduction in the area of ulcerative defects by 63.7%, TE=2.75 ($p<0.05$ compared to the control). When the extract dose was increased to 100 mg/kg, the area of ulcerative defects was reduced by 89.65%, TE=12.88 ($p<0.05$ compared to the control). When the reference preparation of omeprazole was administered at a dose of 20

Table 4

EFFECT OF THE GLYCOSIDE FRACTION FROM THE FRUIT OF JAPANESE PAGODA TREE (SOPHORA JAPONICA) ON EXPERIMENTAL RAT STOMACH ULCERS CAUSED BY THE ADMINISTRATION OF ETHANOL

Group of animals, n=8 (dose, mg/kg)	Dose, mg/kg, (oral)	Rats with ulcers, %	Average ulcer surface area	Pauls index / deviation in %	Therapeutic effect (TE)
Control	–	100	26.16±2.39	26.16	–
Glycoside fraction	10	100	9.5±0.29*	9.5/63.7	2.75
Glycoside fraction	100	75	2.71±0.019*	2.03/89.65	12.88
Omeprazole	20	100	9.46±0.21*	9.46/63.84	2.76

Table 5

EFFECT OF THE GLYCOSIDE FRACTION FROM THE FRUIT OF JAPANESE PAGODA TREE (SOPHORA JAPONICA) ON EXPERIMENTAL RAT STOMACH ULCERS CAUSED BY THE ADMINISTRATION OF INDOMETHACIN

Group of animals, n=8 (dose, mg/kg)	Dose, mg/kg, (oral)	Rats with ulcers, %	Average ulcer surface area	Pauls index / deviation in %	Therapeutic effect (TE)
Control	–	100	14.1±1.06	14.13	–
Glycoside fraction	10	100	4.2±0.47	4.2/70.3	3.36
Glycoside fraction	100	100	2.0±0.05*	2.0/85.6	6.92
Omeprazole	20	100	1.8±0.03*	1.8/87.1	7.76

mg /kg, the area of ulcerative defects was reduced by 63.84%, TE=2.76.

Thus, according to the results of the experiments, it was found that the glycoside fraction from the fruits of Sophora japonica in doses of 10 mg / kg and 100 mg / kg has a significantly pronounced dose-dependent gastroprotective effect in the experimental model of ethanol ulcer, comparable in activity to the action of the comparison drug.

The results of an experiment to study the effect of the glycoside fraction from Japanese pagoda tree (Sophora japonica) fruit at doses of 10 and 100 mg /kg on an indomethacin experimental model of gastric ulcer are presented in Table 5.

According to Table 5, under the conditions of an indomethacin experimental model of gastric ulcer, when the glycoside fraction was administered at a dose of 10 mg /kg, a gastrosparing effect was revealed i.e. reduction in the area of ulcerative defects by 70,3%, TE=3,36 ($p<0.05$ compared to the control). When the dose of the study object was increased to 100 mg/kg, the area of ulcerative defects was reduced by 85.6%, TE=6.92 ($p<0.05$ compared to the control). When the reference preparation of omeprazole was administered at a dose of 20 mg /kg, the area of ulcerative defects was reduced by 87.1%, TE=7.76. According to the results of the experiments, it was found that the glycoside fraction from the fruits of Japanese pagoda tree (Sophora japonica) at doses of 10 and 100 mg /kg has a significantly pronounced dose-dependent gastrosparing effect in the experimental model of indomethacin ulcer, comparable in activity to the comparison drug.

The study of gastrosparing activity revealed a significant dose-dependent effect of the glycoside fraction of Japanese pagoda tree (Sophora japonica) fruit in the studied doses.

Thus, based on the primary biological screening of samples from Japanese pagoda tree (Sophora japonica) fruit using a NO-synthase specific enzyme biotest system in experiments *in vitro*, it was found that the glycoside fraction has the greatest anti-inflammatory activity. When studying acute toxicity using the Kerber method, it was found that the glycoside fraction is a low-toxic compound. Further study of the glycoside fraction using pharmacological methods on animals proved its anti-inflammatory and gastrosparing effect. As a result of the experiments, it was found that the glycoside fraction from the fruits of Japanese pagoda tree (Sophora japonica) is promising for further study and creation of modern herbal medicines based on it.

CONCLUSIONS

1. As a result of screening studies conducted in conditions of experiments *in vitro* using a specific enzyme biotest system based on

inducible NO-synthase, it was found that the glycoside fraction represented by the glycosides of genistein, quercetin and kaempferol shows anti-inflammatory activity and is recommended for further in-depth pharmacological study.

2. As a result of pharmacological studies conducted in conditions of experiments in vivo on laboratory animals, it was found that the glycoside fraction from the fruits of Japanese pagoda tree (Sophora japonica) is low-toxic and has a significantly pronounced anti-inflammatory and gastrosparing effect.

3. The glycoside fraction from the fruits of Japanese pagoda tree (Sophora japonica) according to the results of the research is a promising object for further in-depth study and the creation of a new effective herbal medicine.

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PREVALENCE OF MILD BRONCHIAL ASTHMA IN THE REGIONS OF THE RUSSIAN FEDERATION AND EVALUATION OF VARIOUS MEDICATION REGIMENS (BASED ON THE RESULTS OF AN OBSERVATIONAL STUDY)

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Objectives of observational studies were to estimate the prevalence of mild (intermittent/ persistent) bronchial asthma in several regions of the Russian Federation, the frequency of administration of various inhalation drugs in the treatment of mild bronchial asthma and results of their application. The study included the patients who had the correction of their previous therapy of mild asthma by a doctor's decision. In a cohort of patients who received after correction the therapy with a fixed combination of salbutamol and beclomethasone dipropionate in the form of a dosed aerosol inhaler (SabaComb®), the results of the medicine use and patient satisfaction with the therapy were evaluated after 14–28 days of therapy.

Keywords: bronchial asthma, inhaled glucocorticosteroids (iGCS), short-acting beta 2-agonists (SABA)

Currently, bronchial asthma (BA) is one of the most common diseases among all population groups and occurs, according to epidemiological studies, in 1–18% of individuals, depending on the regions of the world [1]. According to the statistics of the Ministry of Health of the Russian Federation for 2016, 1,515,296 patients with BA were officially registered in Russia [2]. However, in reality, according to experts, the number of such patients is 5–6 times more

BA has different degrees of severity and ranges from mild to severe. According to the level of control, BA is divided into well-controlled, partially controlled, and uncontrolled. Achieving the symptom control and prevention of exacerbations is the purpose of modern therapy. Mild BA is a disease that can be well controlled on stage 1–2 therapy (GINA 2018), that is, with the use of short-acting bronchodilators (SABD) or a minimum amount of control maintenance therapy, which includes low doses of inhaled glucocorticosteroids (iGCS), antileukotrien drugs and cromons. Currently, the category of patients with mild BA includes patients who receive therapy corresponding to the 1–2 stages under GINA (intermittent and mild persistent BA), as well as patients who have not previously received treatment, in which the doctor suggests starting therapy corresponding to the 1–2 stages under GINA 2018 [1].

Mild asthma is 50–75% of all cases [3], which is not always obvious for practitioners, since

such patients seek medical care significantly less often than those with severe BA. There are a number of reasons for this: the lack of correlation between symptoms, changes in lung function and severity of inflammatory changes in the respiratory passages [4]; the lack of adequate perception of own condition by the patients themselves, their tendency to significantly exaggerate the level of bronchial asthma control [5]; low level of adherence to treatment, especially to the basic (maintenance) therapy with iGCS – only 1/3 of patients are going to take medication constantly, and half of them independently control the volume of basic therapy, often increasing only the dose of SABA in case of exacerbation [6,7].

It is important to emphasize that a mild course of asthma in itself does not guarantee the high effectiveness of therapy and the well-being of patients. In Russia, from 2010 to 2011, a multicenter study of NIKA was conducted, the main goals of which were to assess the real level of BA control among patients visiting a medical treatment facility, as well as to compare the perception of BA control by patients themselves with its assessment by the attending physician. According to this study, the disease was actually controlled in only 20% of patients with mild BA diagnosed at the time of treatment and therefore receiving therapy [5]. The mild course of the disease can be accompanied by severe exacerbations and even fatal outcomes. Failures in the treatment of mild asthma are due to two main reasons. On the one hand, doctors underestimate the potential risks associated with the disease, and, as a result, an insufficient volume of therapy (SABA, cromons). On the other hand, due to minimal symptoms, patients often refuse to constantly take medications for maintenance therapy and remain without medication for a long time.

Studies have shown that the lack of proper control of the course of BA can have serious consequences, leading to the development of severe exacerbations of the disease in 30–40% of patients with mild BA, while 53% of patients who sought emergency care were those who did not receive basic therapy containing iGCS [8].

All of the above confirm the provision that mild BA is an urgent problem that requires finding an optimal solution and new approaches to the problem of patient compliance and adherence to iGCS therapy. According to the GINA 2019 recommendations, the preferred basic therapy of the 1st and 2nd stages is now the use of iGCS both in the form of a combination of low-dose of iGCS (budesonide) with long-acting beta2-agonists (formoterol), and in the form of using of low doses of iGCS each time before using SABA as an alternative [9]. To date, fixed combinations of iGCS with SABA have also become available in Russia.

Purposes of this study:

a) assessment of the incidence of mild BA in various regions of the Russian Federation;

b) analysis of the nature of inhalation therapy initially received by patients included in the observational study;

c) determining the effect of therapy on the level of control of BA symptoms;

d) assessment of the clinical effectiveness of the combined drug SabaComb® to achieve control of symptoms of mild BA that is the end point of the study.

SabaComb® is an inhaled combination drug, which is a combination of IGCS -beclomethasone dipropionate at a dose of 250 μg and SABA – salbutamol at a dose of 100 μg.

MATERIALS AND METHODS

The observation program lasted from July to December 2018 and included 2 visits of patients selected according to the criteria for inclusion in the program. Questionnaires were filled out based on the results of both sessions. The first presentation was always face-to-face.

The patient's questionnaire included metric data, etiological factors of exacerbation, period of pollination in the region, history of allergies, complaints, data from physical examination and respiratory functions, the severity of BA and the level of its control. The previous therapy that was prescribed by the doctor earlier, before the patient was included in the study, the date of its change, the dosage regimen of the combination of beclomethasone dipropionate and salbutamol were indicated. The second visit was carried out in the range from the 14th to the 28th day from the date of initiation of SabaComb® therapy, both face-to-face, during a care encounter, and by telephone contact in case of satisfactory health of the patient, which was provided for by the program design. During the survey at the second visit, data on existing complaints, the medicine received, the level of asthma control, and the assessment of treatment results by the patients themselves using a five-point system were recorded. The results of using SabaComb® (if prescribed to patients) and satisfaction with the medicine used were also evaluated, and adverse reactions to SabaComb® were recorded.

3612 questionnaires from 23 cities of the Russian Federation were selected for statistical processing. Data collection for subsequent analysis was carried out using a specially developed questionnaire, which included: the frequency of prevalence of mild forms of asthma depending on the region of the Russian Federation; the frequency of use of various inhalation drug regimens for the treatment of mild asthma, determining the causes and seasonal features that affect the occurrence of symptoms. Then, based on the results of statistically processed data obtained in the answers to the questions posed in the questionnaires, the assessment was carried out. Mathematical and statistical data processing was performed using standard software packages Statistica (V7. 0) and SPSS Statistics (V17.0).

Patient adherence and satisfaction were assessed by filling out a questionnaire – the Likert scale – a psychometric ordinal rating scale used in questionnaire studies. When answering the questions asked, the degree of agreement or disagreement of the patient with the set statement is indicated. The total score of each individual judgment allows you to identify the attitude (opinion) of the trial subject on any question.

RESULTS AND DISCUSSION

1. General characteristics of patients

Analysis of 3612 questionnaires from 23 cities in Russia showed that the average patient with mild BA was 38.56+14.55 years old, with a slight predominance of women. The average number of patients monthly visiting the doctor with mild BA was 33.98+37.67 people. Analysis of the average indicator in particular cities showed that the lowest number of visits to a doctor was in Omsk and Ulyanovsk (6.74+8.44 and 12.00+0.00, respectively). Chelyabinsk was in the first place for the monthly visits of patients with mild BA to a specialist (227+5.28). In Abakan and Krasnoyarsk, patients with mild BA visited the doctor less frequently (80.79+68.77 and 90.00+0.00, respectively). Patients with mild BA in Moscow and Krasnodar visited the doctor with approximately the same frequency (53.9+53.60 and 54.16+39.06, respectively).

In the structure of the main diagnoses in accordance with the ICD-10 codes, the patients with allergic bronchial asthma prevailed (J45, 0–53, 1%). Most often, the patients with mild persistent bronchial asthma sought help (63.40%). Almost half of the patients or their relatives had a history of atopic allergic diseases. A third of patients previously recorded a decrease in the peak expiratory flow rate (PEFR), the forced expiration volume per 1 second (FEV1) and increased eosinophil content in peripheral blood. The most common triggers for BA exacerbation were causal allergens (55.23%), plant pollen

(45.04%), physical activity (39.65%), cold air (35.41%), and inhalation of irritants (36.66%).

At the first visit, almost all patients (99.31%) presented various complaints, 76.30% had auscultative symptoms in the form of dry wheezing, and the study of external respiratory function (ERF) in 1/3 of patients revealed obstructives.

2. Characteristics of therapy in patients with mild BA when they are included in the study

The distribution of patients with mild BA depending on the medicines used and previously prescribed is shown in Table 1.

As follows from the data presented, 51.5% of patients received only short-acting bronchodilators (salbutamol, fenoterol, a combination of fenoterol with ipratropium bromide). Less frequently, basic iGCS therapy in combination with SABA (10.16%), SabaComb® was received by 8.22% of patients. When evaluating the impact on achieving the control, it was found that the best results in maintaining a good level of control of mild BA symptoms were demonstrated by SabaComb® in the "on-demand" regimen (35.00% of patients). When prescribing the iGCS maintenance therapy, the number of patients with good symptom control was lower, that may be due to non-compliance with the recommendations for continuous use of iGCS, because the patients were often limited to the use of SABA only (Table 2).

Table 1

THE DISTRIBUTION OF PATIENTS WITH MILD BA DEPENDING ON THE MEDICINES USED

Options of BA therapy at the first visit	Number	%
Salbutamol	1288	35.66
Fenoterol	158	4.37
Ipratropium bromide + fenoterol	417	11.54
iGCS	159	4.40
iGCS + salbutamol	197	5.45
iGCS + fenoterol	44	1.22
iGCS + ipratropium bromide + fenoterol	126	3.49
SabaComb®	297	8.22
Other therapy	271	7.50
Therapy has not received before	655	18.14
Total	**3612**	**100**

In the framework of the observational program the correction of BA therapy was provided. Prior to inclusion into the study, 297 patients received SabaComb®, both as basic therapy and as on-demand; after inclusion into the study, they continued taking the medicine in the same regimens. After the first visit, SabaComb® was prescribed to a total of 3,556 patients. Of these,

Table 2

THE DISTRIBUTION OF PATIENTS DEPENDING ON THE LEVEL OF CONTROL OF BRONCHIAL ASTHMA SYMPTOMS AND TAKING INTO ACCOUNT THE INITIAL THERAPY (FIRST VISIT)

Symptom control level	SABA on-demand	Basic iGCS	Basic iGCS + SABA on-demand	SabaComb®	
				basic	on-demand
uncontrolled	46.10%	17.61%	27.80%	12.35%	6.67%
partially	51.85%	77.36%	62.94%	67.90%	58.33%
good	2.05%	5.03%	9.26%	19.75%	35.00%

Table 3

DYNAMICS OF THE EFFECTIVENESS OF BA SYMPTOMS CONTROL IN PATIENTS AT THE TIME OF INCLUSION INTO THE STUDY (FIRST VISIT) AND AFTER 14–28 DAYS OF ADMINISTRATION OF SABACOMB® (SECOND VISIT) (COMPARISON WITH PREVIOUS IGCS BASIC THERAPY + SABA ON DEMAND)

Symptom control level	Visit 1 iGCS basic therapy + + SABA on demand		Visit 2 SabaComb®	
	number	%	number	%
uncontrolled	102	**27.79**	28	**0.78**
partially controlled	231	**62.95**	317	**8.92**
good controlled	34	**9.26**	3211	**90.30**
Total	367	100	3556	100

1,186 (33.35%) patients received it on demand, and 2,370 (66.65%) received it as basic therapy. The administration regimen is defined by the attending physician, taking into account the patient's condition. During the second visit the level of control of bronchial asthma symptoms were assessed in patients who had used SubComb®.

Comparison of the results of using iGCS as a basic therapy + SABA on-demand (data obtained during the survey and examination of patients at the first visit) and the appointment of a fixed combination of iGCS/SABA (Sabacomb®) at the second visit after a 14–28-day course of treatment in various regimen in patients with mild BA revealed the following (Table 3).

The results of using a fixed combination of SabaComb® in various regimens at the second visit were quite high that is the disease was controlled in 90.30% of patients 2–4 weeks after the first visit. While in patients, who were initially on basic therapy with low doses of iGCS with the use of SABA on-demand, the disease control was mostly partial, i.e. 62.95%. The data obtained indicate the adherence of patients with mild BA to inhalation therapy with a fixed combination of SABA + iGCS. The advantage of this type of therapy is that while the patient is relieved by

Table 4

LEVEL OF MILD BA CONTROL DEPENDING ON THE REGIMEN OF USE OF THE SABACOMB® INHALER (SECOND VISIT)

Level of BA control	Maintenance therapy		On-demand	
	number	%	number	%
uncontrolled	20	**0.84**	8	**0.67**
partially controlled	237	**10.00**	80	**6.75**
good controlled	2113	**89.16**	1098	**92.58**
Total	2370	100	1186	100.00

Table 5

PATIENTS' ASSESSMENT OF THE RESULTS OF MILD BA TREATMENT USING SABACOMB® (AVERAGE SCORE OF THE PSYCHOMETRIC LIKERT SCALE)

	Valid N	Mean	Median	Minimum	Maximum	Std. Dev.
Therapeutic effectiveness	3556	4.82	5	1	5	0.44
Convenience of the dosage form	3556	4.81	5	1	5	0.45
Assessment of tolerability	3556	4.85	5	1	5	0.42
Patients' adherence	3556	4.74	5	1	5	0.53
Availability of a medicine	3556	4.59	5	1	5	0.67
Availability in pharmacies	3556	4.46	5	1	5	0.80

inhalation, they also receive anti-inflammatory therapy.

Comparison of the effect of different SabaComb® administration regimens ("constantly" and "on-demand") did not reveal statistically significant differences. However, the trend towards the better control of symptoms was observed in the group of patients using the inhaler in the "on- demand" regimen (Table 4).

The questionnaire developed for this observation program included a survey of patients to identify their satisfaction with the treatment, which is one of the important factors for successful therapy. The survey used criteria such as therapeutic efficacy, the convenience of the dosage form, tolerability, affordability, availability in pharmacies, and willingness to continue treatment with SabaComb®. The criteria were evaluated by scores from 1 to 5. The average data obtained showed a fairly high satisfaction of patients with the therapy. The therapeutic efficacy of SabaComb®, its tolerability, ease of use, and, importantly, significant adherence to treatment were evaluated positively (table 5).

The medicine showed good tolerability: out of 3556 patients treated with SabaComb®, only 0.14% of cases (5 patients) had adverse reactions during therapy.

CONCLUSIONS

1. The average monthly medical aid appealability of patients with mild BA in 23 cities of the Russian Federation was 33.98+37.67 people. Most often, residents of Chelyabinsk sought medical advice, less often – residents of Ulyanovsk and Omsk. Both men and women visited the doctor equally often. The average age of patients was 38.56 years, more than half of them were patients with persistent BA.

2. The most common exacerbation of BA was caused by a cause-significant allergens, plant pollen, physical activity, cold air, and inhalation of irritants.

3. The most common treatment option for mild BA, according to the first visit results, was SABA in the form of monotherapy (51.5%); much less often a combination of basic iGCS therapy + SABA on-demand or iGCS monotherapy was used. 297 patients received a fixed combination of SabaComb®.

4. The use of SABA in the form of monotherapy for mild BA made it almost impossible to achieve control of the disease. With iGCS monotherapy, despite the powerful anti-inflammatory effect of this group of pharmaceuticals, the partial control of symptoms was mainly achieved. The combination of basic iGCS therapy with periodic

use of SABA also allowed achieving the partial control of BA. The group of patients with a good level of BA control was the largest among patients receiving a fixed combination of iGCS/SABA – Sabacomb® by the time of inclusion into the study.

5. The use of SabaComb® for 2–4 weeks, both as a permanent basic therapy and as the terapy on-demand, allowed us to achieve better results in controlling the symptoms of BA compared to the initial data in patients, who received basic iGCA + SABA on-demand at the time of the first treatment.

6. Comparison of the effectiveness of different SabaComb® prescribing regimens in the treatment of mild BA did not reveal statistically significant differences, however, when using SabaComb® in the "on-demand" regimen, a certain trend towards more frequent achievement of a good level of BA control was revealed.

7. The therapeutic efficacy, ease of use, adherence to treatment, and tolerability of SabaComb® were rated by patients as quite high i.e. 4.74–4.85 out of 5 points. Adverse reactions were very rare – in 0.14% of cases and did not pose a threat to human health.

The obtained conclusions allow us to recommend SabaComb® as a maintenance therapy for patients with mild BA to achieve good control, both in the regimen of constant administration and in the "on-demand" regimen.

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ORGANIZATION AND OPTIMIZATION OF PREPARATION FOR THE BIOLOGY UNIFIED STATE EXAM IN THE SYSTEM OF INTEGRATION OF NATURAL SCIENCE KNOWLEDGE AND PROFESSIONAL ORIENTATION IN PRE-VOCATIONAL TRAINING

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In this article the authors present the long experience of teaching and preparing for the unified state exam in biology, which is aimed at filling in gaps on difficult topics, generalizing, expanding and deepening the knowledge in all parts of biology. There are a number of problems that limit high-quality and useful preparation for the exam: the difficulty of students' reproducing the material from previous years of study, low ability to integrate and understand in an integrated manner, and therefore memorize the significant amount of natural science knowledge. To overcome the difficulties that arise along this path, a number of methodological techniques are proposed that allow you to optimally develop intelligence and erudition, to synthesize the natural science knowledge, which significantly improves the result of joint activity of the teacher and student. Clear work management, the home work schedule, and the use of creative notebooks in which the material is structured and clearly presented, give the emotional coloring to the learning process as a result and provide the better acquisition of knowledge by the student. Interval repetitions, "annual" and "parallel" tasks, acquiring of the applied knowledge and skills in various fields of natural science will allow the students to expand their creative potential and make a choice of profession.

Keywords: integrative approach, natural scientific education, interval repetitions, structural-logical schemes, creative notebook, professional orientation, Unified State Exam (USE)

Biology is not only an important discipline of the natural science cycle of secondary and senior high school, contributing to the development of the cognitive and intellectual sphere of the students, expanding the horizons of a holistic vision of the world around them, but also one of the most interesting, entertaining and promising sciences in terms of choosing a future profession.

The main task of natural science education is to provide students with the necessary conditions for acquiring of the basics of the knowledge that is accumulated today in the Earth Sciences. Natural science knowledge is very diverse – it also contains historical data of various studies and behind these studies there are scientists,

their biographies and discoveries, scientific facts and theories, as well as methods that science uses to apply rich theoretical material to important areas of human existence: medicine, breeding, agriculture, pharmaceuticals, and molecular genetics.

In modern school, all parts of biology are quite competently and logically built, starting from the 5th to the 11th grade, and, taking into the account the above, students are very willing and motivated to choose biology as a subject to prepare for the exam at their choice. The relevance of the unified state examination is confirmed by FSES of basic general education, that build "a portrait of a high school graduate", whose one of the personal characteristics is the desire "to understand the world, to realize the value of labour, science and creativity" [4]. Thus, the unified state exam in biology becomes an important and necessary stage of natural science education, which allows:

- to repeat the studied material;
- to fill in the gaps on the most difficult topics ("Genetics", "Breeding and biotechnology", "Life cycles of plants and animals", "Intracellular processes of plastics and energy");
- to concentrate, summarize the material of all parts of biology, studied for five years in middle and senior high school;
- expand and deepen knowledge for better understanding of the natural science picture of the world and high-quality preparation for admission to higher school to the medical, environmental, pharmaceutical, veterinary, and pedagogical faculties.

Despite many years of experience in the Uniform State Exam implementation into the elective exam practice and logically enough systematic and structured material of task options there are a number of problems that limit the ability of high-quality and productive training of students for the exam and do not allow to lay into their educational, intellectual baggage the integrated and holistic knowledge, aimed at the idea of creative self-expression. Overcoming these problems is an important task, and its solution is necessary for the future development and formation of students as specialists and highly qualified professionals in the field that they choose as the main one for realizing their potential.

These types of problems include:

- difficulty in repeating of the previous – year material by students. Knowledge in the field of systematics, morphology, features of plant and animal life cycles, their diversity and lifestyle, ways of infection and prevention of infections and infestations; anatomy and physiology of the nervous, circulatory, endocrine, urinary systems and their pathologies; structural and functional features of plant and animal tissues become "outliers". Some important aspects of micro – and macro-evolution, as well as ecology, often cause difficulties;
- difficulties in integrating of natural science knowledge in the step-by-step study of biology parts, which create prerequisites for their separate perception, and therefore prevent the memorization and translation of the information into long-term memory registers;
- presentation of the material according to the "template" leads to decrease in interest in the discipline being studied, a lack of creativity in the process of accumulating the knowledge, and deterioration in memorization and performance during the exam. In addition, this approach "suppresses" the desire for self-development, which, in turn, reduces the motivation of students to study and causes problems with their future professional orientation.

Overcoming the difficulties of preparing for the exam is the task of the methodology of teaching biology, which should be aimed at filling in the gaps in learning, and at generalizing the knowledge and skills accumulated over the years of study in secondary school, at optimizing them in the course of integration in order to gain

new competencies and develop the creative potential of the student, his motivation and intelligence.

Teaching experience shows that there are several approaches that allow not only to train students in grades 10–11 to pass the Unified State Exam in biology, using methods aimed at repeating the facts, theories, laws and obtaining the skills for completing the tasks of different levels of complexity, but also to bring the knowledge base into a single, interconnected system that expands, deepens and integrates the disparate and "lost" information elements of various parts of this subject. It should be noted that such systematization and integration is very important not only in biology itself. It is necessary to understand that comprehension of general biological laws includes understanding the laws of physics, for example, the laws of thermodynamics when studying the intracellular processes of catabolism and anabolism, circulation of substances and energy flow at the biosphere level; laws of chemistry, knowledge of which is widely used to understand the functioning of biological systems, i.e. at all levels of organization of living matter; laws of mathematics, in particular, the acquisition of the ability to operate with the concept of "probability" in genetics, to perform statistical analysis of variation curves of modification variability, to accomplish mathematical actions when performing certain tasks of the Unified State Exam.

One of the essential points of preparation for the exam, of course, is clear and well-coordinated organization of the student's work both in the classroom and at home. Success in the accumulation and synthesis of knowledge and their revision depends largely not only on professionalism and personal qualities of the teacher, but on properly coordinated activities of a student and a teacher in the school, and on the structured homework. For this purpose, we recommend students to learn at home in accordance with a schedule that provides for the distribution of material for the specified topics in different parts of biology by days (3–4 days in the 10th grade and 5 days in the 11th grade).

This work should not last too long: provided time for learning and repetition of theory is 30–40 minutes and time for the practical training, i.e. an appeal to the tasks of the first part of the Unified State Exam variant (for example, FIPI Unified State Exam collection by Rohloff V.S. [7]), exercises of the portal "РЕШУ ЕГЭ" (SOLVE USE) is 25 minutes. Prolonged studies often "wash away" information from memory and reduce interest in the subject. Verification of theoretical knowledge can also be carried out through other control and measurement materials such as tests, creative tasks in the form of making the tables, graphs, illustrations and drawings with appropriate captions to them. Tasks of Part "C" which are the most difficult for learning the topics, or their aspects (so – called parallel-accessible and understandable, independently studied material) should be worked out in a creative home notebook at the specified time on the 4th or 5th day of the home schedule.

The mandatory and most essential component for full and lasting memorization of fairly extensive factual material on biology is not only working in the classroom using classical teaching methods and the ability of the teacher to establish effective communication relationships, but also actively working for development of the creative potential of a senior high school student.

The following techniques proved to be the most effective for revealing such abilities of the student and integrating the natural science knowledge.

First, this is the use of so-called creative notebooks in the educational process, which serve as a reliable support for strengthening the student's knowledge not only in the classroom, but also at home. In these notebooks, the student learns to concisely, shortly and consistently

present the text, compiling it from two or three sources. This activity should be creative, bring your own vision of the phenomenon or process, without distorting its essence and cause-and-effect relationships. This can be either the thesis presentation of information, or the creation of structural and logical schemes that allow you to illustrate and describe the process at any level of organization of living matter. In addition, the independent solution of such a problem is an important stage of the ability to generalize and integrate the knowledge of different topics and parts of biology, which develops intelligence, erudition and intuition. Structuring the material is usually accompanied by a short text that is necessary for better understanding of the natural phenomenon to be explained. Children often add figurative illustrations which emotionally color the material, make it attractive and easy for remembering (Fig. 1).

Another key technique for memorizing and summarizing the new material and consolidating the studied material is to repeat it three times during the preparation for the exam. As is well known, in the psychology of memory there is a problem of quickly forgetting of the novel information. Initially, the processes of forgetting were studied in experiments by the German psychologist Hermann Ebbinghaus in 1895. He derived a graph of dependence, later called as the Ebbinghous curve, which reflects the process of forgetting the memorized information in the person's memory over time (Fig. 2):

To solve the problem of rapid forgetting, in the psychology of memory there are various methods for increasing the strength of knowledge and transferring it to the registers of long-term memory. We recommend using the method of interval repetitions [9], which consists in repeating the studied material in certain, constantly increasing intervals. For long-term memorization of information, the following method is used: the first repetition must be performed one day after the explanation of the material, the second repetition – in a month, and the third repetition – in six months. In preparation

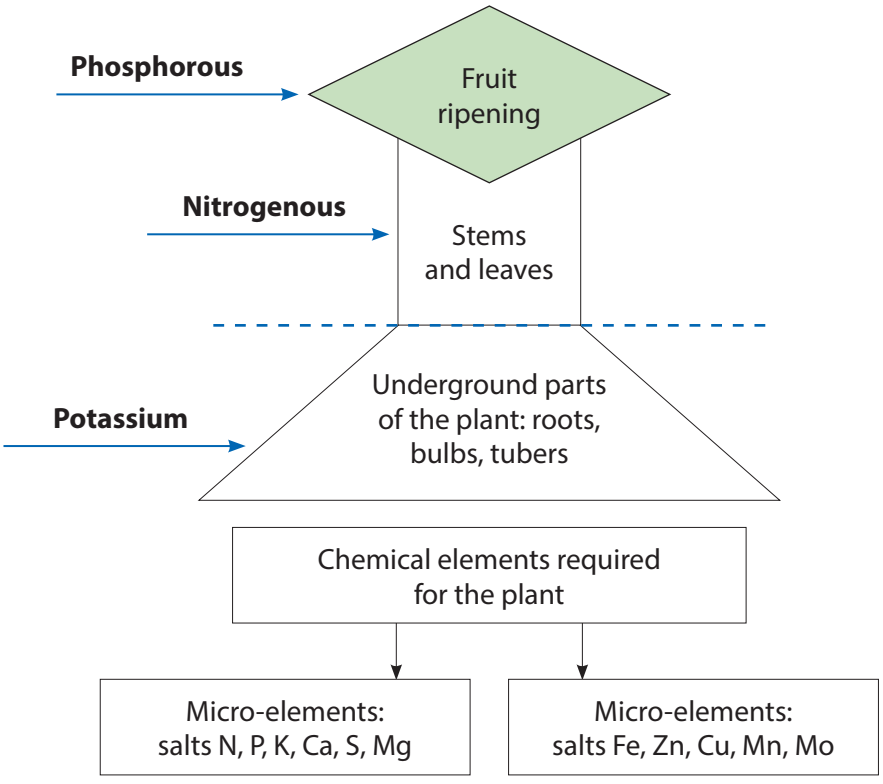

FIG. 1. *Effect of fertilizers on the growth of different plant organs*

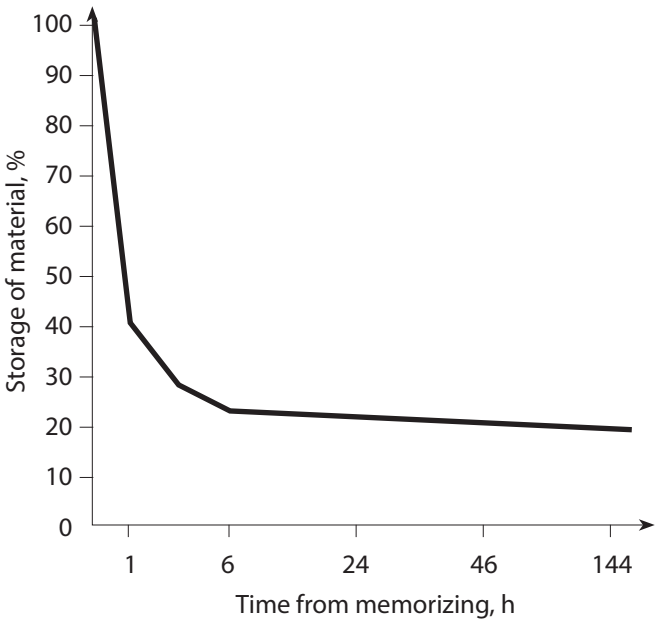

FIG. 2. *Forgetting curve or Ebbinghous curve [8]*

for the unified state exam, the time frame for repetition can be changed depending on the time specified for it. Experience shows that if the material is integrated and repeated several times, the output of knowledge (efficiency of learning) will be high enough, which is reflected in the final result – a USE good score. At the same time, it is mandatory to include all types of memory such as visual, aural and motor (creative work in notebooks at home and in the classroom) into the learning process.

In addition, the completeness and thoroughness of a holistic approach to nature depends on the quality of the study of the so-called annual tasks i.e. learning of complex topics distributed over several lessons of the curriculum and requiring in-depth study. These topics include, in particular, "the History of Life on the Earth" ("Geochronological scale"), the study of the features of monocotyledonous and dicotyledonous plant families, the tree of life and the diversity of flora and fauna of the Earth.

In conclusion, we note that this article presents a number of methodological techniques that are aimed at preparing the students for the unified state exam in biology, related to the ability to get the conceptual framework of the biology course of secondary school, knowledge of biological processes and phenomena at different levels of organization of living matter, to learn skills to solve quantitative and qualitative biological problems of different levels of complexity. These methods teach the ability to generalize various materials, integrate the vast informational background of natural science knowledge, and fully and deeply understand nature, thus contributing to the expansion of horizons and erudition. Applied knowledge and skills in the field of biotechnology, genetics, molecular biology, breeding, nature protection, hygiene and efficient use of natural resources will also help the student in choosing a profession and self-determination in society.

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WET GRANULATION PROCESS PARAMETERS INFLUENCE ON GSB-106 TABLET PROCESS CHARACTERISTICS

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The influence of the parameters of the wet granulation process, such as the type of binder component, its content in the humidifier solution and the residual moisture of the granulate, on the strength and disintegration of GSB-106 tablets has been studied.

Keywords: disintegration, Heckel equation, GSB-106, porosity, strength, wet granulation.

The wet granulation method is widely used in the technology of dosage forms (DF), in particular, in the production of tablets, since this way it is possible to achieve uniform filling of the matrix with the tablet mixture and prevent its delamination under various kinds of vibrations [1,2].

It is known that the wet granulation process is controlled by a number of parameters, such as the compacting pressure, the amount of binders, their content in the solution and the residual moisture. By varying their values, you can change the most important technological characteristics of the resulting tablets [2–4].

So, for example, increasing the mechanical strength of tablets by increasing the compacting pressure or by using high-viscosity binding solutions can lead to a decrease in porosity and thus worsen their disintegration [5–7].

In this regard, **the purpose** of this study is to identify the effect of the parameters of the wet granulation and tableting process on the strength and disintegration of tablets containing the pharmaceutical substance (PHS) GSB-106 with antidepressant activity.

MATERIALS AND METHODS

Materials: PHS GSB-106 (bis- (N-monosuccinyl-L-seryl-L-lysine) hexamethylenediamide [8], lactose monohydrate (Lactochem Fine Powder, DFE Pharma, Germany), microcrystalline cellulose (Microcel MC 101, Blanver Farmoquimica Ltda, Brazil), polyvinyl copolymer alcohol and polyethylene glycol (Kollicoat IR, BASF, Germany), polyvinylpyrrolidone (Kollidon 25, BASF, Germany), partially pregelatinized corn starch (Starch 1500, Colorcon Ltd., United Kingdom), purified water (FS.2.2.0020.15), magnesium stearate (magnesium stearate, EP 01/2008: 0229).

Methods: The appearance of the GSB-106 granules was evaluated based on images of a Phenom XL scanning electron microscope (Phenom-World, Netherlands). Determination of the technological characteristics of tablet mixtures was carried out according to standard methods using the following equipment: moisture analyzer Sartorius MA-35 (Sartorius AG, Germany), flow testers GDT and bulk density SVM-10 (Erweka, Germany) [9]. Based on the obtained values of the bulk density, the Carr compressibility index, Hausner coefficient, true density and porosity were calculated [6,10].

Tablets weighing 0.1 g were obtained using a PRG 1–50 manual hydraulic press (VNIR, Russia) with a punch diameter of 6.0 mm. For GSB-106 tablets, characteristics were determined using a TBF-1000 tablet strength tester (Copley Scientific, United Kingdom) and a PTZ-S tablet disintegration tester (Pharma Test, Germany) [9]. The optimal compacting pressure was selected using the Heckel mathematical model in accordance with the equation:

$$\ln\left(\frac{1}{1-D}\right)=k\times P+A,$$

где D – relative density of the tablet at compacting pressure P; k – the inverse of the slope of the graph that reflects the ability of a material to deform under pressure; A – constant value characterizing the filling of the matrix and the redistribution of particles in the period before their deformation and binding [11].

RESULTS AND DISCUSSION

Model tablet mixtures were prepared by the wet granulation method, pierced through a sieve with a hole diameter of 2 mm, dried in an oven (Binder, Germany) at temperature of 45°C, after which sieve calibration and dusting were performed. Model compositions of tablet mixtures are given in Table 1.

At the stage of preparing binding solutions, it was found that Kollidon 25 and Kollicoat IR form concentrated solutions, in contrast to partially pregelatinized starch, which is associated with the high content of fines in the tablet mixture of the 3rd composition. Tablet composition 1 and 2 consisted of larger granules, however, the granules of tablet composition 1 varied significantly in size and shape.

At the tabletting stage, for the tablet mixture of the 2nd composition, the most uniform filling into the matrix was noted, and for the tablet mixture of the 1st composition, pre-pressing was required, which is a consequence of their technological characteristics (Table 2).

The degree of flowability of the tablet mixture of the 1st composition is assessed as satisfactory, the 2nd composition is very good, and the 3rd composition is good.

Table 1

MODEL COMPOSITIONS OF GSB-106 TABLET MIXTURES

Compo-sition	PHS GSB-106, mg	MC 101, mg	Lactochem, mg	Kollidon 25, mg	Kollicoat IR, mg	Starch 1500, mg	Magnesium stearate, mg
1	1.0	60.0	33.0	5.0	–	–	1.0
2	1.0	60.0	33.0	–	5.0	–	1.0
3	1.0	60.0	33.0	–	–	5.0	1.0

Table 2

TECHNOLOGICAL CHARACTERISTICS OF GSB-106 TABLET MIXTURES

Composition	Carr Index, %	Hausner ratio	Flowability, g/s
1	23.72	1.30	4.83 ± 0.02
2	13.70	1.15	8.74 ± 0.01
3	19.30	1.23	7.91 ± 0.04

The tablets were obtained at three compacting pressures (4, 8, and 15 kN), after which their strength and disintegration were measured, the porosity values were calculated, and graphs of the dependence of the parameters on the compacting pressure were plotted (fig. 1–3).

As expected, tablet hardness increases with increasing compression pressure and decreases with increasing tablet porosity. Tablets of the 1st and 2nd compositions have similar results. Tablets of the 3rd composition, on the contrary, are characterized by low strength with a higher porosity.

Tablets of all compositions disintegrate within 1 minute at known compacting pressures. The increased rate of disintegration of tablets of the

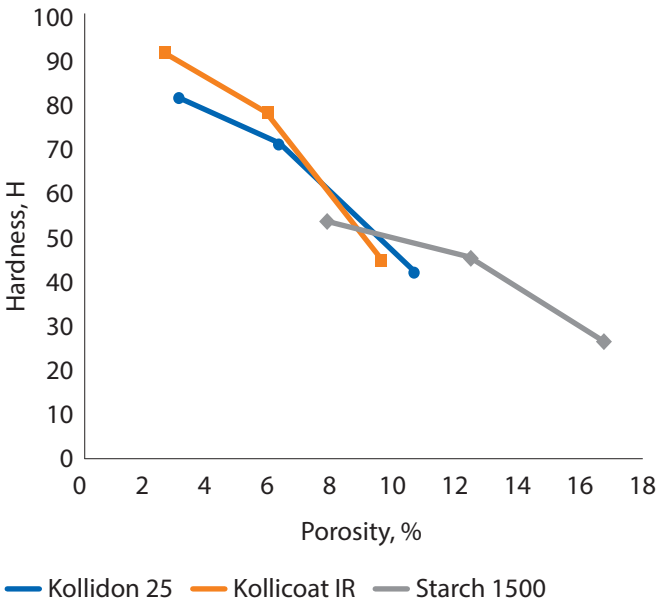


FIG. 2. *Dependence of the strength on the porosity of the GSB-106 tablets*

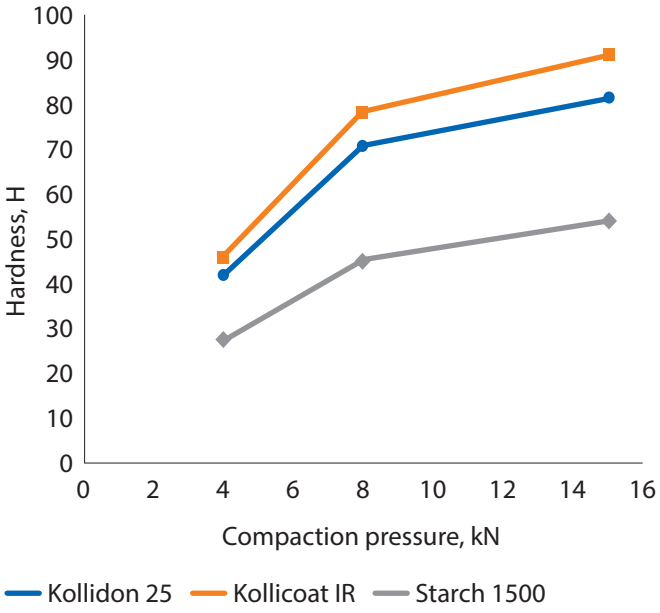


FIG. 1. *Dependence of the strength of the GSB-106 tablets on the compacting pressure*

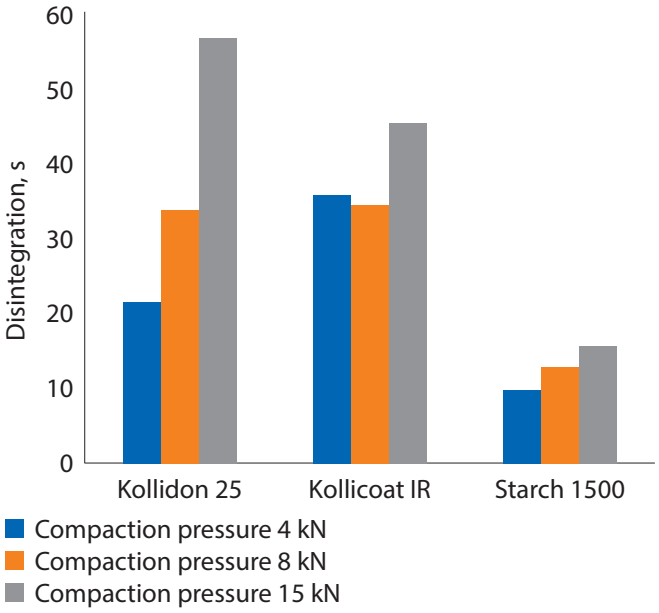


FIG. 3. *Dependence of the disintegration time of the GSB-106 tablets on the compacting pressure*

Table 3

MODEL COMPOSITIONS OF GSB-106 TABLET MIXTURES

Composition 4	Composition 5	Composition 6
6% aqueous solution Kollicoat IR	10% aqueous solution Kollicoat IR	18% aqueous solution Kollicoat IR

3rd composition is probably associated with the physicochemical properties of starch, which acts as both a binder and a disintegrating component, which contributes to an increase in the porosity of the tablets. [12].

Thus, tablets of the 1st and 2nd compositions meet the requirements of SPh XIV edition in terms of strength and disintegration; however, tablets of the 1st composition have the worst flowability, as a result of which Kollicoat IR was chosen as a binder.

The selection of the optimal concentration of the binder solution was carried out on the basis of comparison of model compositions of tablet mixtures (Table 3).

The obtained granules of all compositions have a dense structure with a granular surface; however, the tablet mixture of the 5th composition has the most uniform fractionation (fig. 4).

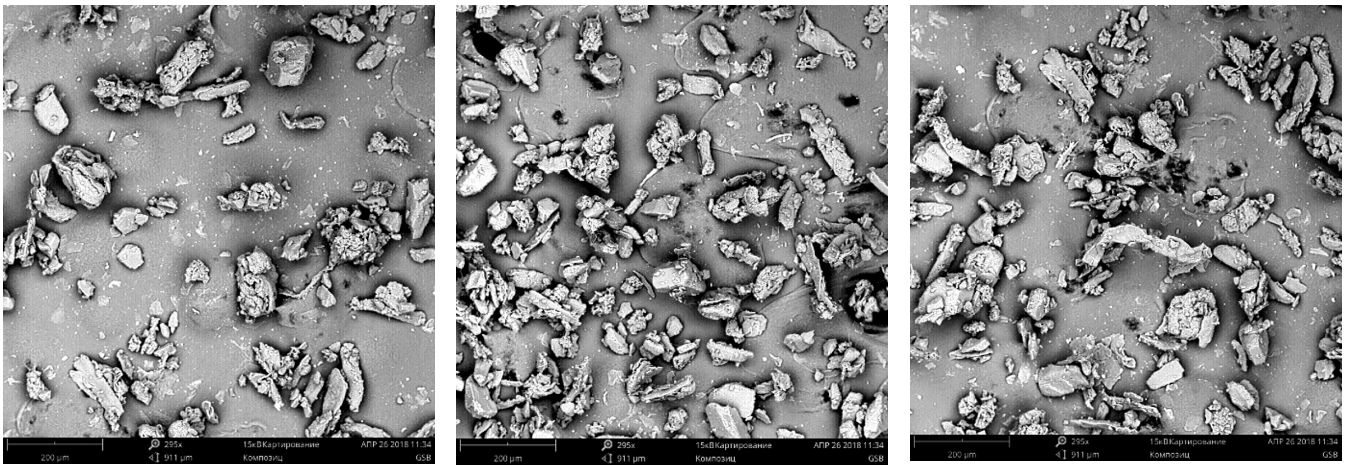
The tablets were prepared at a compacting pressure of 8 kN. The tablet mixture of the 5th composition was poured into the matrix much easier than the mixtures of 4th and 6th compositions. The tablets of the 5th and 6th compositions did not visually differ or did not differ significantly, the tablets of the 4th composition turned out to be fragile. An increase in the ejection force from the matrix for tablets of the 6th composition was noted [13].

The results of measuring the process characteristics of tablet mixtures and tablets are presented in Table 4.

The degree of flowability of the tablet mixture of the 4th composition is assessed as unsatisfactory, the 6th composition is good, the 5th composition is very good.

A joint assessment of the process characteristics of tablet mixtures and GSB-106 tablets made it possible to recognize the composition containing 10% aqueous solution Kollicoat IR as optimal.

It was found that with an increase in the moisture content in the mixture of the selected



Composition 4 **Composition 5** **Composition 6**

FIG. 4. *Electron microscopy of GSB-106 granules*

Table 4

PROCESS CHARACTERISTICS OF GSB-106 TABLET MIXTURES AND TABLETS

Composition	Visual estimate	Flowability, g/s	Carr Index, %	Hausner ratio	Crushing strength, N	Disintegration, s
4	Chipping in places	2.45 ± 0.02	26.60	1.33	26.72 ± 0.03	46.88 ± 3.91
5	Smooth flat surface	10.82 ± 0.04	13.85	1.15	73.19 ± 0.07	157.21 ± 11.16
6	Smooth flat surface	7.34 ± 0.02	19.80	1.23	184.83 ± 0.12	492.73 ± 37.13

composition, the strength increases and the porosity of the tablets decreases, while their disintegration slightly increases (Table 5).

As a result, a moisture content was considered desirable above which the degree of flowability of the tablet mixture is assessed as satisfactory (weight loss on drying 5%).

During the experiment on the selection of the compacting pressure, a graph of the dependence of the natural logarithm of porosity ln (1 / ε) on the applied pressure was plotted (fig. 5).

The straight section of the graph is located in the pressure range from 260 to 350 MPa (8.5–11.0 kN/m^2), and the optimal compression pressure for obtaining GSB-106 tablets was 312.5 MPa, which corresponds to 8.83 kN/m^2.

Thus, the optimal composition of the PHS GSB-106 tablets was selected: lactose monohydrate and MCC in a 1:2 ratio, 10% aqueous solution of Kollicoat IR, magnesium stearate.

CONCLUSION

A 10% aqueous solution of a copolymer of polyvinyl alcohol and polyethylene glycol Kollicoat IR was selected as a binder solution.

It has been established that the moisture content of the tablet mixture after drying should not exceed 5%.

The optimal compacting pressure was selected, which was 8.83 kN/m^2.

Table 5

DEPENDENCE OF THE PROCESS CHARACTERISTICS OF GSB-106 TABLET MIXTURES AND TABLETS ON MOISTURE CONTENT

Process characteristics	Moisture content, %		
	7,31 ± 0,02	5,04 ± 0,01	2,50 ± 0,01
Flowability, g/s	4.56 ± 0.03	6.98 ± 0.01	9.51 ± 0.05
Carr Index, %	23.17	19.70	13.74
Hausner ratio	1.30	1.23	1.14
Porosity, %	4.12 ± 0.07	6.47 ± 0.02	7.71 ± 0.02
Crushing strength, N	105.71 ± 0.06	87.03 ± 0.01	82.96 ± 0.03
Disintegration, s	64.31 ± 3.45	47.98 ± 2.01	44.66 ± 2.06

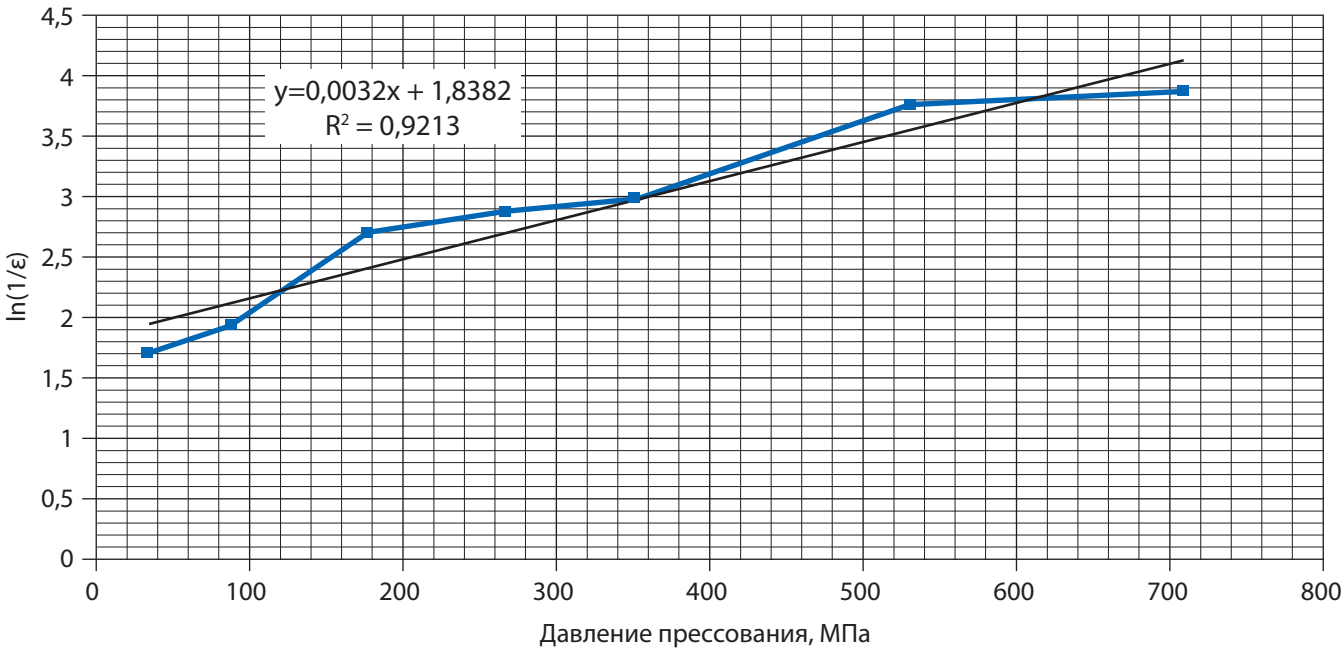


FIG. 5. *Dependence of the natural logarithm of the porosity of GSB-106 tablets on the compacting pressure*

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Happy Anniversary!

November twenty-first, 2020 marked the 80th anniversary of the Honored Scientist of the Russian Federation, full member of the Russian Academy of Education (RAE), Doctor of Pharmacy, Doctor of Education, Professor Vladimir Andreevich Popkov.

In 1963, V. A. Popkov graduated from the Pharmaceutical Faculty of the I. M. Sechenov First Moscow Medical Institute (now Sechenov University). Since 1982, he was the Head of the Department of Physical and Colloid Chemistry, which was transformed into the Department of General Chemistry in 1987. In 1983 V.A. Popkov was awarded a degree of Doctor of Pharmacy and in 1984 he achieved a rank of Professor. In 1999 V.A. Popkov was elected an Academician of the Russian Academy of Education, and in 2001 he was awarded the degree of Doctor of Education.

Under the leadership of Vladimir Andreevich, a scientific school was formed, his numerous students conduct research on the standardization of medicines and biologically active substances. V.A. Popkov completed a series of works devoted to the detection and quantitation of residual pesticides in medicinal plant raw materials and medicines obtained from them.

Vladimir Popkov is a highly qualified teacher and methodologist with fundamental knowledge and professional skills.

For many years, V.A. Popkov has given lectures on chemistry and conducted laboratory classes with students of medical, pediatric and dental faculties, as well as given lectures for students of the faculty of pedagogical education of the Lomonosov Moscow state University. He is the author of a number of programs that were used by students of medical universities of the USSR, and currently students of medical higher educational institutions of the Russian Federation are also studying. He developed and implemented the chemistry training programs for specialized medical and biomedical classes that are part of the School - University complexes. The textbook for pre-university training "Fundamentals of chemistry", which he co-authored, went through 16 editions.

The training complex, consisting of a textbook, a laboratory guide and a problem book, has passed 10 editions.

In 2005, the Fund for Development of National Education awarded V.A. Popkov a diploma for the best scientific book.

For many years Vladimir Andreevich was the Chairman of the Central Academic and Methodological Commission on Chemistry of Roszdravnadzor, a member of the Scientific-and-Methodological Council in Chemistry of the Federal Education Agency, the Deputy Chairman of the Academic Board of the RAE on psychological and pedagogical issues, a member of the Academic Board of RAE on the problems of higher pedagogical education, the Chairman of the Central Problem Academic and Methodological Commission on Chemistry of the Ministry of Health of the Russian Federation, a member of the Scientific-and-Methodological Council in Chemistry of Ministry of Education of the Russian Federation, a member of the Editorial Board of journal «Chemistry in school», a member of the Council of Elders of the I.M. Sechenov University.

For ten years, V.A. Popkov was the Chairman of the Expert Commission of the annual all-Russian competition in the field of education science and work with children and youth under twenty years «For the moral feat of a teacher». For his personal scientific and organizational contribution to the development of enlightenment, the Moscow Patriarchate of the Russian Orthodox Church awarded Vladimir Andreevich the Order of St. Macarius, Metropolitan of Moscow, 3rd class.

For a long time, V. A. Popkov worked as an expert of the Higher Attestation Commission, and was awarded a Certificate of Honor for his great achievements in the certification of scientific and academic and teaching staff.

For the development of the concept «New approaches to the interaction of secondary and higher schools in the field of chemical education. Concept and practical implementation» he won the Russian Presidential Award in the field of education.

V. A. Popkov has 15 author's certificates and patents of the USSR and the Russian Federation. Under his leadership, 25 PhD and 10 habilitational theses were prepared and defended for the degree in pharmaceutical, chemical, medical and pedagogical sciences.

He is the author of more than 300 scientific papers, including 18 monographs, 13 author's certificates and patents on the following problems:

- physical and chemical methods of analysis of pharmaceutical substances;
- methods of teaching chemistry in medical schools;
- history and philosophy of education.

In addition, Vladimir Andreevich is the author of 8 educational programs, 19 textbooks and study guides.

V.A. Popkov conducts researches related to the development of modern methods and forms of chemical education for medical, biological and pharmaceutical specialists.

V. A. Popkov revealed that the most significant in terms of professional self-improvement is developing in university professors a special form of cognitive activity such as a critical thinking style. He also formulated the main provisions of the methodology for formation of such thinking, identified the stages and levels of its maturity, laid down the concept of professional improvement of a university professor, where the general pedagogical approaches to solving this problem are presented in a generalized form - through the formation of a set of evaluative and reflexive skills that allow the teacher to deeply and comprehensively conceptualize various fragments of university pedagogical reality and on this basis successfully carry out professional activities.

V.A. Popkov pays great attention to the problems of higher school didactics. His textbook «Higher school didactics» has passed 3 editions, and the textbook «Theory and practice of higher professional education «was published in the series «Classic university textbook», dedicated to the 250th anniversary of Lomonosov Moscow State University.

Currently, Vladimir Andreevich continues to work actively at the Russian Academy of Education. Vladimir Andreevich is a kind and sympathetic person. His students, who work in different parts of our country and abroad, speak warmly about their teacher and mentor and thank him for versatile knowledge obtained from him.

The pharmaceutical and educational community, colleagues and numerous friends heartly congratulate Vladimir Andreevich on his glorious anniversary, wish him good health, further creative success and great personal happiness!

ARTICLE SUBMISSION GUIDELINES

The Editorial Board of the Journal of Pharmaceuticals Quality Assurance Issues request the authors to comply with the following rules when preparing articles for publication.

Editorial ethics. The data presented in the paper shall be original. It is not allowed to send to the editorial office the works that have already been printed in other publications or accepted for publication by other editorial offices.

The article shall be accompanied by an official referral from the institution where the work was performed, have a scientific supervisor's visa, and a seal. The article shall be signed by all authors (on the last page), which enables to publish it and place it on the publisher's website

The Editorial Board reserves the right to shorten and edit accepted works. The date of receipt of the article is the time of receipt of the final (revised) version of the article.

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2. Size of the original article shall not exceed 10 pages of typewritten text, lecture – 8–10 pages, literature review – 18–20 pages, reviews, chronicles – 4–5 pages, personalia – 2–3 pages. When preparing review articles, please, include mainly recent publications in the references.
3. Materials shall be prepared in Microsoft Word (" DOC text" parameter); computer typing shall be performed without formatting and hyphenation in ANSII text format, 14 – font size, with 1.5 line spacing (double typewriting spacing).
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