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EDITORIAL

The most significant achievements in the field of natural sciences are reached in joint collaboration, where important roles are taken by biology and chemistry. Therefore publication of a Journal, displaying results of current studies in the field of biology and chemistry, facilitates highlighting of theoretical and practical issues and distribution of scientific discoveries.

One of the basic goals of the Journal is to promote the extensive exchange of information between the scientists from all over the world. We welcome publishing original papers and materials of biological and chemical conferences, held in different countries (after the process of their subsequent selection).

Creation of special International Journal of Biology and Chemistry is of great importance, because a great amount of scientists might publish their articles and it will help to widen the geography of future collaboration. We will be glad to publish also the papers of the scientists from the other continents.

The Journal aims to publish the results of the experimental and theoretical studies in the field of biology, biotechnology, chemistry and chemical technology. Among the emphasized subjects are: modern issues of technologies for organic synthesis; scientific basis of the production of physiologically active preparations; modern issues of technologies for processing of raw materials, production of new materials and technologies; study on chemical and physical properties and structure of oil and coal; theoretical and practical issues in processing of hydrocarbons; modern achievements in the field of nanotechnology; results of studies in the fields of biology, biotechnology, genetics, nanotechnology, etc.

We hope to receive papers from a number of scientific centers, which are involved in the application of the scientific principles of biology, biotechnology, chemistry and chemical technology on practice and carrying out research on the subject, whether it relates to the production of new materials, technology and ecological issues.

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Diversity, morphology and karyotypes of species from genus *Neogobius* (*Perciformes; Gobiidae*) at coastal zone of the Caspian Sea (Aktau city)

Abstract: The paper presents the results on the diversity, morphology and karyotypes of species from the genus *Neogobius* collected during the summer seasons 2012-2016 in the shallow coastal zone of the Caspian Sea (Aktau city). A total of 6 species of goby fish belonging to two genera were found: *Neogobius* Iljin, 1927 and *Ponticola* Ilijn, 1927. Most species are widespread in the Northeast and Middle Caspian. The results of the morphological analysis did not reveal any significant differences between the populations of goby fish of the Northern and Middle Caspian, and also in comparison with the literature data. Chromosome kits and karyotypes detected in the observed gobies *N. fluviatilis pallasi*, *N. melanostomus affinis*, *N. caspius*, *P. gorlap* and *P. syrman eurystomus* are represented in the diploid set mainly by 46 acrocentric chromosomes, and in one species previously identified by us as *N.sp* (*P.iljini*) 2n = 40 chromosomes with a number of chromosome arms (NF) 46.
Key words: diversity, morphology, karyotypes, goby fish, Caspian.

Introduction

The biological diversity of animals in the Caspian Sea basin is characterized by the greatest number of endemics of a diverse taxonomic rank – from subspecies to families [1]. A significant number of endemics from all taxa were found in the Middle Caspian. According to Kazancheev E.N., the number of endemics at the genus level is 8.2%, species – 43.6%, subspecies – 100% [2].

The fauna of the fish of the Caspian Sea and adjacent areas of deltas and freshened bays and lagoons (continuously occurring species, migrating or occasionally setting) include 119 species and subspecies (including controversial status) belongin Qg to 52 genera from 15 families, of which 35 are from 12 genera belong to the family *Gobiidae* order *Perciformes* (*Perciformes*) [1; 2].

The fish of the *Gobiidae* family are one of the most complex in the taxonomic group of the Ponto-Caspian region [3]. Representatives of this family are not fully studied, and the status of some species is subject to discuss. This is mainly due to the large variability and in some cases to the difficulty of analyzing the external morphological features used in the taxonomy of gobies. The greatest difficulties are usually encountered in determining the species, some groups and genera contain many closely related and hardly distinguishable forms [4].

According to the literature, at present the family of gobies in the Caspian Sea includes the following genera: *Anatirostrum* Iljin, 1930, *Babka* Iljin, 1927, *Asra* Iljin, 1941, *Benthophilus* Eichwald, 1831, *Caspiosoma* Iljin, 1927, *Chasar* Vasilieva, 1996, *Hyrsanogobius* Iljin, 1928, *Knipowitschia* Iljin, 1927, *Mesogobius* Bleeker, 1874, *Neogobius* Iljin, 1927, *Ponticola* Iljin, 1927, *Proterorhinus* Smitt, 1899 [1]. The largest discussions about the taxonomic composition arose around the genus *Neogobius*. For the genus the Black Sea-Caspian gobies (*Neogobius* Iljin, 1927) are the senior synonym for the simultaneously published names of the generic group (*Neogobius* and *Apollonia*), *Neogobius* chosen by Berg L.S. [5]. The genus *Neogobius* now includes four species, three of which live in the Caspian Sea: *N.caspius* (Eichwald, 1831) – caspian goby, *N.melanostomus affinis* (Eichwald, 1831) – caspian round goby, *N.pallasi* (Berg, 1916) – caspian monkey goby. In the genus *Ponticola* Ilijn, in 1927 there are at least 10 species, in the Caspian Sea there are three: *P. goebelii* (Kessler, 1874) – caspian ratan goby, *P.gorlap* (Iljin, 1949) – caspian big-head

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goby, *P.syrman eurystomus* (Kessler, 1877) – caspian shirman goby [6; 7; 8].

The ichthyofauna of the coastal zone of the Kazakhstan part of the Middle Caspian is poorly understood. This applies to almost all taxa of fish fauna. However, several publications have recently appeared on the distribution, morphology, biology and karyology of the gobies of the coastal zone of Aktau [8, 9, 10]. In the present work, in addition to these studies, the results are presented for the species diversity of the gobies, as well as morpho-biological and karyological characteristics for 4 species.

Materials and methods

The material was collected in summer seasons of 2012-2016, in the coastal zone of the Caspian Sea near the city of Aktau (Mangistau region of the Caspian Sea), on Figure 1 the collection points are marked with the sign (▲).

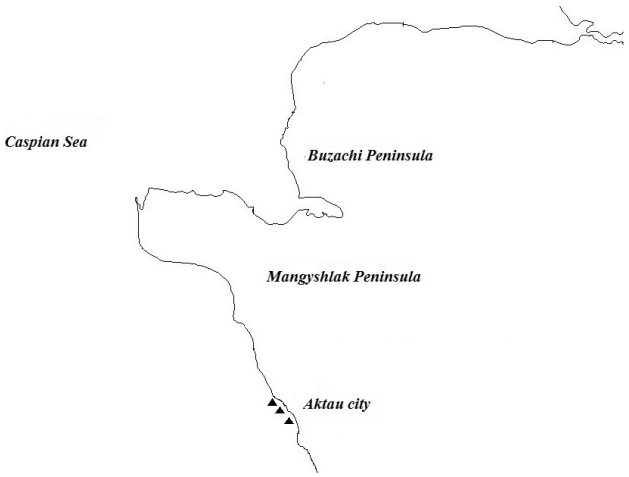

Figure 1 – Collection points within Aktau city.

During the period of research, 200 specimens of goby fish of various species were collected, of which 30% had a bridal detail. In determining the fish of the genus *Neogobius* and *Ponticola*, standard sources, descriptions and determinants were used [1; 2; 11; 12].

Fish catching was done with a net (mesh 8 mm, width 55 cm). The captured material was fixed in a 4% formalin solution. Methods of morphological and cytogenetic analysis of fish were applied. In the collection and analysis of the material, the methods commonly used in ichthyological studies were used [13]. The analysis included 4 biological indexes, 4 meristic and 22 plastic signs. Statistical processing of primary data was carried out according to standard methods of variation statistics and included: *max* – maximum, *min* – minimum, calculation of mean values of the indicator for each indicator (*M*), their error (*m*) [14]. Chromosome preparations were prepared from fixed pieces of tissue according to *Kligerman A., Bloom S.* [15]. Chromosomes were classified according to the Levan system, distinguishing four main types of chromosomes [16]. The analysis of the preparations was carried out with the help of the Microscope *MicroOptix* MX 300 T, metaphase photographs were taken with a digital camera *MicroOptix* C600. The search for metaphase plates was carried out at low magnification; the best metaphases were photographed at 100x under the immersion oil. The layout of karyotypes was made using the *Adobe Photoshop* 12.0.3 program.

Results and their discussion

During the research period near the city of Aktau, we found 6 species of goby fish: caspian monkey goby, caspian round goby, caspian goby, caspian bighead goby, caspian shirman goby and *N.sp* (*P.Iljini*). The most numerous for the whole period of observations were two species of gobies – eastern-caspian big-head goby and the caspian round goby. At the same time, their ratio over the years was the same (Table 1). The catches dominated the above species, which occur with a wider range of salinity than, for example, caspian monkey goby and caspian goby.

Morphological analysis of meristic and plastic signs revealed no significant differences between the genera *Ponticola* and *Neogobius*. Of the 22 plastic signs calculated for the length of the body, significant differences were noted only for 7. At the same time, of the 10 plastic signs of the head part, significant differences were observed in 6 indices. The percentage of differences in meristic characters from the total number of indicators studied was 15%. This indicates that the external morphology of gobies belonging to different genera is sufficiently similar, the latter circumstance complicates their species identification.

Biological characteristics, morphological and karyological analysis were carried out on 4 species of gobies, of which 3 species belong to the genus *Neogobius*, and one, previously described by us as *N.sp*., is referred to the genus *Ponticola* Ilijn, 1927. Morphological and biological features and karyology of gobies we described earlier [10]. It is shown that the caspian round goby is characterized by high morphological variability depending on the conditions and habitat [17]. According to several au-

thors [18; 19] in the populations of caspian round goby, males predominate. Analyzing the sex structure of goby fish in our catches, the ratio of males and females goby close to 1:0, juvenile specimens also was not found. This is due to the biology of breeding gobies, males in a bridal detail guard the oviposition with caviar, without leaving it even for nutrition, while females after oviposition leave for a longer distance.

There is no complete morphological and karyological data on the caspian goby, which is poorly studied in the Kazakhstan part of the sea. Probably, this is due to laborious and incorrect definition of a species or morphological similarity with a caspian round goby.

For all the goby species of the Mangistau region of the Caspian Sea, we conducted cytogenetic analysis for the first time, with the subsequent determination of karyotypes and formulas. The chromosome data on six species of goby fishes are presented, from which more than 300 metaphases have been studied and a "modal" number, i.e., the number of chromosomes most common for a given fish species was esteemed (Table 1).

Table 1 – Species diversity of goby fish (*Gobiidae*) in different years near Aktau, the number of chromosomes and the fundamental number of arms

Species, subspecies	Frequency of occurrence by years					2n	NF
	2012	2013	2014	2015	2016		
P. Iljini	+	+	+	+	+	38-40	46
N. fluviatilis pallasi	-	-	-	-	+	46	46
N. melanostomus affinis	+	+	+	+	+	46	46
N. caspius	-	-	+	+	+	46	46
P. gorlap	+	+	-	-	-	46	46
P. syrman eurystomus	+	-	-	-	-	38	46
Total	16	30	42	31	81		

Note: 2n is a diploid set of chromosomes; NF is the fundamental number of chromosomal arms

A karyotype of 46 acrocentric chromosomes is considered to be generic or basic for many gobies, including the genus *Neogobius* [4]. According to the results of conducted studies on goby fish of some authors, including ours, the chromosome set of the caspian round goby consists of 46 acrocentric chromosomes, with a fundamental number of chromosomal arms 46 [4; 9; 10; 20; 21].

Our research has shown that in the studied gobies karyotype is represented mainly by 2n = 46 acrocentric chromosomes, with the exception of *N.sp*. (*P.iljini*) in which the karyotype is represented by 38-40 chromosomes, at the same time NF = 46 (Fig. 2).

Previously obtained long-term data also allows us to separate the goby which was originally identified as the caspian big-head goby or *N. kessleri gorlap* because of similar morphological features, however, cytogenetic analysis showed that it has characteristic differences in the chromosome set. So, according to the literary sources *N. kessleri gorlap* in the diploid set has from 43 to 46 with NF = 46 [9]. In the population of *N.sp.* from the coastal zone of Aktau we found three cariomorphs with 2n = 38, 2n = 39; 2n = 40; with the number of arms NF = 46. Thus, in all the studied cariomorphs, from 1 to 4 large metacentric and submetacentric chromosomes are present. The discrepancy in the number and morphology of the chromosomes is explained by the large number of Robertsonian translocations that significantly reduced the number of chromosomes in its karyotype. Thus, the goby *N.sp.* has in the diploid set no more than 40 chromosomes, which significantly differs from the caspian big-head goby, which implies its species independence [22].

According to molecular genetic studies conducted abroad, the data obtained make it possible to classify Black Sea-Caspian gobies on a fundamentally new and higher taxonomic level and contribute to the solution of some issues at the level of genera. Thus, a certain morphological similarity between the species of the modern genera *Gobius, Neogobius, Babka, Ponticola* and *Proterorhinus* caused them to be considered within the same genus *Gobius*, i.e. to unite representatives of two different subfamilies into

one genus [23]. However, many authors [24; 25] returned to the scheme of Ilyin B.S. [26], which singled out subgenera in the genus *Neogobius* in 1927. At the same time, molecular data made it possible to impart a phylogenetic context to Ilyin's classification and substantiated the genus status of the subgenus *Ponticola*.

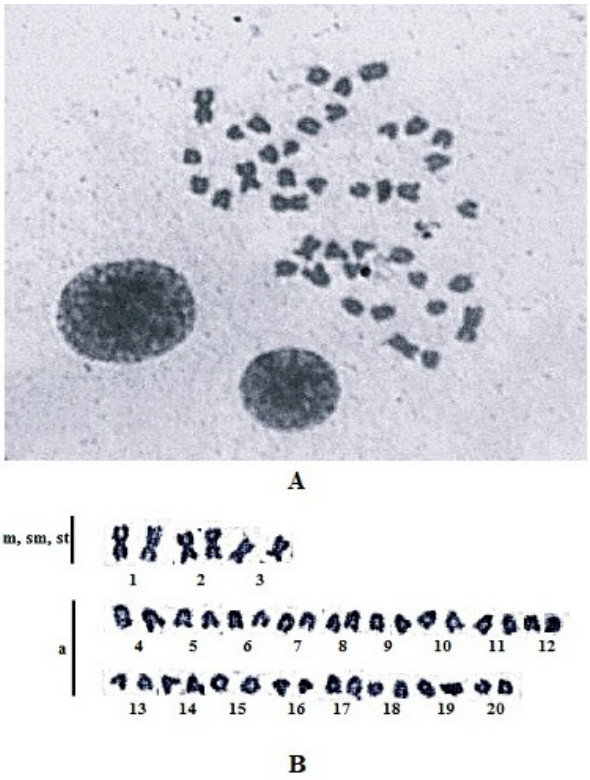

Figure 2 – A is the metaphase plate of *P. iljini*; karyotype formula: 2n = 40; 3m + 3sm + 34a; NF = 46, B – karyogram.

Recent results of the karyological and molecular genetic analysis of Russian scientists allowed separating into an individual genus, giving validity to the species *P. iljini*, or the so-called «eastern-caspian bighead goby», whose range is limited by the coast of the Mangyshlak Peninsula. On a phylogenetic tree constructed from the variability of the mitochondrial cytochrome b gene in the main species of Ponto-Caspian gobies, *P. iljini, P. gorlap* and *P. kessleri* form independent phyletic lines within the clade of the genus *Ponticola* [8].

At the same time, our long-term studies confirm the above thesis [22]. It should be noted that this species was previously registered by us as *N.sp.*

Thus, according to our latest and literary data in the Caspian Sea there are not three but four species of gobies of the genus *Ponticola* Ilijn, 1927 of the 10 existing species.

The existence of the ecosystem of the sea sections under study is explained by the vast area of well-warmed habitats of representatives of the goby family. There are still many new species and subspecies waiting to be described.

The endemism of species and intraspecies forms of the family of goby species attests to a particularly energetic process of speciation [1; 27; 28].

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Study of the effect of cadmium, lead, zinc salts on the rat blood cells

Abstract. Changes were detected in the blood of rats caused by heavy metal compounds. The results show that the salts of heavy metals poisoning demonstrates in damage in the blood cells in the animal body and making weaker their immunity. As a result, various changes occur in animals. In addition, material was collected on immune depressive exposure to heavy metals in the body of rats 25, 50, 75, 100 times more compounds of zinc ions, lead and cadmium. Animal disorders were observed in the nervous system, including tearing, inflammation conjunctive sac and certain groups even with bleeding. Leukogram, which was made by the blood of rats, showed that the poisoning caused by the impact of three types of heavy salts, has led to significant changes in the health and behavior of the animals. When increasing by 100 times more poisoning three compound salts in animal organisms have been found strong signs of poisoning, finally, all experimental rats died within 5-6 days. It is important to note, animals poisoned by 75-100 times the maximum permissible concentration of heavy metal salts of the three compound salts have died. Leukogram of experimental rats showed that, in the blood of rats, poisoned by three salts, during neutrophilia and lymphopenia, leukopenia was found. Neutrophils are characterized by granulated toxicity and gipersigmatich core.

Key words: Rats, blood, neytrofil, heavy metals, leykogramma.

Introduction

Today the decrease and destruction of immune system of an organism are considered as important problems of modern physiology. In scientific-technical and industrial century, the increase of man-made and heavy metals exposure has an influence on psychological health of human. The response of an organism to environment's adverse factors is its increased defensive reaction and as a result changes of blood immunological indexes can be seen in physiological tendency of an organism [1; 2]. In a time of stress blood's leukogram and leukocytal index changes at one definite range that depends on organism's immunological demands [3; 4].

When affected by heavy metals human and animal organisms' adaptive abilities are decreased and organism's protective system is exhausted and weakened by exerting pressure on and disturbing organism's vital functional systems. To restore the individual and cumulative impacts of negative factors of metals such as zinc, lead and cadmium, the search for new biologically active compounds that determine the cellular mechanisms of immune-boosting properties and effects on the body is the basis for the research work. Industrial development of economy and agricultural chemistry led to a sharp increase in the number of transport vehicles in big cities in recent years and which overall leads to environmental pollution. Not only the production of chemical substances harm human body, but everyday use of household chemicals increase the risk of harm to human health [5].

In many countries, including Kazakhstan, the increased industrial development neglects the responsibility to nature protection and wastes like toxins, heavy metals, pesticides and other harmful substances lead to the environmental pollution. Adverse climatic factors interact with chemicals and further enhance the strength of their insalubrities [6; 7].

Many chronic diseases are considered to be the result of the negative influence of environmental factors on the human body. The occurrence of disease is due to decreased activity of immune system [8; 9].

If allowed concentration is increased, it may produce toxic effects in human and animal body. In all cases, when heavy metals enter the body of an organism it leads to development of various pathologies, gives rise to a distortion of defensive system, which leads to a decrease of organism's functional structures [10].

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The Republic of Kazakhstan is paying careful attention to water pollution. Research results show that rivers Irtysh and Ural are polluted [11]. In Semipalatinsk region groundwater pollution can cause an ecological disaster to the area of military airport. Three largest cities of Kazakhstan – Oskemen, Pavlodar, Semipalatinsk, where there are 900 water users, nonferrous metallurgical and chemical industries, engineering and oil-gas factories, food and water agricultures are provided with water from the artery of the country – river Irtysh. This river has always been polluted by copper, zinc and other metals, and water volume required for the population is 4.82 km^3 which is 20% of all water in the country. In Oskemen city the main sources of groundwater pollution are: lead, zinc, manganese, fluoride, cadmium. In addition, for the past time there is a lot of concern about Caspian Sea. For the flora and fauna of the water reservoir it is a universal geocenosis [12; 13].

The industrial metal ions apart from gas produced by vehicles are considered as separate wastes to the environment. It is known how heavy metal ions of the elements that are thrown into the environment are gradually collected and how they enter human body from the water and atmosphere. Then they accumulate in gastrointestinal tract for a long time which cause major changes in the activity of a single individual organs or body [14].

At the moment, there is a large amount of heavy metals in the environment. Every day human body is being exposed to heavy metals, the immunological and physiological influence of which is the main focus of the research works. But the cases of excess amount of metal ions in the immune system of animals and humans have not been studied. In this regard, in the course of poisoning by heavy metals the relevance and importance of the work will be substantiated.

During the experiment changes in blood cells of rats caused by excess amount of heavy metals will be determined. Tasks: to study the changes in rat blood's leukogram after 10-days exposure to 25, 50, 75, 100 times admissible concentration limit (ACL) of zinc, lead, cadmium salts; to study and observe general changes in rat's leukogram for 60 days following 20-days exposure to 50 times admissible concentration limit (ACL).

Materials and methods

In order to achieve goals and objectives faculty of Biology and Biotechnology in the Al-Farabi Kazakh National University provided in vivarium grown laboratory rats. Weight range was 220-250 g, rats were fed with standard food, adult rats were about 5-6 months, different sex, and in total of 50 rats. To determine the effect of salts of heavy metals toxic to laboratory rats were divided into 5 experimental groups. Each group of 10 rats.

Water with 25 times ACL of salts of heavy metals (mixture of zinc sulfate, lead acetate and cadmium chloride with concentrations of 125, 0.75, 0.025 mg/l respectively) was given to the animals in group 1.

Dose of 50 times ACL of salts of heavy metals (mixture of zinc sulfate, lead acetate and cadmium chloride with concentrations of 0.05, 1.5, 250 mg/l respectively) was given to the animals in group 2.

Dose of 75 times ACL of salts of heavy metals (mixture of zinc sulfate, lead acetate and cadmium chloride with concentrations of 0.075, 2.25, 375 mg/l respectively) was given to the animals in group 3.

Dose of 100 times ACL of salts of heavy metals (mixture of zinc sulfate, lead acetate and cadmium chloride with concentrations of 0.1, 3.0, 500 mg/l respectively) was given to the animals in group 4. After 10 days of poisoning the blood of rats was studied.

0.38 ml of 3% of acetic acid was poured into clean test tube and mixed with 0.02 ml of blood. White cell count techniques: counting chamber was filled with that solution. Then the biggest indicator is replaced [15]. The number of identified cells is calculated by using this formula:

$$X = \frac{B \cdot 4000 \cdot 20}{400}$$

X – number of leukocytes in 1mm^3 of blood; 1/4000 –volume of small square; dilution ratio is 20; The number of small squares is 400; B-25 number white blood cells on a large square. Mathematical data was analyzed using the program Excel Microsoft Office. All the statistical work and comparison of $p \leq 0.05$ *, ** $p \leq 0.01$, and *** $p \leq 0.001$ were done using Student (t) program. ANOVA was used to determine the accuracy of the method.

Results and discussion

Rats that were given salts of heavy metals with 25 times ACL did not show any immunological or physiological change that is physiological parameters like size, weight, appetite, skin and basic animal instincts are kept within normal index.

Groups of rats poisoned with a mixture of salts showed 3 times lower leukocyte indexes. It must be noticed that these groups of animals has sharp in-

crease in the number of segment and core neutrophils and there was a release of immature neutrophils into peripheral blood. Absolutely no eosinophilia was found. The number of basophils was for 5-6 times higher and the number of lymphocytes was also increased.

Groups of animals that were poisoned with the mixture of salts of heavy metals also showed low level of leukocytes. In particular the number of polynuclear cells: medullocells – up to 7% ($p < 0.05$), metamedullocells- up to 6% ($p < 0.05$). The number of segment core neutrophils increased and stick-core neutrophils increased by 5-6 times. No granulocyte white blood cells were found, but the number of agranulocyte white blood cells was up to 9% that is decreased by 4-5 times.

Leukogram of rat blood that were poisoned with all three salts heavily showed significant changes in blood. Because after poisoning the development of blood cells in the bone marrow has significantly changed. During lymphopenia and leucopenia, neutrophylesis was registered. Neutrophils were characterized as toxic and hyper segmented.

Leukogram of peripheral blood showed increased number of medullocells by 16% and metamedullocells by 4%. Stick-core neutrophils were 16% and segment core neutrophils were 62% but the number of lymphocytes has reduced comparing with normal level of leukocytes. However, monocytes and basophiles were lost (Table 1).

The result of poisoning animals by 50 times ACL showed no change in physiology and physical activity of the rats. Animal's appetite is normal, skin covering is smooth, conjunctiva is clean and all the basic instincts are reserved.

Poisoning groups of animals with all three salts of heavy metals resulted in reduced number of leukocytes. In particular the number of polynuclear cells: medullocells – up to 7% ($p < 0.05$), metamedullocells- up to 6% ($p < 0.05$). The number of segment core neutrophils increased and stick-core neutrophils increased by 5-6 times. No granulocyte white blood cells were found, but the number of agranulocyte white blood cells was up to 9% that is decreased by 4-5 times.

The number of leukocytes significantly reduced (Table 1). The leukogram of rat blood registered neutrophylesis along with lymphopenia and leucopenia. Neutrophils were characterized as toxic and hyper segmented.

The effects of poisoning animals by 75 times ACL were decreased appetite, disturbance of psychomotor response, lowered physical activity and other physiological changes. Also there were registered serious physiological changes. Rats were not paying any attention to the food, skin coverings are very dirty, and at some places there was no hair on the body, not clean conjunctive, red gums and swelling of the chin, swelled abdomen (Table 1).

Interestingly, lymphocytes were up to that is increased by 2-2.5 times. Moreover, there was a sharp decline in the total number of leukocytes, by 5-6 times. Lymphocytes and eosinophils count 2.0 ± 0.01 ($p < 0.05$), monocytes and basophiles were largely absent. Neutrophils were characterized as toxic and hyper segmented. In comparison to other groups the result of poisoning animals by 100 times ACL showed much more significant complications.

Animals poisoned with the mixture of salts after 5-6 trials began to die. The body of an animal was exposed to severe stress, no hair on some parts of the skin, tearing eyes, not clean conjunctiva and red swelled area around teeth some of them were bleeding, swelling of the chin. Total number of leukocyte decreased for 3-4 times compared to normal, whereas numbers of stick core and segment core neutrophils increased, immature granulocytes are decreased. The nuclei of neutrophils are hyper segmented. Also, in all poisoned groups of animals the red blood cells granulated with basophils. All animals with poisoning concentration of 75-100 times ACL died.

Poisoning with a mixture of heavy metals resulted in 1.5-fold increased neutrophil cells compared with the control (Table 1).

Three groups of animals poisoned with salts of heavy metals were observed: neutrophils number fell for 3.8 times.

Moreover, in blood of rats poisoned with heavy metals along with neutrophylesis and lymphopenia, leucopenia was registered. Neutrophils were characterized as toxic and hyper segmented.

Lymphocytes increased for 2.46 times, the size of immature lymphocytes increased for 1.5 times. Animals that were poisoned with concentration of 100 times ACL compared to control group showed a number of signs of heavy poisoning and which eventually died on 5^{th} and 6^{th} day. The study showed that significant changes in white blood cells and leukograms of poisoned animals were observed, and decrease of the immune system of animals was also noticed. As a result, there are different varieties of changes in animal body. The body of an animal was exposed to severe stress, no hair on some parts of the skin, tearing eyes, not clean conjunctiva and red swelled area around teeth, some of them were bleeding, swelling of the chin.

Table 1 – Total number of white blood cells and data of leukograms made by analyzing control group and groups where rats were poisoned with salts concentrations of 25, 50, 75, 100 times higher than ACL.

Concentration	Leukocytes	Medullocells	Metamedullo-cells	Neutrophils		Eosinophils	Basophils	Lymphocytes	Monocytes
				Stick core	Segment core				
	M± m	M± m	M± m	M± m	M± m	M± m	M± m	M± m	M± m
Control	11182.2±12.2	0 0	0 0	477.5±2.7 5.2±0.3	.5692.96±72.0 62.0±0.1	376.5±5.2 4.1±0.03	165.3±6.3 1.8±0.1	2020.4±6.1 22.0±1.9	560.1±4.2 6.1±0.5
25x ACL	9680.2±56,4	440.8±9.2 8.2±0.2	327.2±7.4 7.2±0.2	908.8±5.7 18.3±0.5	2356.4±13.2 70.2±3.1	113.6±1.7 4.1±0.03	0 0	568.4±0.4 12.3±0.9	0 0
50x ACL	3800.7±17,9	266.2±8.6 7.8±0.01**	228.0±1.8 6.5±0.2	570.0±5.2 15.3±0.3	2356.4±12.5 62.6±2.8	38.3±6.7 1.1±0.03*	0 0	342.2±2.1 9.0±0.2	0 0
75x ACL	1130.8±26,9	67.8±5.6 6.3±0.4	67.8±5.1 6.9±0.1	169.5±6.2 15.0±1.2	723.2±5.6 64.0±2.9	22.6±0.7 2.0±0.01**	0 0	79.1±2.7 7.1±0.8	0 0
100x ACL	0	0	0	0	0	0	0	0	0

Note – part–total number of cells in1μl of blood; the content of the cells is presented in %, the statistical reliability of results in comparison with control * p <0.05, ** p <0.01.

Leukograms of rats before and after poisoning with zinc, lead, cadmium salts were compared with each other, where at the beginning number medullocells was 0.5% but after it is increased up to 15% ($p < 0.001$) (Fig. 1, 2), the same with core segment neutrophils, which before poisoning were 23.5% and after increased up to 30% ($p < 0.01$), no immature neutrophils in peripheral blood were observed (Fig. 2).

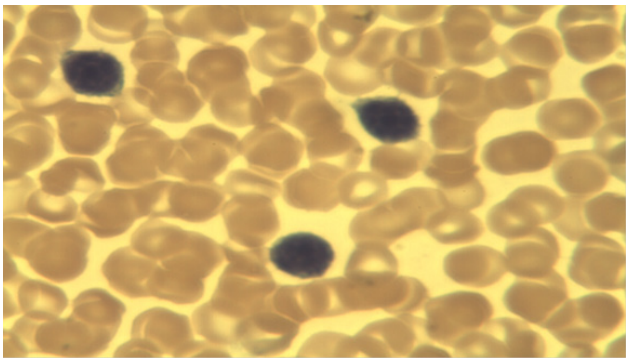

Figure 1 – Lymphocytes before poisoning

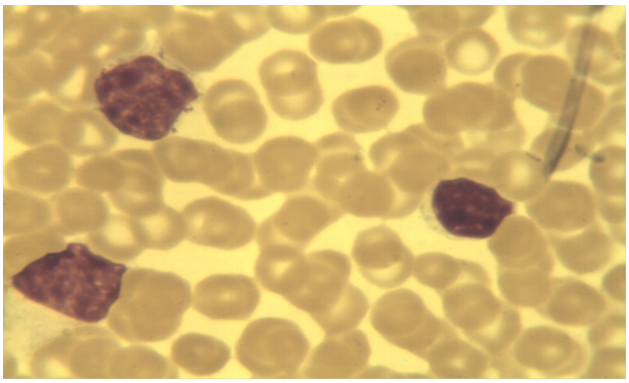

Figure 2 – Medullocells after poisoning with zinc, lead, and cadmium salts

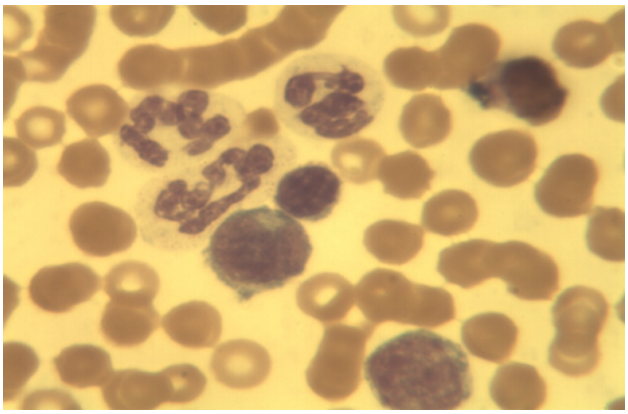

Figure 3 – Core segment neutrophils and monocytes after poisoning with zinc, lead, and cadmium salts

Therefore, cadmium salt decreases the number of lymphocytes, increases the number of immature lymphocytes and reduces activity of the immune system.

To conclude, zinc, lead and cadmium ions concentrations of 25, 50, 75, 100 times ACL hugely impact on immune system of rats which have an immunosuppressive effect on animals.

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Protective and damaging role of electromagnetic influences on biomembranes

Abstract. The paper presents the results of studies of the effect of an alternating low-power electromagnetic field and light irradiation with monochromatic light from a laser source on the state of biological membranes. It was shown that the effect of these factors on the erythrocyte membrane causes a different effect and affects its resistance differently, which is reflected in the change in the erythrogram of acid hemolysis in the experiment. It has been established that the action of an ultrahigh-frequency electromagnetic field leads to a decrease in the resistance of erythrocytes and an increase in the rate of lysis of red cells. Laser irradiation with a low-power helium-neon laser at low exposure increases the resistance of red blood cells to acid hemolysis. Increasing exposure reduces the effect noted. With simultaneous processing of blood samples by UHF (ultra high frequency electromagnetic fields) source and laser light, a significant decrease in the negative effect of the UHF field on the resistance of biomembranes is noted. Possible mechanisms of marked biological effects are discussed.
Key words: laser, electromagnetic fields, blood, erytrocytes, biomembrane.

The functional state of a living cell is mainly determined by the structure and associated work of cell membranes. This dependence becomes clear when one considers that the structure of a cell is a set of membranes that form all intracellular structures of the organism interacting with each other in the volume of the cytosol.

Factors of the external environment affecting the body change functional characteristics of the function of various systems and organs, which ensures the adaptation of the organism to these factors. At the heart of all the adaptive, physiological and pathological reactions of the body is always the changes occurring at the cellular subcellular level. Therefore, it is the change in the characteristics of cell membranes that is one of the defining moments in the development of physiological and biochemical abnormalities in the body.

Among the factors influencing the organism, the study of the action of alternating electromagnetic fields has recently taken on great importance. This is due to the fact that in the last century the influence of electromagnetic fields of artificial origin on the nature of our planet has increased manyfold [1-3].

Electromagnetic fields are created by numerous electric motors, transformers, computer systems, and communication devices, electrical devices. All that makes modern life comfortable and without which we do not represent our normal existence [1; 2].

The study of the effect of electromagnetic fields on biological objects began almost simultaneously with the introduction of electricity in technology and life.

For a long time it was believed that electromagnetic fields do not have any significant effect on living structures. However, a deeper understanding of the structure of the tissues, the development of biophysical studies and the facts noted by specialists from various clinical disciplines made it necessary to look at the problem differently [5-7].

It turned out that the electromagnetic fields have a pronounced effect on virtually all living organisms. However, the effect value depends on many chartes characterizing this factor. These characteristics include wavelength, radiation intensity, frequency response, radiation mode (intermittent, impulsive), area of irradiated surface and exposure. Specific and individual differences influe on observed effects.

The reason for the influence of variable electromagnetic fields is that biological fluids that fill the intracellular space have the properties of electrolytes. Under the action of a periodically changing field voltage, the charged particles advance and the polarization characteristic of the subcellular structures changes [3; 4].

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The reason for the effect of variable electromagnetic fields is that biological fluids that fill the intracellular space have the properties of electrolytes. Under the effect of a changing field, the polarization characteristic of the subcellular structures changes [1; 3; 4].

Depending on the parameters of the current field and the characteristics of the irradiated biological object, both the stimulation of the systems and organs of the animal organism and the suppression of their functional characteristics can occur. It turned out that at the cellular and tissue levels, a variety of and sometimes an opposite reaction is possible.

Under certain irradiation regimes, the regeneration process can increase, while changing the parameters of the effect can lead to changes in the genetic apparatus of the nucleus and change the nature of the cellular metabolism, leading to cell death and the development of degenerative changes at the tissue level.

So we have sad before, the intensity of the impact on the human body of various variable electromagnetic fields with a varied power and frequency response has increased tens of times [1; 4].

Actually, the influence of variable fields on living organisms is a natural component of our natural environment. Living beings for a million years have adapted to the effects of electromagnetic fields, as a natural component of the environment.

However, the anthropogenic electromagnetic pollution of the environment occurring in recent decades is one of the factors whose consequences can not currently be predicted.

The most widespread and least studied is the effect of low-power high-frequency electromagnetic fields.

This problem on the one hand attracts great attention from both scientists and the public. This is due to the widespread use of cellular telephony. At present it is difficult to meet a person who does not use this system of communication.

However, as it is not paradoxical, there is still no consensus on the nature of the effect of such irradiation on biological structures [7].

We decided to analyze the effect of UV radiation on the structure of biomembranes. As a test system, erythrocyte membranes were used, and as an analysis method, an analysis of the kinetics of acid hemolysis was used.

This choice is due to the fact that the structure of the erythrocyte membrane is largely identical to the structure of other cell membranes of the body and for this reason is used as an integral indicator of the influence of various factors on biomembranes.

Irradiation was carried out by a source of electromagnetic oscillations with a frequency of 47 MHz for 15 seconds and 60 seconds for distances of 5 cm.

Studies were performed on blood samples taken from nonlinear rats in the morning before feeding from the tip of the tail.

20 μl of blood was mixed with 20 ml of saline, thus obtaining a suspension of erythrocytes in a dilution of 1:1000.

Sampling and work with them was carried out at room temperature.

The measurement of the optical density of the test sample of the erythrocyte suspension was carried out on CKK-2 at a wavelength of 670 nm.

To determine the rate of acid hemolysis, 2 ml of erythrocyte sediment were placed in a cuvette of working width of 10 mm and 2 ml of a solution of HCl in a concentration of 0.004 N.

After placing the cuvette in the cuvette holder, measurements were taken every 30 seconds.

The change in extinction caused by a decrease in the number of blood elements due to developing hemolysis was recorded as a difference between the initial (E_0) and the next result. Measurements were carried out until two identical values were obtained, i.e. until the end of hemolysis (E_n).

The resulting series of decreasing extinctions characterizes the degree of hemolysis at the time of measurement. The difference $E_0 - E_n$ was taken as 100%.

On the basis of the data obtained, erythrograms of acid hemolysis and graphs of kinetics of erythrocyte destruction have been compiled.

To construct the erythrogram, the percentage of erythrocytes subjected to hemolysis was calculated by the formula

$$E = (D_0\text{-}D_n) / \Delta Di * 100\%,$$

where:

E – the number of erythrocytes subjected to hemolysis, %

ΔDi – difference of the two following values of optical density

ΔDi = Di – Di + 1, where:

D_0 is the initial value of the optical density,

D_n is the final value of the optical density

The results of the experiments are shown on Figure 1.

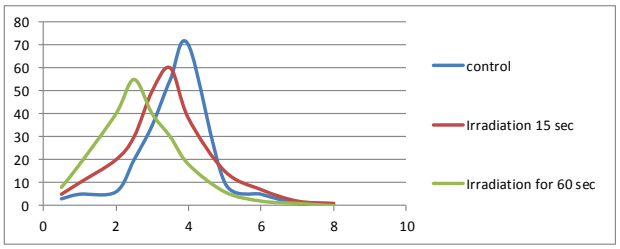

Figure 1 – Acid erythrograms upon irradiation of the same duration

As can be seen from the presented graphs, blood irradiation with the selected field parameters lead to a shift of the erythrogram to the left. This shift is not very pronounced with short-term irradiation (within 15 seconds) and is much more pronounced when irradiated within the first minute. The changes noted indicate a decrease in the resistance of the cell membrane under the influence of the UHF field.

Another widespread factor of influence on the body is the coherent monochromatic measurement of laser sources. Lasers are widely used in industry, household appliances, and their distribution in medicine has turned them into a widely used medical technology.

Despite their large amount of research in this field, there is no single concept of the mechanism of the effect of laser measurement on biological objects. It is known that the biological effect depends on the intensity of radiation, exposure, the wavelength of the light flux, power density, energy density [8.9].

The result of the action of irradiation with the use of low-power sources can be manifested in the form of stimulation of regeneration processes, an increase in the immune status, and in suppression of immune reactions, suppression of the inflammatory process. Earlier in our works, the effect of helium-neon laser irradiation on the processes of carbohydrate metabolism, cellular respiration, and immune status was shown.

Taking into account the earlier expressed thesis that any changes at the organ or tissue level have a cellular and subcellular basis, including changes in light biomembranes, it was of some interest to analyze the effect of laser radiation on the course of acid hemolysis.

The results of studies are shown on Figure 2.

As can be seen from the presented graph, the intensity of hemolysis after irradiation with a helium-neon laser with a radiation wavelength of 752 nm and a power of 20 mW decreases.

In this case, short-term irradiation promotes a marked increase in the resistance of the cell membrane, while a longer exposure gives a much less pronounced effect.

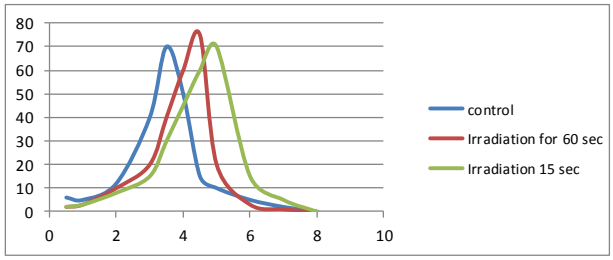

Figure 2 – Acid erythrogram at irradiation with helium-neon laser with various exposure

Taking into account the obtained results, it was of interest to analyze the simultaneous effect on the biomembranes of UHF fields and laser irradiation.

For the experts of this series, the same sources of radiation were used, as before, the duration of processing by the UHF field emitter was 60 seconds, and the helium-neon laser with a laser was 15 seconds.

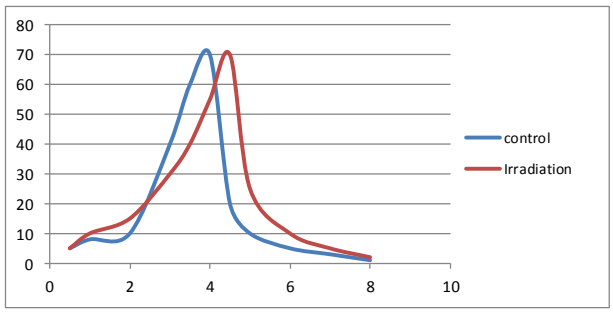

Figure 3 – Acid erythrograms in the complex effect of laser and UHF radiation

From this graph it can be seen that with the combined action of UHF radiation and laser irradiation, the hemolysis course slows down somewhat compared to the action of one UHF radiation. Moreover, the erythrogram graph is shifted to the right even in comparison with the control curve.

The reasons for the observed experimental results are related to the participation of two mechanisms of the action of the electromagnetic field. First, with the transition of electromagnetic energy into thermal energy. Such a transition is associated with the excitation of the molecule's rotations due to the absorption of quantum energies. The energy of quanta is much lower than the kinetic energy of the molecules, and therefore such a result of this absorption is funda-

mental. Secondly, the electromagnetic field can cause a reorientation of molecules, which is possible only in the constant, or its power should be sufficiently [10-12].

The manifestation of the thermal effect is related to the output power of the source and the flux density (the intensity of the electromagnetic field). The degree of heating is related to the frequency of the field. This to some extent explains the observed effect of reducing the resistance of membranes shown in the experiment. This decrease may be due to an increase in the activity of oxidizing processes and the movement of the concentration of peroxides.

At the same time, to explain protective laser irradiation, we need to recall the features of the organization of biomembranes. The liquid crystal structure of biomembranes is formed by separate clusters (membrane "rafts").

It should be emphasized that water, which is the main component of intracellular content and forms extracellular space, also has a cluster organizing structure [8].

Among the hypotheses for explaining the biological effect of low-intensity laser radiation, two are distinguished. One of them is the stimulation of biological processes through a photoregulatory system, that is, a quantum-mechanical approach [9-12].

Another suggests that laser radiation realizes the process of biostimulation by absorbing the energy of laser radiation (Inyushin V.M. – energy-information approach) [12; 13].

In our opinion, an increase in the resistance of biomembranes when irradiated with laser light is associated with the ordering of the cluster structure of biomembranes involving water molecules when light-quantum energy is absorbed.

This conclusion is supported by the results of experiments with irradiation of water which introduced a suspension of erythrocytes. In this case irradiation of the aqueous medium, rather than cells, increased the resistance of erythrocytes in hypotonic hemolysis [14-16].

The role of the water component in maintaining the stability of membranes was also confirmed in experiments with the determination of the optical characteristics of biomembranes in connection with the change in their functional characteristics [17; 18].

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Study of the functional state of cardiorespiratory system of students of the al-Farabi Kazakh National University

Abstract. Integration of the national higher education system of Kazakhstan into the international education puts forward increased requirements for the training of specialists whose quality is determined by the level of physical health of students. In the conditions of modernization, today's student's educational activity characterized by saturation, intensity, high level of psychoemotional tension and complexity which is carried out against a background of decreased motor activity and physical health [1, 2, 3, 4]. Therefore, today in the system of higher education of our Republic there is a contradiction between the need to improve the quality of education and the lack of physical health of future specialists. The universities face a problem when a future specialist with insufficient physical health is not quite ready for the tough demands of the modern labor market. The preparation of highly qualified and competitive new generation specialists who is capable of adapting to rapidly changing social and economic conditions is impossible without good health. There we find out that the one of the most important conditions for the disclosure of student labor potential is the strengthening and preservation of their health [5]. One of the main factors determining the level of physical health of student is the functional state of a vital cardiorespiratory system that combines external breath, heart and hemodynamics.

This article presents the results of a study of the functional readiness of the cardiovascular and respiratory systems of students of the KazNU named al-Farabi, who studies in the academic lessons of physical education in the main educational department.

Key words: functional state, cardiorespiratory system.

Introduction

The physical health of a person is determined by a complex of interrelated factors, where the functional state of external respiration and the cardiovascular system plays an important role. They are the main systems of the oxygen transportation system, which provide the body with oxygen [6].

The cardiovascular system is a system of organs that together provide circulation of blood in the human body and provide its homeostasis [7; 8].

The functions of the cardiovascular system are very global and are important for the human body as a whole: it is fully responsible for the process of blood circulation in the body and for the enrichment of all cells of the body with the necessary minerals, vitamins, and oxygen (O_2). Reserve of CO_2 (carbon dioxide), organic and inorganic substances worked out by the body, is also carried out with the help of the cardiovascular system.

The lymphatic system, being an auxiliary mechanism of the cardiovascular system, supplements the movement of fluid (lymph) in the human body.

The human respiratory system is a set of organs that provide the human body with oxygen (an important chemical element involved in oxidation-reduction processes) [9; 10].

Normal and full-fledged functioning of all organs and systems of the human body can be achieved only if the energy balance is quickly and timely restored. The organism is supplied with energy due to oxidation of the basic organic substrates – carbohydrates, fats, proteins.

Thus, it can be stated that the activity of all body structures, beginning with the cell and ending with the system of organs, is coordinated and subordinated to a single whole. Each structural unit contributes to the functioning of the body.

The functional state of the cardiorespiratory system is one of the main factors that have the greatest impact on a person's physical health.

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At the same time, the functional readiness of the studying system of human, reflecting the level of functioning of the cardiovascular and respiratory system comes as a result of physical training, which is carried out in the process of exercising.

Materials and methods

To study the function of external respiration of students (21 men and 27 girls) of Al-Farabi KazNU, we used the method of *spirography* using the spirograph "BTL-08 SpiroPro" which measures and records on paper the following basic parameters of respiration [11-13]:

- ERV and IRV (ml);
- VC (ml);
- LI (life index);
- FVC (ml);
- FEV_1 (ml);
- FEV_1 / FVC (%);
- PEF (l/s);
- FEF_{25}, FEF_{50}, $FEFC_{75}$ (l/s);
- MVV (l/min).

Complex evaluation of the cardiovascular system of the surveyed contingent was carried out according to indicators:

- heart rate (HR), systolic (SBP) and diastolic blood pressure (DBP) in a state of relative dormancy with the calculation of Kvass indices, cost-effectiveness factors and self-regulation of blood circulation [14; 15];
- reactions of cardiovascular system for standard physical activity – a functional test of Martine-Kushelovsky [16; 17].

To prove objectivity and reliability, the results were processed in the computer program "Excel" with the calculation of the following statistical characteristics: arithmetic mean, average squared difference and coefficient of variation [18; 19].

Results and discussion

Assessment of the external respiration state of the surveyed contingent was carried out using the methodology of RF. Clement [20], based on age and height. The average age-growth indicators of our surveyed students are: 18.5 years and 172.1 cm in boys; 17.5 years and 161.7 cm in girls.

The results of the study of the state of external respiration of students (boys and girls) are shown in tables 1, 2.

Table 1 – Results of the study of the state of external respiration of young men of the basic educational department of KazNU named al-Farabi

Indicators	Actual average ($\bar{x} \pm \sigma$)	Factor of variation of actual indicators (V, %)	Due average (by Clement) ($\bar{x} \pm \sigma$)	Deviation of actual averages from due average in %
IRV (ml)	2320 ± 350	10.5	2634.5± 350	- 11.9
ERV (ml)	1560 ± 120	10.8	1676.0± 120	- 6.9
VC (ml)	4790± 490	10.2	4670 ± 297	+ 2.5
LI	77.2 ± 5.5	7.1	67.5 ± 2.5	+ 14.4
FVC (ml)	4790 ± 518	10.4	4530 ± 297	+ 5.7
FEV_1 (ml)	4470 ± 717	16.0	3960 ± 221	+ 12.9
FEV_1 / FVC (%) Gensler's Index	83.58 ± 7.0	8.4	87.5 ± 2.5	- 4.5
PEF (l/s)	9.66 ± 1.63	17.0	8.46 ± 0.6	+ 14.2
FEF_{25} (l/s)	3.10 ±1.21	39.2	2.67 ± 0.1	+ 16.1
FEF_{50} (l/s)	5.96 ± 2.0	33.7	5.55 ± 0.4	+ 7.4
FEF_{75} (l/s)	8.64 ± 2.08	24.2	8.28 ± 0.8	+ 4.3
MVV (l/min)	107.0 ± 22.2	20.7	106.5± 8.1	+ 0.5

Analyzing the average statistics of external respiration according to Table 1, we can say the following:

– IRV reduced by 11.9%, and ERV by 6.9%; LI and VC exceed the required norms by 14.4% and 2.5%, respectively;

– FVC is higher than the required regulatory requirements by 5.7%;

– FEV_1 is above the standards by 12.9%; the Gensler's index is only slightly reduced by 4.5%;

– PEF is more than normal by 14.2%; FEF_{25}, FEF_{50} and FEF_{75} increased by 16.1, 7.4 and 4.3 percent respectively;

– MVV is slightly higher than due norms (0.5%).

Table 2 – Results of the study of the state of external respiration of girls in the main educational department of KazNU named al-Farabi

Indicators	Actual average ($\bar{x} \pm \sigma$)	Factor of variation of actual indicators (V, %)	Due average (by Clement) ($\bar{x} \pm \sigma$)	Deviation of actual averages from due average in %
IRV (ml)	1760.8 ± 284.6	24.5	1931.3 ± 101.1	- 8.8
ERV (ml)	868.9 ± 257.5	45.3	965.7 ± 50.5	- 10.0
VC (ml)	3836.8 ± 447.2	18.4	3511.5 ± 183.7	+ 8.5
LI	54.5 ± 6.4	14.4	57.5 ± 2.5	- 5,2
FVC (ml)	3717.7 ± 555.6	26.2	3441.2 ± 170.5	+ 8.0
FEV_1 (ml)	3139.4 ± 393.0	20.2	3057.3 ± 129.5	+ 2.7
FEV_1 / FVC (%) Gensler's Index	81.1 ± 19.6	24.1	90.0 ± 5	- 9.8
PEF (l/s)	7.6 ± 0.9	24.1	6.7 ± 0.2	+ 13.4
FEF_{25} (l/s)	6.4± 0.9	25.1	6.1 ± 0.2	+ 4.9
FEF_{50} (l/s)	4.7 ± 1.0	36.4	4.6 ± 0.2	+ 2.2
FEF_{75} (l/s)	2.7 ± 0.8	40.9	2.4 ± 0.1	+ 12.5
MVV (l/min)	59.2 ± 12.0	20.2	82.5 ± 4.0	- 28.2

Analyzing table 2, we can state that the average statistical indicators for girls are as follows:

– IRV is reduced by 8.8%, and ERV by 10.0%; LI is reduced from the due norms by 5.2%, while the VC is higher than the standards by 8.5%;

– FVC is higher than the corresponding regulatory requirements by 8.0%;

– FEV_1 is also above the norm by 2.7%; the Gensler's index is only reduced by 9.8%;

– PEF is higher than the established rate by 13.4%; FEF_{25}, FEF_{50} and FEF_{75} also exceed them by 4.9; 2.2 and 12.5 percent respectively;

– MVV is below the due standards (28.2%).

The parameters of the functional state of the cardiovascular system at rest of the surveyed contingent are presented in Table 3.

Analyzing the functional parameters of the cardiovascular system in the students of the main educational department at rest, it can be noted that:

– HR, SBP, DBP and PP parameters correspond to the due norms of sex and age, both in girls and boys, but the pulse and its pressure are at the upper limit of the norm;

– TSBC equal to 93.6 in girls and 94.7 in boys, is characterized as a cardiovascular type, also within the proper norms;

– CEBC, exceeding the upper limit of the norm, indicates the initial stage of the state of fatigue;

– EF according to the Kvass formula, above the due norms, which indicates the weakening of the cardiovascular system and the reduction of the aerobic mechanism of energy supply of the body.

Table 3 – Average statistical indicators of the functional state of the cardiovascular system at rest in students of the main educational department of KazNU named al-Farabi

Indicators	Girls	Boys	Estimated rate	
	M ± σ	M ± σ	Lower bound	Upper bound
Systolic blood pressure (SBP), mmHg	117.0 ± 9.9	119.1 ± 9.4	110	126
Diastolic blood pressure (DBP), mmHg	76.5 ± 6.8	73.9 ± 6.4	60	80
Pulse pressure (PP), mmHg	40.5 ± 10.1	37.4 ± 12.6	35	40
Heart rate (HR), beats / min	79.8 ± 13.1	78.6 ± 7.8	60	80
Type of self-regulation of blood circulation (TSBC)	93.6 ± 19.1	94.7 ± 13.5	90	110
The coefficient of efficiency of blood circulation (CEBC)	3104.9 ± 1095.2	3390.4 ± 1077.0	2500	3000
Endurance factor (EF)	21.0 ± 6.1	19.6 ± 7.5	16	

Thus, by systematizing the obtained average statistical results of the cardiorespiratory system research in a state of relative rest in the surveyed contingent, it can be concluded that:

• all indicators of young male correspond to the age-sex physiological norms, exception: endurance factor, blood circulation economy index (CEBC), reserve volumes of breath in and out (IRV, ERV), which indicates a weakening of the heart and characterizes unreadiness of their respiratory system for physical exertion;

• almost all major girls indicators of external respiration also within the proper standards, exception inspiratory and expiratory reserve volumes (IRV, ERV), the life index (LI), the index of Gensler and maximum ventilation indicators of lungs (MVV), which doesn't correspond to standards and shows the weakness of their breathing apparatus for physical activity. The research of the cardiovascular system shows that in a state of relative rest the functional state of students fit properly in age-sexual norms, except of profitability ratios circulation (PRC) and endurance factor (EF).

Due to the fact that the above mentioned results indicate the insufficient readiness of the cardiorespiratory system of young people for physical activity, we evaluated its response to the standard physical load in the form of a Martine-Kushelovsky functional test (20 sit-ups in 30 seconds), the results of which are shown in Table 4.

Table 4 – Average rates of cardiovascular system reaction of students of KazNU named after Al-Farabi for the Martine-Kushelovsky trial

At rest			Recovery period					
			1 minute		2 minutes		3 minutes	
	HR	AP	HR	AP	HR	AP	HR	AP
Girls	75	117/77	136	126/77	109	122/75	90	116/72
Boys	72,3	112/74	135	122/63	109	120/64	98	115/64

Based on the analysis of cardiovascular system reaction of the students right after the exercise (the first minute of recovery) in comparison with the baseline, we can say:

– the systolic pressure rises weakly, which is 3-4 times lower than the norm; it reflects the weakening of the systole force – the contraction of the left heart of the heart;

– the diastolic pressure of the girls remains unchanged, indicating a decrease in the tone of the arterioles, which ensures better access of oxygen to the periphery, while in young men, having a tendency to a sharp decrease, contributes significantly higher than the required standards to a rise in pulse pressure;

– a significant increase in heart rate, indicates that the increase in blood circulation, even with a low-intensity load, is achieved by increasing the heart rate, and not increasing the stroke volume of the heart, which is irrational for the heart.

– The AP on the third minute of rest is restored to the initial value, and the period of recovery of heart rate is delayed.

Acknowledgements

Thus, on the basis of a generalization of the results of our study, we can briefly summarize:

– in rest the functional state of the cardiovascular system of practically healthy students corresponds to the proper age and sex standards;

– for standard physical activity, the cardiovascular system in both boys and girls reacts with a significant increase in heart rate, a slight increase in systolic and unchanged diastolic pressure in girls but decrease DP in boys, an increase in the recovery time of heart rate, which can be characterized as unfavorable – asthenic (hypotonic) type of reaction.

All this testifies to the physical detraining of the body of students and justifies the actual need for the development, scientific and methodological substantiation and introduction of the most rational methods and means of physical training that have the greatest health and training effect into the practice of physical education.

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Characteristics of miRNA binding sites in mRNAS of human and mouse titin gene

Abstract: We have studied characteristics of miRNA (microRNAs) binding sites in mRNAs (matrix RNAs) of human, primate and mouse titin gene. miRNAs are small non-coding RNAs with the length about 21-22 nucleotides binding with mRNAs of genes and blocking or disturbing their translation. Titin is the largest protein of heart muscle tissue that is a base of myofibril. Defects of titin synthesis lead to malfunction of muscle tissue, for example, to the heart failure which is one of the widest reasons of the death in the world. We have found differences and similarities of characteristics of miRNA binding sites in human and mouse titin gene mRNAs. The differences are the following: different number of binding sites, different values of binding energy and different nucleotide sequences of orthologous human and mouse miRNAs. The similarities are concluded in that all of these sites are located in protein-coding part of mRNA and they all have particular complementarity. But changing some nucleotides can help to get artificial miRNAs with ideal complementarity and maximal effect on expression. We have noticed that characteristics of miRNA binding sites in mRNAs of titin gene between different species of primates are more similar than between human and mouse. It can be explained by different evolutionary distance between these species. So the model of miRNA regulation of mouse titin synthesis is not completely adequate for human titin gene, but weakness of miRNA interaction with mRNA of mouse titin gene can be compensated by increasing of miRNA concentration in relation to mRNA.

Key words: miRNA, mRNA, binding, sites, titin, gene, human, primates, mouse.

Introduction

Titin is a protein of muscle tissue. It is the largest protein in the nature and plays enormous role in providing elasticity and structural integrity of sarcomers [1]. For example, the longest transcript variant of human titin gene encodes 35991 amino acids and includes all 363 exons of this gene. Disturbance of titin synthesis causes the development of serious cardiovascular diseases, such as dilated cardiomyopathy, heart failure, ischemic heart disease, myocardial infarction and etc. Different titin isoforms are synthesized in various types of muscle tissue (heart, smooth and skeletal striated muscle tissue) and are encoded by different combinations of exons [2].

Recently miRNA binding with mRNAs of different genes, participating in the development of cardiovascular diseases, was studied [3-4]. For example, it was proved, that high level of miR-208b expression leads to cardiac hypertrophy in titin-based dilated cardiomyopathy [5]. But characteristics of miRNA interactions with mRNAs of titin gene were not studied. So it was important to establish what kind of miRNAs bind with mRNAs of titin gene? Since miRNA binding sites in mRNAs of orthologous genes can differ it is necessary to study characteristics of miRNA binding with mRNAs of orthologous genes, especially with mRNAs of human and mouse titin gene because mouse is used in experimental research. So it was important to compare characteristics of miRNA binding sites in mRNAs of titin gene. It is possible that differences can be observed in miRNA binding with mRNAs of different titin isoforms.

Materials and methods

Materials of research are titin mRNA nucleotide sequences of *Homo sapiens, Pan troglodytes, Pongo abelii, Macaca fascicularis, Papio anubis, Pan paniscus, Colobus angolensis, Chlorocebus sabaeus, Rhinopithecus roxellana, Callithrix jacchus, Aotus nancymaae*, *Saimiri boliviensis*, *Gorilla gorilla, Nomascus leucogenys* and *Mus musculus*. These sequences were taken from the Genbank (*https://www.*

ncbi.nlm.nih.gov/genbank/). Nucleotide sequences of 2568 miRNAs were taken from the miRBase (*www.mirbase.org/*) and 3707 other miRNAs were discovered in 2015 by Eric Londina and other scientists [6]. Human miRNAs have abbreviation "hsa" (*Homo sapiens*) in the beginning of their names. Mouse miRNAs have abbreviation "mmu" (*Mus musculus*). Free energy of miRNA-mRNA binding (ΔG), ΔG/ΔG_m ratio (%), positions and schemes of potential miRNA binding sites were calculated by the program MirTarget [7-8]. ΔG_m (maximal ΔG) is free energy of miRNA binding with nucleotide sequence that is absolute complementary to this miRNA. ΔG/ΔG_m ratio was used as comparative criterion of miRNA-mRNA interaction. ΔG/ΔG_m ratio should be equal or more than 85% because 15% of ΔG_m correspond to three nucleotides of miRNA sequence which can encode one amino acid. Binding site with value of ΔG/ΔG_m ratio, that is less than 85%, is counted as unreliable because it has low specifity and can bind different miRNAs. Diagrams of evolutionary conservation of olygopeptides encoding by miRNA binding sites were created by the program WebLogo (https://www.weblogo.berkeley.edu/). The information about microRNA expression was taken from Human miRNA tissue atlas (*https://ccb-web.cs.uni-saarland.de/tissueatlas*) and TiGER: Tissue-specific Gene Expression and Regulation (bioinfo.wilmer.jhu.edu/tiger/). Positions of miRNA binding sites were compared with single nucleotide polymorphisms (SNPs) of human titin gene (https://www.ncbi.nlm.nih.gov/snp/?term=TTN*)*.

Results and their discussion

We have studied binding of 6271 human miRNAs with mRNA of human titin gene. There were found 23 binding sites of 18 miRNAs with value of ΔG/ΔG_m that is more than 85% (Table «a»). These sites have "bubble" in the structure of miRNA-mRNA duplex with the exception of hsa-miR-11-28905-3p and hsa-miR-14-24215-3p. The density of miRNA binding sites in mRNA sequence of human titin is approximately equal to one site for five thousands nucleotides. hsa-miR-6861-5p, hsa-miR-494-5p, hsa-miR-374b-3p, hsa-miR-374c-3p, hsa-miR-34a-3p and hsa-miR-4495 are synthesized by intergenic regions. hsa-miR-578, hsa-miR-3714, hsa-miR-1278, hsa-miR-544b, hsa-miR-4738-3p, hsa-miR-136-3p and hsa-miR-4693-5p are synthesized by host genes (*CPE, PLCL2, CDKN1A, CDK4, CDK6, CDC73, UMPS, UNK, RTL1* and *RP11,* respectively) [9-11]. hsa-miR-19-36945-3p, hsa-miR-1-1585-3p, hsa-miR-11-28905-3p, hsa-miR-14-24215-3p and hsa-miR-12-32366-3p are novel human miRNAs and absent in the miRBase [12]. hsa-miR-6861-5p and hsa-miR-14-24215-3p have three binding sites each. These sites are located from 177th exon to 197th exon inclusively and contain so called PEVK-repeats. The rest miRNAs have only one binding site each. Only hsa-miR-374b-3p, hsa-miR-374c-3p, hsa-miR-3714, hsa-miR-4738-3p, hsa-miR-136-3p and hsa-miR-4495 have relatively high level of expression in heart and muscle tissues in comparison with other tissues [13-16]. In this way, no one binding site of these miRNAs doesn't coincide of known pathological mutations of human titin gene.

Four mouse miRNAs (mmu-miR-34a-3p, mmu-miR-136-3p, mmu-miR-374c-3p and mmu-miR-494-5p) are orthologous to corresponding human miRNAs (Table «b»). Differences of mouse miRNA nucleotide sequences and corresponding human sequences are one-three nucleotides. For example, the sequence of hsa-miR-34a-3p has a cytosine (C) in the beginning that is absent in the sequence of mmu-miR-34a-3p. So the length of hsa-miR-34a-3p is 22 nucleotides while the length of mmu-miR-34a-3p is 21 nucleotides. The sequence of hsa-miR-136-3p has nucleotide C in the beginning that is absent in the sequence of mmu-miR-136-3p. But it hasn't an uracil (U) that is present in the sequence of mmu-miR-136-3p. So the length of these miRNAs is the same. hsa-miR-374c-3p is three nucleotides longer (22 nucleotides) than mmu-miR-374c-3p (19 nucleotides) because hsa-miR-374c-3p has nucleotide C in the beginning and nucleotides A (adenine) and U at the end that are absent in the sequence of mmu-miR-374c-3p. hsa-miR-494-5p has nucleotide U at the end of sequence that makes it longer than mmu-miR-494-5p. The length of hsa-miR-494-5p is 23 nucleotides and the length of mmu-miR-494-5p is 22 nucleotides.

We have calculated interaction between mmu-miR-34a-3p, mmu-miR-136-3p, mmu-miR-374c-3p, mmu-miR-494-5p and mRNA of human titin gene. Only mmu-miR-494-5p bound with mRNA of this gene with value of ΔG/ΔG_m that is not lower than 90 %. It has ΔG/ΔG_m that is equal 93 % (Table «a»).

Than we have calculated binding of 18 human miRNAs and mmu-miR-34a-3p, mmu-miR-136-3p, mmu-miR-374c-3p, mmu-miR-494-5p with mRNA of mouse titin gene. Only eight miRNAs bind with mRNA of mouse titin gene with values of ΔG/ΔG_m equal 85 % and more (hsa-miR-19-36945-3p, hsa-miR-11-28905-3p, mmu-miR-34a-3p, hsa-miR-1278, hsa-miR-544b, hsa-miR-34a-3p, hsa-miR-4693-5p and hsa-miR-4495) (Table «c»).

Table «a» – The characteristics of human and mouse miRNA binding sites in the mRNA of human titin gene, *$\Delta G/\Delta G_m$ ratio is more than 85 %*

miRNA	Position in the mRNA	Position in exon[1]	Energy[2] (kJ/mole)	$\Delta G/\Delta G_m$ (%)	Length of miRNA, nt	Exon	Schemes of miRNA-mRNA interaction[3]
hsa-miR-494-5p	1301	161	-108	90	23	7	5′ – UGAGAGAGACAACGCUGACAACCU – 3′ |||| |||||||| |||||||| 3′ – UCUCU-UCUGUUGUGCCUGUUGGA – 5′
mmu-miR-494-5p	1302	162	-108	93	22	7	5′ – GAGAGAGACAACGCUGACAACCU – 3′ |||| |||||||| |||||||| 3′ – CUCU-UCUGUUGUGCCUGUUGGA – 5′
hsa-miR-578	1960	72	-98	90	21	11	5′ – ACAGUCCCGGGAGCUCAAGAAG – 3′ |||||||||| ||| ||||||| 3′ – UGUUAGGAUC-UCGUGUUCUUC – 5′
hsa-miR-19-36945-3p	3271	204	-102	92	20	18	5′ – AGUGCUGUAAAUGAGGCUGGA – 3′ ||||||| |||||||||||| 3′ – UCACGAC-UCUACUCCGACCC – 5′
hsa-miR-1-1585-3p	8609	3	-96	92	21	36	5′ – UCAAGAUCAUUAAAAAGCCAAA – 3′ |||||| ||| |||||||||| 3′ – AGUUCUUGUA-UUUUCCGGUUU – 5′
hsa-miR-374b-3p	17239	110	-98	90	22	58	5′ – AAAGAUAACACAAUCCUGCGAAG – 3′ || ||||||||| ||||| ||| 3′ – UUACUAUUAUGUU-GGACGAUUC – 5′
hsa-miR-374c-3p	17241	112	-98	90	22	58	5′ – AGAUAACACAAUCCUGCGAAGUG – 3′ | |||||||| ||||| ||||| 3′ – UAUAUUAUGUU-GGACGAUUCAC – 5′
hsa-miR-11-28905-3p	17446	38	-117	89	23	59	5′ – UCCCUCCGGGGAGGCACAGCUGC – 3′ ||||||||| |||||||||||| 3′ – AAGGAGGCCGCCCUGUGUCGACG – 5′
hsa-miR-3714	17450	42	-110	90	22		5′ – UCCGGGGAGGCACAGCUGCCUUC – 3′ | |||||| ||| |||||||| 3′ – UGUCCCCUC-GUGACGACGGAAG – 5′
hsa-miR-34a-3p	22116	208	-104	91	22	75	5′ – AGGGCAGUAUUCCUGCGAGAUUG – 3′ ||||||||| ||||| ||||| 3′ – UCCCGUCAUAUGAACGA-CUAAC – 5′
hsa-miR-1278	24928	197	-98	90	22	85	5′ – AUAGAGGAUUAUGCACAGUACAG – 3′ ||||| ||| |||||||||| | 3′ – UAUCUACUA-UACGUGUCAUGAU – 5′
hsa-miR-544b	26044	179	-104	93	22	89	5′ – CUGGAAAUGCACAAUCUCAGUGU – 3′ ||||||||||||||||||| || 3′ – AAUCUUUACGUGUUGGAGUC-CA – 5′

Continuation of table «a»

hsa-miR-14-24215-3p	37245	61	-104	93	22	177-178*	5′ – AGAAGCUCCAAUUGUCCCAGUG – 3′ 3′ – UCUUCGAGGUUUAUAGGGUCAU – 5′
hsa-miR-6861-5p	37324	59	-115	92	22	178-179*	5′ – CCAGAAGCCCCACCUGCCACAGU – 3′ 3′ – GGACCUCGGGGUGGAUGG-GUCA – 5′
hsa-miR-14-24215-3p	37998	61	-102	91	22	186-187*	5′ – AGAAGCUCCGAUUGUCCCAGUG – 3′ 3′ – UCUUCGAGGUUUAUAGGGUCAU – 5′
hsa-miR-6861-5p	38077	59	-115	92	22	187-188*	5′ – CCAGAAGCCCCACCUGCCACAGU – 3′ 3′ – GGACCUCGGGGUGGAUGG-GUCA – 5′
hsa-miR-14-24215-3p	38751	61	-104	93	22	195-196*	5′ – AGAAGCUCCAAUUGUCCCAGUG – 3′ 3′ – UCUUCGAGGUUUAUAGGGUCAU – 5′
hsa-miR-6861-5p	38830	59	-115	92	22	196-197*	5′ – CCAGAAGCCCCACCUGCCACAGU – 3′ 3′ – GGACCUCGGGGUGGAUGG-GUCA – 5′
hsa-miR-136-3p	71469	1528	-102	91	22	326	5′ – GGACCCACCUGAGAACGAUGGUG – 3′ 3′ – UCUGAGUAAACUCU-GCUACUAC – 5′
hsa-miR-12-32366-3p	71984	2043	-108	90	22		5′ – UGGCUCUGGAUCCCAUUGACCCA – 3′ 3′ – ACCGAGAC-UGGGGUCACUGGAU – 5′
hsa-miR-4738-3p	74955	5014	-113	93	22		5′ – UCCUCCUGGCACUCCAGUUGUCA – 3′ 3′ – AGGAGGUCCGCGAGGUCAA-AGU – 5′
hsa-miR-4693-5p	92464	86	-108	94	23	339	5′ – GGUGGCAGUGAAAUUCAACAGUAU – 3′ 3′ – ACACUGUCACUUUAAGU-GUCAUA – 5′
hsa-miR-4495	93909	1531	-93	90	21		5′ – AGCAGGAAGCCCAUUUACCAUU – 3′ 3′ – UCGUUUUUCGGACAAAUG-UAA – 5′

Notes:
1. Distance from exon beginning (from the first exon beginning if miRNA binding site is located on the border of two exons).
2. Energy of miRNA-mRNA interaction.
3. In each scheme upper sequence is the sequence of mRNA site, lower sequence is miRNA strand.
* – miRNA binding site is located on the border of two exons.

Table «b» – Differences between orthological human and mouse miRNAs binding with mRNAs of human and mouse titin genes

MiRNAs of human	MiRNAs of mouse
hsa-miR-34a-3p 22 nt. **C**AAUCAGCAAGUAUACUGCCCU	mmu-miR-34a-3p 21 nt. AAUCAGCAAGUAUACUGCCCU
hsa-miR-136-3p 22 нт. **C**AUCAUCGUCUCAAAUGAGUCU	mmu-miR-136-3p 22 nt. AUCAUCGUCUCAAAUGAGUCU**U**
hsa-miR-374c-3p 22 нт. **C**ACUUAGCAGGUUGUAUUAU**AU**	mmu-miR-374c-3p 19 nt. ACUUAGCAGGUUGUAUUAU
hsa-miR-494-5p 23 нт. AGGUUGUCCGUGUUGUCUUCUC**U**	mmu-miR-494-5p 22 nt. AGGUUGUCCGUGUUGUCUUCUC
Note: different nucleotides of corresponding human and mouse miRNA sequences are signed by bold letters.	

Table «c» – The characteristics of human and mouse miRNA binding sites in the mRNA of mouse titin gene, *$\Delta G/\Delta G_m$ is equal or more than 85 %*

miRNA	Position in the mRNA, nt.	Energy[1] (kJ/mole)	$\Delta G/\Delta G_m$ (%)	Length of miRNA, nt	Exon	Position in exon[2]	Schemes of miRNA-mRNA interaction[3]
hsa-miR-19-36945-3p	3288	-100	90	20	18	204	5′ – AGCGCCGUGAAUGAGGCCGGG – 3′ ||||||| || ||||||||| 3′ – UCACGACUCU-ACUCCGACCC – 5′
hsa-miR-11-28905-3p	16760	-113	86	23	58	37 nt.	5′ – CUCCCUCCGUGGGGGCACAGCCGC – 3′ |||| |||| |||||||||||| 3′ – AAGG-AGGCCGCCCUGUGUCGACG – 5′
	16761					38 nt.	5′ – UCCCUCCGUGGGGGCACAGCCGC – 3′ |||||||| |||||||||||| 3′ – AAGGAGGCCGCCCUGUGUCGACG – 5′
mmu-miR-34a-3p	19463	-93	86	21	66	203 nt.	5′ – UGGACAGUAUACUUGCCAAGUU – 3′ |||||||||||||||||| || 3′ – UCCCGUCAUAUGAACGACU-AA – 5′
hsa-miR-1278	24243	-96	88	22	83	197 nt.	5′ – AUAGAGGAUUAUGCACAGUACGC -3′ ||||| ||| ||||||||||| 3′ – UAUCUACUA-UACGUGUCAUGAU -5′
hsa-miR-544b	25359	-100	89	22	87	179 nt.	5′ – CUGGAGAUGCACAGUCUCAGUGU – 3′ |||||||||||||||||||| || 3′ – AAUCUUUACGUGUUGGAGUC-CA – 5′
hsa-miR-34a-3p	27077	-100	87	22	93	208 nt.	5′ – AGGGCAGUACAACUGCUACAUUG – 3′ ||||||||||| |||||| |||| 3′ – UCCCGUCAUAUGAACGAC-UAAC – 5′
hsa-miR-4693-5p	90243	-98	85	23	282	86 nt.	5′ – GGUGGCAGUGAGAUUCAACACUAC – 3′ |||||||||||||||| || ||| 3′ – ACACUGUCACUUUAAGU-GUCAUA – 5′
hsa-miR-4495	96348	-91	88	21	295	245 nt	5′ – GGCAAGAAGACCGUUUACAUU – 3′ ||||||||| |||||||||| 3′ – UCGUUUUUCGGACAAAUGUAA – 5′

Notes:
1. Energy of miRNA-mRNA interaction; 2. Distance from exon beginning; 3. In each scheme upper sequence is the sequence of mRNA site, lower sequence is miRNA strand.

Table «d» – miRNA binding sites in mRNAs of human and mouse titin gene and their artificial miRNAs, different nucleotides are signed by bold letters

miRNA	Position, nt.	Nucleotide sequence of miRNA binding site	Artificial miRNA
hsa-miR-494-5p	1301	UGAGA**G**AGACAACGC**U**GACAACCU	AGGUUGUCAGCGUUGUCUCUCUCA
mmu-miR-494-5p	1302	GAGA**G**AGACAACGCUGACAACCU	AGGUUGUCAGCGUUGUCUCUCUC
hsa-miR-578	1960	ACAGUCCCGG**G**AGC**U**CAAGAAG	CUUCUUGAGCUCCCGGGACUGU
hsa-miR-19-36945-3p	3271	AGUGCUG**U**AAAUGAGGCUGGA	UCCAGCCUCAUUUACAGCACU
hsa-miR-1-1585-3p	8609	UCAAGAUCA**U**UAAAAAGCCAAA	UUUGGCUUUUUAAUGAUCUUGA
hsa-miR-374b-3p	17239	AA**A**GAUAACACAA**U**CCUGC**G**AAG	CUUCGCAGGAUUGUGUUAUCUUU
hsa-miR-374c-3p	17241	A**G**AUAACACAA**U**CCUGC**G**AAGUG	CACUUCGCAGGAUUGUGUUAUCU
hsa-miR-11-28905-3p	17446	UCCCUCCGG**G**GAGGCACAGCUGC	GCAGCUGUGCCUCCCCGGAGGGA
hsa-miR-3714	17450	**U**C**C**GGGGAG**G**CACAGCUGCCUUC	GAAGGCAGCUGUGCCUCCCCGGA
hsa-miR-34a-3p	22116	AGGGCAGUAU**U**CCUGCG**A**GAUUG	CAAUCUCGCAGGAAUACUGCCCU
hsa-miR-1278	24928	AUAGA**G**GAU**U**AUGCACAGUAC**A**G	CUGUACUGUGCAUAAUCCUCUAU
hsa-miR-544b	26044	CUGGAAAUGCACAAUCUCAG**U**GU	ACACUGAGAUUGUGCAUUUCCAG
hsa-miR-14-24215-3p	37245	AGAAGCUCCAA**U**UGUCCCAGUG	CACUGGGACAAUUGGAGCUUCU
hsa-miR-6861-5p	37324	CC**A**GAAGCCCCACCUGCC**A**CAGU	ACUGUGGCAGGUGGGGCUUCUGG
hsa-miR-14-24215-3p	37998	AGAAGCUCCGA**U**UGUCCCAGUG	CACUGGGACAAUCGGAGCUUCU
hsa-miR-6861-5p	38077	CC**A**GAAGCCCCACCUGCC**A**CAGU	ACUGUGGCAGGUGGGGCUUCUGG
hsa-miR-14-24215-3p	38751	AGAAGCUCCAA**U**UGUCCCAGUG	CACUGGGACAAUUGGAGCUUCU
hsa-miR-6861-5p	38830	CC**A**GAAGCCCCACCUGCC**A**CAGU	ACUGUGGCAGGUGGGGCUUCUGG
hsa-miR-136-3p	71469	GGACCCACCUGAGAA**C**GAUGGUG	CACCAUCGUUCUCAGGUGGGUCC
hsa-miR-12-32366-3p	71984	UGGCUCUG**G**AUCCCA**U**UGACCCA	UGGGUCAAUGGGAUCCAGAGCCA
hsa-miR-4738-3p	74955	UCCUCC**U**GGCACUCCAGUU**G**UCA	UGACAACUGGAGUGCCAGGAGGA
hsa-miR-4693-5p	92464	**G**GUGGCAGUGAAAUUCAA**C**AGUAU	AUACUGUUGAAUUUCACUGCCACC
hsa-miR-4495	93909	AGCAGGAAGCCCAUUUAC**C**AUU	AAUGGUAAAUGGGCUUCCUGCU
hsa-miR-19-36945-3p	30584	AGCGCCG**U**GAA**U**GAGGCCGGG	CCCGGCCUCAUUCACGGCGCU

These miRNAs have nine binding sites. Only hsa-miR-19-36945-3p bind with mRNA of mouse titin gene with value of $\Delta G/\Delta G_m$ equal to 90%. hsa-miR-4693-5p has the lowest value of $\Delta G/\Delta G_m$ ratio that is equal to 85%. It has binding site in the 282nd exon of mouse titin gene. hsa-miR-19-36945-3p, hsa-miR-11-28905-3p, hsa-miR-544b and hsa-miR-34a-3p have values of miRNA-mRNA interaction energy equal to -100 kJ/mole and lower. Other miRNAs have higher values of binding energy than these miRNAs. hsa-miR-11-28905-3p has two binding sites in the 58th exon with the lowest level of interaction energy (-113 kJ/mole) and ΔG/ΔGm ratio that is equal 86%. hsa-miR-4495 has the highest level of miRNA-mRNA interaction energy that is equal -91 kJ/mole. Binding site of this miRNA is located in the 295th exon of mouse titin gene. The single mouse miRNA binding site in mRNA of mouse titin gene is binding site of mmu-miR-34a-3p that is located in the 66th exon. It has value of $\Delta G/\Delta G_m$ that is equal 86 %. Binding site of hsa-miR-34a-3p is located very far from binding site of mmu-miR-34a-3p in the mRNA of mouse titin, in the 93rd exon, and has value of $\Delta G/\Delta G_m$ that is equal 87 %. hsa-miR-1278 has binding site in the 83rd exon of mouse titin gene and value of $\Delta G/\Delta G_m$ that is equal 88 %. hsa-miR-544b has binding site in the 87th exon of mouse titin gene and value of $\Delta G/\Delta G_m$ that is equal 89 %.

One of the evidences of miRNA binding site existence is presence of it in mRNAs of orthologous genes. We have studied binding of 6271 human miRNAs with mRNAs of titin gene of 14 species of primates (*Homo sapiens, Pan troglodytes, Pongo abelii, Macaca fascicularis, Papio anubis, Pan paniscus, Colobus angolensis, Chlorocebus sabaeus, Rhinopithecus roxellana, Callithrix jacchus, Aotus nancymaae*, *Saimiri boliviensis*, *Gorilla gorilla, Nomascus leucogenys*). It was found that only hsa-miR-494-5p and hsa-miR-578 bind with mRNAs of primate titin gene with values of $\Delta G/\Delta G_m$ equal to 90% and more. Although orthologous miRNAs of various primate species are different, primates have conservative genes of miRNA synthesis and conservative miRNA binding sites. For example, Figure1: binding sites of hsa-miR-494-5p in titin mRNAs of primates encode conservative heptapeptide **RETTLTT**, in which the last aminoacid changed (threonin is changed to alanin or serin). Figure2: binding sites of hsa-miR-578 in titin mRNAs of primates encode conservative heptapeptide **TVPGAQE**. As we can see, flanking amino acid sequences are even more conservative than these olygopeptides themselves. Conservation of olygopeptides, encoding by miRNA binding sites, proves conservation of these mRNA regions. Characteristics of miRNA binding sites in mRNAs of primate titin gene are more similar than in mRNAs of human and mouse gene because of different evolutionary distance between these species.

miRNAs can't effectively block translation of titin because they are not absolutely complementary to their sites. But artificial miRNAs, that would be absolutely complementary to these sites and have $\Delta G/\Delta G_m$ ratio equal 100 %, could effectively bind with mRNAs of titin genes and block their translation (Table «d»). There are 25 miRNA binding sites in this table that have ΔG/ΔGm ratio equal 90% and more. Nucleotides of miRNAs, decreasing energy of miRNA-mRNA interaction, are signed by red color. For example, we need to change three nucleotides in the sequence of hsa-miR-374b-3p binding site that is located in the position 17241 of human titin gene to synthesize absolutely complementary artificial miRNA to this site. In other case, we need to change only one nucleotide in the sequence of mmu-miR-494-5p binding site located in position 1302 to get ideal artificial miRNA. In the case of hsa-miR-494-5p binding site, located in position 1301 of human titin gene, two nucleotides are needed for synthesis of complementary sequence for this site. In relation to mouse titin gene mRNA, it is needed to change two nucleotides in the sequence of hsa-miR-19-36945-3p located in position 30584 to get artificial miRNA.

Conclusions

We have found differences and similarities of characteristics of miRNA binding sites in mRNAs of human and mouse titin gene. Differences are the following:

1. Human titin mRNA has 23 miRNA binding sites whereas mouse titin mRNA has only nine sites.
2. 19 miRNAs (18 human miRNAs and one mouse miRNA) interact with mRNA of human titin but only eight miRNAs from this number bind with mRNA of mouse titin gene.
3. $\Delta G/\Delta G_m$ ratio of miRNA binding sites in the mRNA of human titin gene varies from 89% to 94% but in the mRNA of mouse titin gene it varies from 85% to 90%.
4. Orthologous human and mouse miRNAs have different nucleotides in their sequences.

Similarities of characteristics of miRNA binding sites in human and mouse titin mRNA are the following:

1. Positions of miRNA binding sites in the mRNA of mouse titin gene are very close to such positions in the mRNA of human titin.

Figure 1. Pinsky I., Labeit S., Labeit D., Ivashchenko A.

Figure 1 – Diagram of evolutional conservation of olygopeptides encoding by binding sites of hsa-miR-494-5p in mRNAs of primate titin genes. Horizontal axis is positions of amino acids in the sequence of this olygopeptide, vertical axis is a frequency of their appearing in this sequence

Figure 2. Pinsky I., Labeit S., Labeit D., Ivashchenko A.

Figure 2 – Diagram of evolutional conservation of olygopeptides encoding by binding sites of hsa-miR-578 in mRNAs of primate titin genes. Horizontal axis is positions of amino acids in the sequence of this olygopeptide, vertical axis is a frequency of their appearing in this sequence

2. All miRNA binding sites are located in protein-coding part of these mRNAs.

Thus characteristics of miRNA binding sites in mRNAs of human and mouse titin gene are different and it should be taken into account. So the model of mouse titin synthesis regulation by miRNAs is not completely adequate for human titin gene. Nevertheless, increasing of miRNA concentrations in relation to mRNA of mouse titin gene can cause the same effect on expression of this gene as in human. In something's totality, obtained results permit to suppose that expression of titin gene is weekly regulated by miRNAs in human and mouse organisms.

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Ecological assessment of the territory of the projected State Nature Reserve 'Bokeyorda' in West Kazakhstan region

Abstract. Presented paper deals with the environmental protection problems on the example of the project of the State Natural Reserve 'Bokeyorda' in West Kazakhstan region. During the development and implementation of the project the main goal was to assess the impact of natural and anthropogenic factors on the unique natural ecosystems and their biological diversity. The natural conditions were investigated and the key plant and animal species were identified, their current status and the human impact were evaluated as well as recommendations for the conservation of species and their habitats were made. Based on the ecosystem analysis and GIS technology the most important areas for biodiversity conservation were revealed, the Reserve boundaries were defined, zoning of functional areas were performed and maps were prepared.

Key words: ecological assessment, ecosystem, protected areas, state natural reserve, steppe, flora and fauna.

Introduction

The territory of Kazakhstan has a unique variety of landscapes – from deserts to high mountains and marine ecosystems. As tempo of the natural resources use is rapidly increasing in such economically developing country as Kazakhstan, the more urgent is the question of further improving of the system of the Nature protection areas, effective for biodiversity conservation.

A large part of the world natural steppe areas, more than 120 million ha, is located in Kazakhstan, which steppe ecosystems are the habitat of the unique steppe vegetation and the globally endangered species of steppe fauna. Kazakhstan steppe ecosystems are the most significant for the more than 2000 plant species, including about 30 endemics.

Steppes are the least protected ecosystems in Kazakhstan, and highly under-represented in the system of strictly protected areas (SPA). This is due to low volume of humus horizon of soils and scarcity of biological productivity of vegetation.

Currently, the system of SPA in the West Kazakhstan region is represented by three protected areas of national significance and seven protected areas of regional significance. The total area is of 188.7 thousands ha, or 1% of the total area of the region. No protected areas with a strict regime of protection and with the status of a legal entity are present in this region (1-25).

One of the reasons of creation of strictly protected area at West Kazakhstan Region is that it is the habitat of the Ural population of saiga antelope (*Saiga tatarica*). Not so long ago, the mass death of this animal from pasteurellos is has occurred. 12 thousand individuals deceased in May 2010, and more than 400 individuals – in May 2011. As a result, the number of the Ural population of saiga antelope has declined from 39 thousand to 27 thousand individuals (27).

The purpose of this study was to evaluate the ecological state of ecosystems in the area between the rivers Volga and Ural, to provide the scientific justification for the creation of the State Natural reserve 'Bokeyorda' of West Kazakhstan region, that is necessary for the conservation of habitats of rare plant and animal endemic species, including the Ural saiga antelope population *Saiga tatarica*, as well as the conservation of the whole region biodiversity.

Materials and Methods

Geobotanical, soil, floral, faunistic and ecosystem researches in the project area were performed with the use of commonly accepted methods (4, 6, 8, 11, 12, 14, 15, 21, 23, 24, 28-31). The monitoring

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areas were distinguished to fix the data in the system of GPS. We mapped areas of distribution of plant and animal key species and evaluated the impact of negative factors on biological diversity (10).

Distinguishing the valuable sites for SPA, we used the ecosystem approach, based on a complex assessment of the ecological state of the natural components, dominating biogeocoenoses and zoocenoses of steppe environment, using remote sensing data and GIS technology. It will allow to assess the quality potential of biodiversity, based on the existing correlation between the ecotope (type of habitat), the type of vegetation and animal population. Ecological potential of habitats and the potential diversity of biota were determined on the base of gradation of ecosystem components (relief, soil, vegetation and associated animal population) (7).

Results and Discussion

The project area has a complex composition of land users. According to preliminary data of 'Scientific-Production Centre of Land Cadastre', the 74 land users are located on the total area of 78 796.2 ha. Based on the need of preserving the unique natural complexes and taking into consideration the interests of the local population as well as the perspectives of the agriculture development in this region, it has been proposed to include in the reserve only the southern part of the project area, with lake Aralsor and adjacent territory (Fig. 1). This is due to the fact that the northern and north-eastern parts of the project area within the boundaries of Borsy and Zhaksybay rural districts, and Koshankol and Karaoba rural districts, were previously seen as a promising area for the development of animal husbandry.

The project area is located in the northwestern part of the Caspian lowland in the West Kazakhstan region within the territory of Zhanibek, Bokeyorda, Kaztalovka raion districts. The total area of the studied region is 690.929 thousand ha, or about 4.5% of the region area (Fig. 1). The nature of its relief is almost a hollow, flat and slightly rolling plain with slight slope to the south. This plain is represented by places of depressions, clayish salt marshes and ancient channels of temporary streams.

The hydrographic network on the territory of the nature reserve is very weakly developed. The river Aschyozek flows on the east of the project area, with several tributaries – the largest of them are the right-bank tributaries Sherembetsay, Tatkensay, Zhamansay, Astausalgan arroyo, Bersharal, and the left-bank tributaries Koldybaysay and Tereksay, flowing into the large lake Aralsor. Due to the frequent alternation of soil conditions, soils are inhomogenic and have different structure. However, within the individual parts of the territory of the future nature reserve in the watershed areas, the zonal types of soils prevail – kastanozems, light-kastanozems and brown earth (5).

The flora of the project area is very diverse and poorly investigated. We have identified 537 species of vascular plants from 66 families and 265 genus. However, this is not complete species composition. In the overall flora of the West Kazakhstan region it constitutes 42.7% of total number of species (537), 54.4% (265) of total number of genera and 56.4% (66) of total number of families. The representatives of 3 families are the most numerous in the studied area – *Asteraceae*, *Poaceae* and *Chenopodiaceae*. *Asteraceae* is represented by 95 (17.3%) species, *Poaceae* – by 54 species (9.8%) and *Chenopodiaceae* – by 42 species (7.6%) (9, 16).

The project area 'Bokeyorda' belong to two natural zones – the steppe zone (subzone of *Gemifructis-gramen-poaceae* desertified steppes on light-kastanozems) and the semi-desert zone (northern subzone of *Artemisia* and long-term *Salsola* semideserts on brown earth). According to the system of phyto-geographical zoning, the project area is represented by the Eurasian steppe and the Afro-Asian desert areas. Steppe is represented by its most arid subzone – Zavolzhye-Kazakhstan *Gemifructis-gramen-poaceae* desertified steppes, which includes the northern part of territories. Desert is represented by the least arid north-western outskirt of the Caspian province – the North Turan steppefied desert, which include the large part of the Caspian depression (17-18). 7 types of vegetation are well represented in the project area: steppe, desert, forest, shrub, meadow, swamp, submerged-water (20).

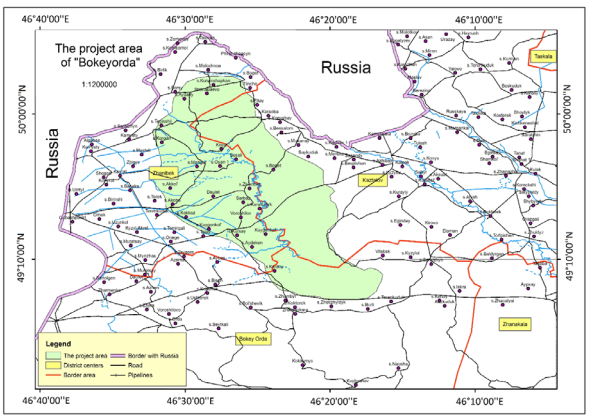

Figure 1 – Map of the projected State Natural Reserve 'Bokeyorda' in West Kazakhstan region

Animals of the project area are represented by steppe and desert zone species. Among rodents are little souslik *Spermophilus pygmaeus*, several species of hamsters (*Cricetidae*), dipodids (*Dipodidae*), gerbils (*Gerbillidae*), voles (*Microtidae*), mice (*Muridae*). Carnivores are represented by wolf *Canis lupus*, red fox *Vulpes vulpes*, korsak *Vulpes corsac*, steppe polecat *Mustela eversmanni*, stoat *Mustela erminea*, badger *Meles meles*, common weasel *Mustela nivalis*, and others. Among even-toed ungulates are two species: saiga antelope *S. tatarica* and wild boar *Sus scrofa.* The very characteristic for the studied territory are such birds as larks (*Melanocorypha*), swallows (*Hirundinidae*), little bustard *Tetrax tetrax*, demoiselle crane *Anthropoides virgo*, and others. Among the small birds of prey are kestrel *Falco tinnunculus* and red-footed falcon *Falco vespertinus*, and among the large ones are common buzzard *Buteo buteo*, hen harrier *Circus cyaneus* and steppe eagle *Aquila nipalensis,* which is rarely observed. The waterbodies are very rich in various waterfowl species. Among reptiles, steppe-runner *Eremias arguta*, sand lizard *Lacerta agilis* and steppe viper *Vipera ursinii* are ordinary to the desert.

We identified 57 individual ecosystems within the planned State Nature Reserve 'Bokeyorda', which were arranged in a hierarchical classification by their typological groups, structural and genetic classification. This classification was the basis of the legend displayed on the map (Fig. 2). This map shows classification of ecosystem ranks (Legend in Fig. 2).

Figure 2 – Map of ecosystems of the projected State Natural Reserve 'Bokeyorda' in the West Kazakhstan region

LEGEND OF MAP FOR ECOSYSTEMS IN PROJECTED STATE NATURAL RESERVE «BOKEYORDA» IN THE WEST KAZAKHSTAN REGION

GROUND
AUTOMORPHIC
Desert-steppe ecosystems on light-kastanozems soils
Gramen-poaceae of flat plains

1(1) – With the predominance of *Poaceae-filipéndula*, *Artemisia-poaceae-festuca*, *Tanacétum-festuca* associations

2(2-5) – With the predominance *Variherbetum-poaceae-agropyron* associations

3(6) – With the predominance of *Poaceae-festuca*, *Artemisia-festuca* assosiations in combination with *Variherbetum-gramen-poaceae* in depressions

Gramen-poaceae of sloping plains dissected by erosion

4(7) – With the predominance of *Poaceae-filipéndula*, *Artemisia-poaceae-festuca*, *Tanacétum- festuca* assosiations

5(8) – With the prevalence of *Artemisia-poaceae-stipa* assosiations with a predominance of *Filipéndula*

6(9) – With the prevalence of *Artemisia-poaceae-festuca* assosiations with a predominance of *Filipéndula* in conjunction with the cultivated lands

7(10) – With the prevalence of *Artemisia-poaceae-stipa* dominated communities Filipéndula in conjunction with *Sálsola-artemisia* pauciflora, and cultivated lands

8(11) – With the predominance of *Poaceae-festuca* with in conjunction with *Sálsola-artemisia* pauciflora

9(12) – With the prevalence of *Artemisia -poaceae-festuca* dominated communities Filipéndula in conjunction with the *Sálsola*

Artemisia of flat complicated by suffusion slides plains

10(13) – With the prevalence of *Poa praténsis-artemisia pauciflora* sometimes with *Poaceae* and *Sálsola* together with representatives of the *Sálsola-artemisia pauciflora*

11(14) – With the prevalence of *Gramen-poaceae-artemisia pauciflora*, sometimes with *Poaceae* and *Sálsola* together with representatives of the *Sálsola-artemisia pauciflora*

12(15) – With the prevalence of *Artemisia-roaceae-festuca* dominated communities Filipéndula in conjunction with the cultivated lands

Artemisia of sloping plains dissected by erosion

13(16) – With the prevalence of *Gramen-poaceae-artemisia pauciflora* in combination with the black *Sálsola-artemisia pauciflora*

Desert-steppe ecosystems on brown earth soils
Artemisia of sloping plains dissected by erosion

14(17) – With the prevalence of *Artemisia lerchiana* and bluegrass during *Poa praténsis-artemisia lerchiana* community

Sálsola in depressions and depressions

15(18) – With the predominance of *Variherbetum-poaceae-euphórbia* communities with a predominance of *Artemisia* and *Filipéndula*

16(19) – With the prevalence of *Artemisia pauciflora-limonium*, *Poaceae-artemisia lerchiana* and *Artemisia-ruccinelli* communities

Halophytes-artemisia ecosystems on solonetz

Artemisia of flat complicated by suffusion slides plains

17(20) – With the predominance of *Sálsola-artemisia pauciflora* complex with *Festuca-roaceae-artemisia* communities with a predominance of *Filipéndula*

Artemisia of sloping plains dissected by erosion

18(21-22) – With the predominance of *Artemisia pauciflora*, *Sálsola* in complex with *Artemisia-roaceae-agropýron* communities

19(23-25) – With the predominance of *Sálsola-artemisia pauciflora* in the complex, *Ephemerae-proaceae-artemisia lerchiana* communities

20(26) – With the predominance of *Sálsola-artemisia pauciflora* in conjunction *festuca-roaceae-artemisia* communities with a predominance of *Filipéndula*

Sálsola of flat complicated by suffusion slides plains

21(27) – With the predominance *Petrosimonia sibirica (Pall) Bge Ahab Rev* in a complex of *Sálsola-artemisia pauciflora* and *Proaceae-filipéndula* communities

Sálsola of gently rolling plains dissected by erosion

22(28-29) – With the predominance of *Artemisia-sálsola-limonium* communities

SEMIHYDROMORPHIC

Meadow-desert ecosystems on meadow-light kastanozems soils

Poaceae of sloping plains dissected by erosion

23(30) – With the prevalence of *Leymus poaceae* communities

Gramen-poaceae of flat complicated by suffusion slides Plains

24(31) – With the prevalence of *Gramen-poaceae-artemisia-variherbetum* communities

Meadow-desert ecosystems in the meadow solonetz

Artemisia in depressions

25(32) – With the predominance of *Sálsola-artemisia pauciflora* communities in a complex *Artemisia pauciflora-suaeda* and *Artemisia-roaceae*

Sálsola in depressions

26(33) – With the prevalence of *Puccinelli-halimione-artemisia* communities

HYDROMORPHOUS

Meadow-desert ecosystems in the meadow soils

Variherbetum-poaceae in depressions

27(34-36) – with a predominance of *Variherbetum-poaceae* communities sometimes with *Carex*

The hyper halophytic ecosystems of depression

Sors in depressions and in the saline depressions

28(37-43) – Sors, salt marshes with sparse *Sálsola* vegetation

GROUND ANTHROPOGENICALLY DISTURBED ECOSYSTEMS AGRO-ECOSYSTEMS

Anthropogenically transformed ecosystems on light-kastanozems soils

Parturition of arable and cultivated land

29(44-45) – Arable land and cultivated land on the site of *Tanacetum-festuca-stipa* communities

30(46-47) – Arable land and cultivated land on the site of *Variherbetum-stipa* communities

Anthropogenically transformed ecosystems on brown earth soils

Parturition of arable and cultivated land

31(48) – Arable land and cultivated land on the site of *Festuca-artemisia pauciflora*, *Festuca-linosyris* communities

Anthropogenically transformed ecosystems on solonetz

Parturition of arable and cultivated land

32(49-50) – Arable land and cultivated land on the site of *Tanacetum-festuca-stipa* communities

Anthropogenically transformed ecosystems on meadow light-kastanozems soils

Parturition of arable and cultivated land

33(51) – Arable land and cultivated land on the site of *Tanacetum- festuca-stipa* communities

DEGRADED

Anthropogenically disturbed ecosystem on eroded light-kastanozems soils

Parturition of pasture failures around residential housing

34(52) – *Ephemera-ceratocarpus* overgrazing or anthropogenic disturbances

35(53) – *Artemisia lerchiana-ephemeral*

36(54) – *Artemisia procera*. Ruderal thickets

Anthropogenically disturbed ecosystem on eroded solonetzes

Parturition of pasture failures around residential housing

37(55) – *Ephemerae-egetorugit*

38(56) –*Artemisia pauciflora-sálsola* thickets

Note: 1. The first number has specified number of a type of an ecosystem according to the table of a legend

2. The second number (in brackets) has specified serial number of an ecosystem according to the table of a legend

The project area includes the habitat of the Ural population of the saiga antelope *S. tatarica*. Saiga antelope of the Azgir-Urda grouping usually lives here in the spring and autumn periods. In the winter they leave the territory of Atyrau region. The main areas of its habitat are neighborhood of the Sors Khaki, river Aschyozek, lake Aralsor. In some years it rises to the north to the settlements Kaztalovka and Borsy (13, 20).

Areas of the main habitat of the saiga antelope, including wintering, summering and mass calving, currently occupy the neighbourhood of the north-western part of the Volga-Ural interfluve. Basically, these territories are less affected by economic activity and are remote from major population centers. Obviously, the saiga antelope prefer to feed here. In the Volga-Ural interfluve, the most part of saiga antelope population is concentrated now in Aralsor solonchak- lake structural basin, where a desert-solonchak complex of vegetation is predominant, with the most preferred by saiga antelope (*S. tatarica*) plant of the families *Asteraceae*, *Chenopodioideae*, *Cruciferae and Rosaceae* (1-3). These are not only such plant species as *Chenopodium album*, *Kochia*

prostrata, *Potentilla* sp., *Crinitaria tatarica*, *Polygonum patulum* and others, that are usually preferable by herbivorous mammals, but also many weeds and poisonous plants, such as *Lactuca serriola*, *L. tatarica*, *Artemisia austriaca*, *Thlaspi arwense* and others, which other ungulates do not eat by *S. tatarica* (19).

The Volga-Ural interfluve is now a place for mass saiga antelope calving. Now, it is located more to the north than in the past: to the east and north of the lake Aralsor and even in the village Borsy – the most north-western part of the interfluve. The change of the range of mass calving, occurring in recent years, is, no doubt, due to the increase of anthropogenic load on the previous areas of calving. The territories located to the south now include agricultural land, covering an area of 12 785.000 ha, of which arable land – 769,8 ha (6.0%), hayfields – 1.010 mln ha (7.9%) and pastures – 10.106 mln ha (79.0%). The main economic activities in the region of the planned reserve are the sheep farming, cattle husbandry and herd horse breeding. This factor should be taken into account in the development of this project.

Local authorities planned to provide to large farmers the agricultural areas of land for the needs of pasture animal husbandry. With this in mind, it has been recommended to create two types of SPA on the project area – the State Nature Reserve and the State Natural Zakaznik (the complex one). Possible boundaries of these SPA are shown on the map of the Reserve 'Bokeyorda' (Fig. 1.) The area of the Reserve is 239 242 ha, and its protected area is 81 513 ha, Zakaznik – 299 218 ha.

During the development of the project, it was necessary to take into account the recommendations for the conservation of biological diversity of the region. Rare and endangered species with important economic, scientific and aesthetic value were included in The Red Book of Kazakhstan (26, 32).

Our investigation of plant communities showed that the flora of the project area 'Bokeyorda' consists of 104 species of fodder plants. We noted the presence of at least 4 unique and significant plant communities, which represent a kind of original botanical-geographic phenomenon and have an important environmental, water regulation, water protection, soil protection, and other roles. Despite of widespread plowing, the small natural sites of zonal steppe complexes were fragmentary preserved. These are the Sherembetsay tract, Tegisshyl complex steppe, steppe Aralsor steppe, Karaoba *Festuca* steppe.

In the studied area of the planned reserve we also found 21 rare, endemic and relict, species of plants, which occur with various level of abundance and play different roles in the vegetation. They constitute 5.1% of the total flora. Following species are listed in the Red Book: *Calophaca wolgarica*, *Adonis vernalis*, *Centaurea talievii*, *Tulipa schrenkii*, *Tulipa biebersteiniana*, *Tulipa biflora*, *Ornithogalum fischerianum*.

The studied territory is also important for the conservation of habitats of steppe animal species. We revealed 37 species of mammals, 71 species of birds and 7 species of reptiles. 5 species are listed in the Red Book – demoiselle crane *Anthropoides virgo*, little bustard *Tetrax tetrax*, eagle-owl *Bubo bubo*, golden eagle *Aquila chrysaetos* and steppe eagle *Aquila nipalensis*.

Conclusions

The territory of the future State Nature Reserve 'Bokeyorda' has the highest potential as the habitat of *Saiga antelope* and *S. tatarica*. This is due to preserved special conditions with rich biodiversity, necessary for large endemic mammals of steppe and semi-desert areas.

The studied territory is a refuge for many endangered plant and animal species and is very important for migratory species. Here, the significant natural fodder plant resources preserved, they dominate in the pastures and hayfields and are very diverse in its stern properties and seasonal use. Fodder plant communities are the source of highly nutrient food for animals throughout the year.

On the basis of created cartographic materials we tried to define the significance of ecosystems for biodiversity conservation. For that purpose, we revealed key plant and animal species for each ecosystem. Furthermore, for the assessment of environmental significance of the project area we used the richness of species. We also revealed the degree of disturbance of ecosystems in the studied area in connection with anthropogenic load and its impact on natural systems.

The existing natural potential, and, in particular, ecological conditions, allow to particular representatives of flora and fauna to pass all the stages of the biological cycle in the territory of Reserve, including the breeding stage. However, the impact of external factors, both natural and antropogenic, to the representatives of wildlife fauna, continues to grow due to the intensity of the economic development of the territory. Now, it is necessary to take additional and more effective activities for the conservation of species and their habitats.

No strictly protected area is created in the West Kazakhstan region at the present time. Organisation of a new environmental institutions not only will fully ensure the preservation and restoration of steppe biodiversity of the region, but also will improve the socio-economic conditions, the development of ecotourism, etc. Analysis of ecological state of the project area, as well as the study of the habitat of *Saiga antelope S. tatarica*, will contribute to the creation of the large State Natural Reserve 'Bokeyorda' in the west of the West Kazakhstan region.

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Effects of Almaty city ecological factors on students blood indices at Al-Farabi Kazakh National University

Abstract: Environment pollution is a wide-researching problem and it is likely to influence the health of human populations is great. And environmental health is that aspects of public health that is concerned with those forms of life substances, forces and conditions in the surroundings of man that may exert an influence on man's health and well-being. A number of natural and anthropogenic activities and may cause adverse effects on human health and the environment. Increased combustion of fossil fuels in the last century is responsible for the progressive change in the atmospheric composition. Air pollution has both acute and chronic effects on human health, affecting a number of different systems and organs. It ranges from minor upper respiratory irritation to chronic effects on human disease, lung cancer, and acute respiratory infections in children and chronic bronchitis in adults. The effects of environmental factors on student's health and its mechanisms are briefly discussed.

The climatic – geographic conditions of the environmental living habitat is one of the most influential reasons that has always affected and produced physiological changes in our body. Because of this, it gives a chance to determine the change in organisms in time, and give us enough time to organize the appropriate prophylactic events in advance.

In our work, the data which had been measured during laboratory research was statistical analyzed the methods were used in our study, and assessment of the value of the measured data, we paid much more attention to assess correctly the changes because of various climatic – geographical reasons effects to students body. In order to determine the functional status of the students' organisms, we used special pilot tested, and got the effective positive comment on its using. In this study we put significant effort to determine the physiological variations (disorders) to the students' which from the different regions of the republic of Kazakhstan and their adaptation process in immune system because of various climatic – geographical changes in their life.

Key words: environment factors, blood indices, cardiovascular system, blood cell count.

Introduction

The environment affects our health in a variety of ways. The interactions between human health and the environment has been extensively studied and environmental risks have been proven to significantly impact human health, either directly by exposing people to harmful agents, or indirectly, by disrupting life-sustaining ecosystems [1]. Climate change is also posing risks to human population health and well-being and thus is emerging as a serious concern worldwide [2-5].

Although a number of physical activities (volcanoes, fire, etc.) may release different pollutions in the environment, anthropogenic activities are the major cause of environmental air pollution. Air pollutants, such as carbon monoxide (CO), sulfur dioxide (SO_2), nitrogen oxides (NO_X), volatile organic compounds (VOC_S), ozone (O_3), heavy metals, and respirable particulate matter (PM2.5 and PM10), differ in their chemical composition, reaction properties, emission, time of disintegration and ability to diffuse in long or short distances. Hazardous chemicals can escape to the environment by accident, but a number of air pollutants are released from industrial facilities and other activities and may cause adverse effects on human health and the environment. As far as humans are

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concerned an air pollutant may cause or contribute to an increase in mortality or serious illness or may pose a present or potential hazard to human health. The determination of whether or not a substance poses a health risk to humans is based on clinical, epidemiological, and/or animal studies which demonstrate that exposure to a substance is associated with health effects. Contact with unsafe drinking or bathing water can impose serious risks (both acute and delayed) to human health [6; 7].

The main change in the atmospheric composition is primarily due to the combustion of fossil fuels, used for the generation of energy and transportation. Variant air pollutants have been reported, differing in their chemical composition, reaction properties, emission, persistence in the environment, ability to be transported in long or short distances and their eventual impacts on human and/or animal health. However, they share some similarities and they can be grouped to four categories: 1. Gaseous pollutants (e.g. SO_2, NO_X, CO, ozone, Volatile Organic Compounds). 2. Persistent organic pollutants (e.g. dioxins). 3. Heavy metals (e.g. lead mercury). 4. Particulate Matter [8-11].

Sporadic air pollution events, like the historic London fog in 1952 and a number of short and long term epidemiological studies investigated the effects of air quality changes on human health. A constant finding is that air pollutants contribute to increased mortality and hospital admissions [12]. The different composition of air pollutants, the dose and time of exposure and the fact that humans are usually exposed to pollutant mixtures than to single substances, can lead to diverse impacts on human health. Human health effects can range from nausea and difficulty in breathing or skin irritation, to cancer. They also include birth defects, serious developmental delays in children, and reduced activity of the immune system, leading to a number of diseases. Moreover, there exist several susceptibility factors such as age, nutritional status and predisposing conditions. Health effects can be distinguished to acute, chronic not including cancer and cancerous. Epidemiological and animal model data indicate that primarily affected systems are the cardiovascular and the respiratory system. However, the function of several other organs can be also influenced [13-16]. Environmental degradation poses a significant threat to human health worldwide. Harmful consequences of this degradation to human health are already being felt and could grow significantly worse over the next 50 years [17].

Materials and methods

Objects of study: the objects of study were determined by the methods which we had accepted. According to decision, 35 young students (female) who not had pathological abnormalities were examined. They divided into three groups; first group the local students who live in Almaty city, and this group was consists of 13 girls who were 18 – 21 years old, 49 – 65 kg in weight, 149 – 168 cm tall. Second group the students who are from the south part of the republic of the Kazakhstan, and consists of 10 girls who were 18 – 20 years old, 51 – 66 kg in weight, 161 – 169 cm tall. And the third group the students who are from the west part of the republic of Kazakhstan, and consists of 12 girls who were 18 – 22 years old, 55 – 70 kg in weight, 162 – 170 cm tall. Overall 35 students and all of them were girls. The height of the objects of this research was ranges from 149 to 170 cm; the age was ranges from 18 to 22 years old; the weight was between 49 and 70 kg. The object of study was examined before and after the physical workload, between 14:00 and 17:00, in autumn (October to November). They were estimated to make a prediction to their cardiovascular system status.

Methods of study: the objects of study were university students in Almaty, and they were kept normal mode of labor and rest. The research was carried out at Keremet Medical Center in Al-Farabi Kazakh National University; indoor temperature was 22 -25 C° and it was 20 – 29 C° in the environment (outside of room) of the Almaty city. Possible reserve of human blood indices which were dependent on time of year before and after the physical workload was added to their own research work.

The complex study of students started with register their health status, general self-feelings, ages and weights. Anthropometric indices were measured with medical scale and height meter, height (H, cm): students stand on the platform with your back against the wall and your feet together. Stand up as straight as possible with your heels, back, shoulders, and head all touching the wall. Tuck in your chin and look straight ahead. Body weight (M, kg): students stand in the middle of the scale, error is ±50 g. blood pressure was measured with Korotkoff method using stethoscope and tonometer, systolic blood pressure is the pressure on the arteries as the heart contracts and pumps blood, the diastolic blood pressure is the pressure on the arteries when the heart is at rest between each heartbeats. The cardio respiratory system function was carried out with the help of the elec-

trocardiographic device which name was "Merguette Hellige". Physical workload was given by bicycle ergo meter (Proteus Cycle Pec 3000, 2000) and the physical work load was Watts every 5 min, the speed was 12 km/h, distance was 1.1 km. research was carries out in two different stages according to research method. That is, at normal circumstance (before physical workload) and at special circumstance (after the physical workload).

The blood indices analysis was also carried out before and after the physical workload. Therefore, we did 1170 times research among the students. Hematological research included the erythrocytes count in peripheral blood vessel, leukocytes count in peripheral blood vessel and absolute number of lymphocytes was carried out based on N. M. Mykolayiv method. In addition, the shape of leukocytes was determined by the paint method of Popringler – Kryukov. Determining of phagocytic activity of neutrophil leukocytes was carried out and calculated by the Hamburger's phagocytic index.

The students had been used various tests to analyze the functional conditions of the students; the amount of leukocytes and lymphocytes, the reaction of blocking the leukocytes migration, phagocytic activity of neutrophil leukocytes tests were have been studied.

Data were analyzed using the software Microsoft office excel 2010. Significant differences are indicated by asterisks ($P^* < 0.05$; $P^{**} < 0.01$) for comparisons between before and after the physical workload.

Results and discussion

This situation shows the immune system of the students from the southern Kazakhstan wasn't totally adapted to the local climatic – geographical changes. But the average amount of lymphocytes in the peripheral blood vessels of the third group was known that between 26.00 and 27.36.

This means that the adaptation ability of immune system ($P > 0.05$) in the students of third group was weaker than the students of the first group that live in the Almaty city for their entire life.

So it was shown that there were carried out much more obvious changes in the students immune system to adapted to the new Almaty cities climatic – geographical conditions from the foreign climatic – geographical condition.

Therefore, the defense system of the students were endure incredibly pressure ($P < 0.05$) when the students who were come from the atmospheric air temperature was comparable lower and more humidity region, and we had been discovered that their defense system had been undertaken complex adaptation reaction.

It has a magnificent importance to check out the neutrophil leukocytes phagocytic activeness in the peripheral blood vessels of the students that came to Almaty from the other parts of the republic of Kazakhstan for biological adaptation.

So, the materials that we took from our study shows that there was a huge differences in the absolute number of leukocytes and the absolute number of the lymphocytes, the leukocytes migration inhibition factor and Phagocytic activity of neutrophil leukocytes in the Almaty city's local residence and non – residence of the initial condition and after the physical workloads. These changes were depended on the adaptation process of cardio respiratory outputs became lower in the students who came to Almaty city from the other parts of the republic of Kazakhstan.

The adverse condition of Almaty city's environment affects to human body, the cardio immune system shows a significantly reducing of the object of study (second, third groups).

It might have had close contact with the high temperature of the environment and the ecological adverse factors. it should pay more attention to that the absolute number of leukocytes in the peripheral blood vessels of the students who are from the south and west parts of the republic of Kazakhstan were more than the absolute number of leukocytes in the peripheral blood vessels of local students. This implied that the students' bodies that were from south and west parts of Kazakhstan have sensitivity to Almaty cities environmental factors.

So, according to the data above, to adapt to the conditions of Almaty city in the three years, the students who are from the south and west parts of republic of Kazakhstan, there were dynamic changing in their describing indices of peripheral blood.

It was known as the former data, the absolute amount of lymphocytes in the peripheral blood vessels of the local students in Almaty, or the students who are from the other parts of the republic of Kazakhstan was declining in the past three years, however, the other blood indices were increasing. The differences of leukocytes migration inhibition index might depend on the climatic – geographical conditions and ecological adverse conditions which people lived in.

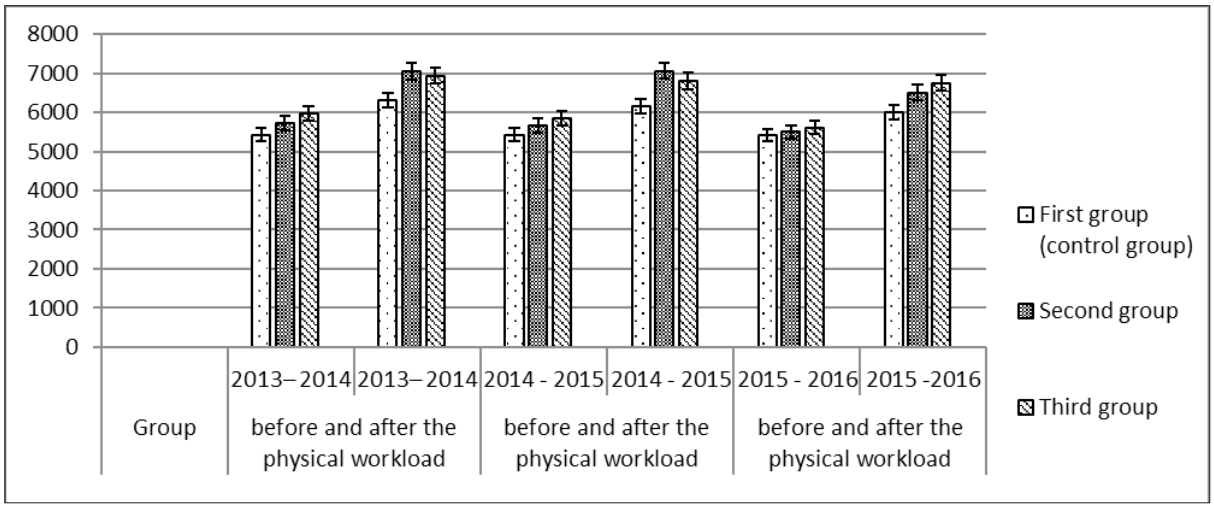

Figure 1 – Leukocytes counts in the peripheral blood vessels of the students that before and after the physical workload (1μl, in blood)

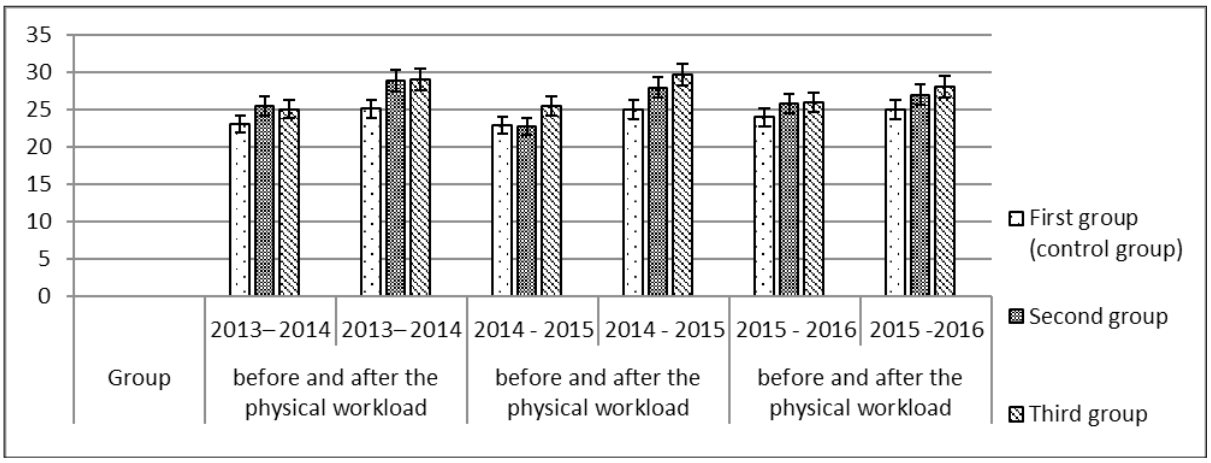

Figure 2 – Lymphocyte counts in the peripheral blood vessels of the students that before and after the physical workload (1μl in blood)

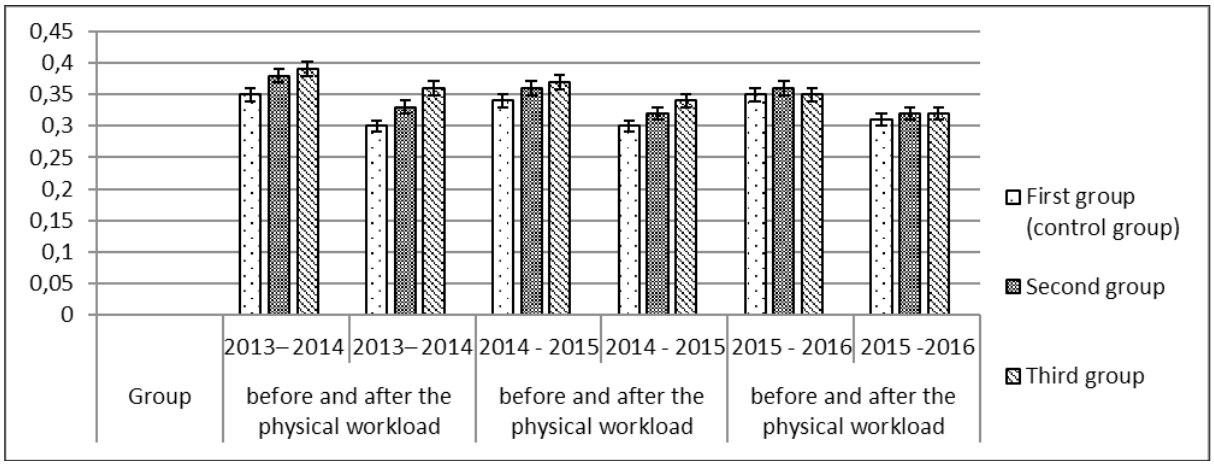

Figure 3 – Research results of the leukocytes migration inhibition factor (1 μl, in blood)

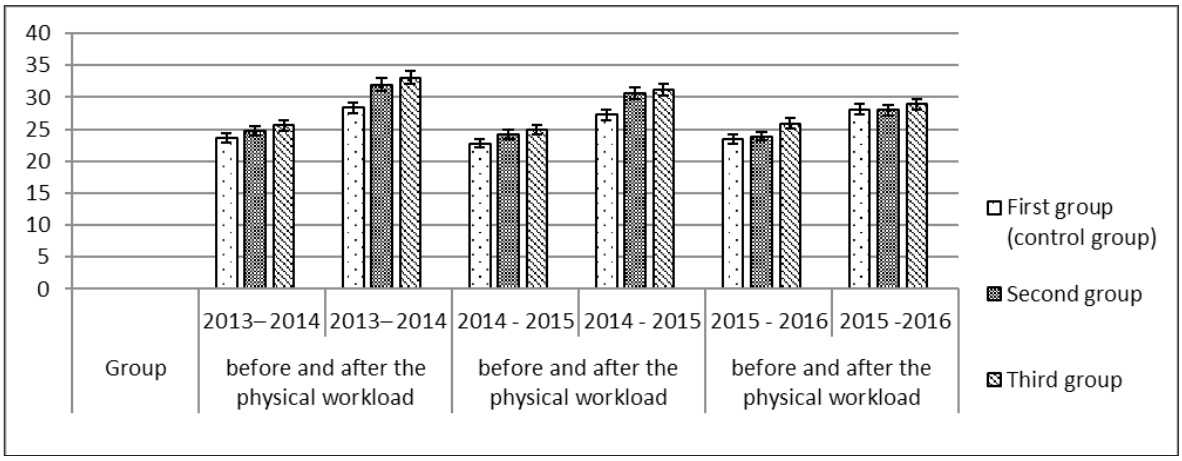

Figure 4 – Phagocytic activity of neutrophil leukocytes in peripheral blood vessel (1 μl, in blood)

Table 1 – the absolute amount of leukocytes in the peripheral blood vessels of the local students in Almaty city (control group) in the past three years and was analyzed before and after the physical workload

Experiment condition	First year	Second year		Third year	
	2013 – 2014	2014 – 2015	P_1	2015 – 2016	P_2
Before the physical workload	5413.6 ± 102.4	5428.7 ± 102.5	>0.05	5399.3 ± 100.2	>0.05
After the physical workload	6292.4 ± 109.8	6157.3 ± 106.9	>0.05	5987.2 ± 90.7	>0.05

Table 2 – the absolute amount of leukocytes in the peripheral blood vessels of the students who are from the south part of republic of Kazakhstan during the past three years and were analyzed before and after the physical workloads

Experiment condition	First year	Second year		Third year	
	2013 – 2014	2014 – 2015	P_1	2015 – 2016	P_2
Before the physical workload	5709.1 ± 106.4	5648.9 ± 107.1	>0.05	5488.5 ± 108.5	>0.05
After the physical workload	7042.7 ± 114.3	6891.5 ± 115.5	>0.05	6489.6 ± 121.6	>0.05

Table 3 – the absolute amount of leukocytes in the peripheral blood vessels of the students who are from the west part of republic of Kazakhstan during the past three years and were analyzed before and after the physical workloads

Experiment condition	First year	Second year		Third year	
	2013 – 2014	2014 – 2015	P_1	2015 – 2016	P_2
Before the physical workload	5958.8 ± 103.7	5837.9 ± 118.2	>0.01	5585.6 ± 114.2	>0.01
After the physical workload	6908.3 ± 82.6	6860.7 ± 126.4	>0.05	6759.4 ± 122.2	>0.05

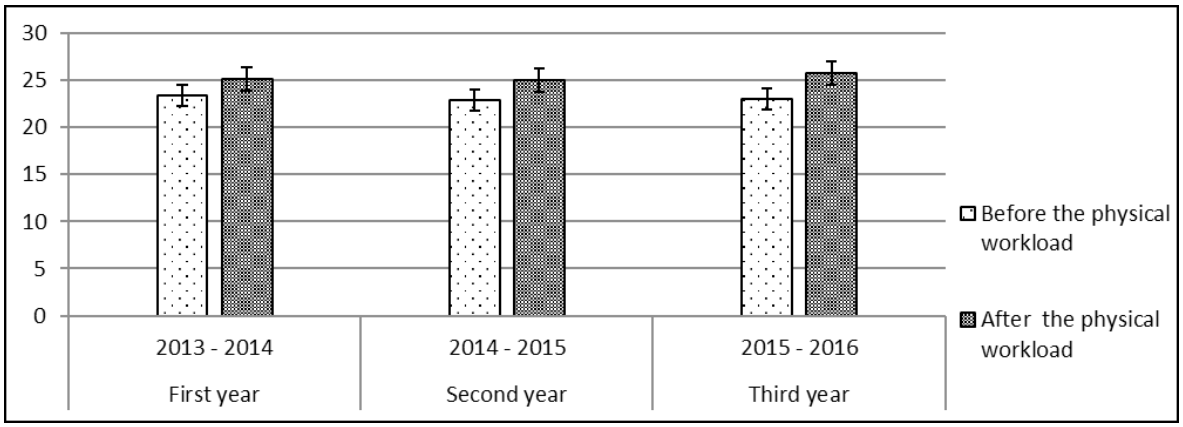

Figure 5 – the absolute amount of lymphocytes in the peripheral blood vessels of the local students in Almaty city (control group) in the past three years and was analyzed before and after the physical workload

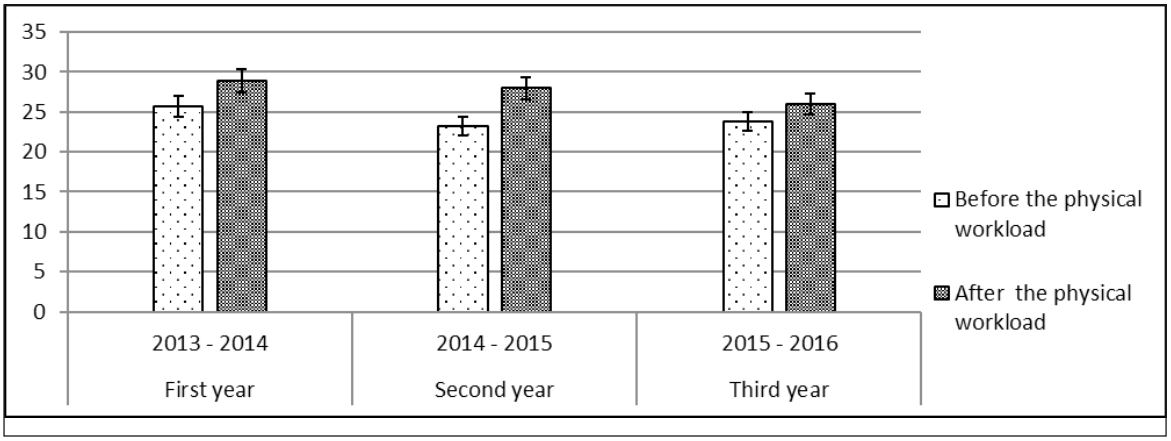

Figure 6 – the absolute amount of leukocytes in the peripheral blood vessels of the students who are from the south parts of republic of Kazakhstan in the past three years and were analyzed before and after the physical workloads

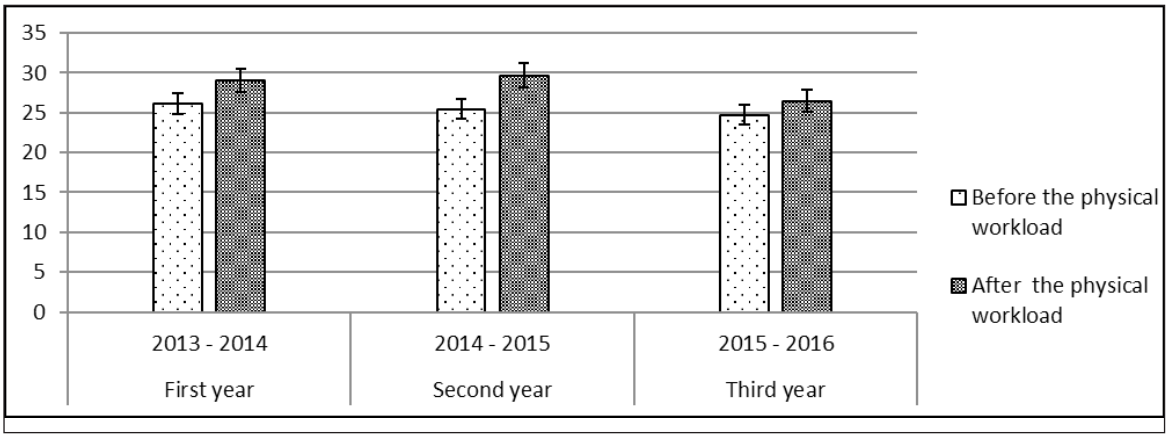

Figure 7 – the absolute amount of lymphocytes in the peripheral blood vessels of the students who are from the west parts of republic of Kazakhstan in the past three years and were analyzed before and after the physical workloads

Table 4 – the phagocytic activity of neutrophil leukocytes in the peripheral blood vessels of the local students in Almaty city (control group) in the past three years that was analyzed before and after the physical workload

Experiment condition	First year	Second year		Third year	
	2013 – 2014	2014 – 2015	P_1	2015 – 2016	P_2
Before the physical workload	21.80 ± 1.16	20.40 ± 0.12	>0,05	21.50 ± 0.96	<0,02
After the physical workload	26.70 ± 1.62	24.20 ± 1.52	>0,05	26.10 ± 1.50	<0,01

Table 5 – the phagocytic activity of neutrophil leukocytes in the peripheral blood vessels of the students who are from the south parts of republic of Kazakhstan in the past three years and were analyzed before and after the physical workloads

Experiment condition	First year	Second year		Third year	
	2013 – 2014	2014 – 2015	P_1	2015 – 2016	P_2
Before the physical workload	23.00 ± 1.34	22.30 ± 1.19	>0,05	21.90 ± 0.90	>0,05
After the physical workload	30.20 ± 2.40	28.30 ± 1.90	>0,05	26.00 ± 1.66	>0,05

Table 6 – the phagocytic activity of neutrophil leukocytes in the peripheral blood vessels of the students who are from the west parts of republic of Kazakhstan in the past three years and were analyzed before and after the physical workloads

Experiment condition	First year	Second year		Third year	
	2013 – 2014	2014 – 2015	P_1	2015 – 2016	P_2
Before the physical workload	24.70 ± 1.72	23.30 ± 1.44	>0,05	22.10 ± 1.20	>0,05
After the physical workload	31.70 ± 2.30	29.50 ± 1.83	>0,05	26.80 ± 1.81	>0,05

At the same time, the phagocytic activity of neutrophil leukocytes in the peripheral blood vessels of the students who are from the south and west parts of the republic of Kazakhstan after the physical workloads are more changeable than the phagocytic activity of neutrophil leukocytes in the peripheral blood vessels of the students who usually live in Almaty city.

This is considered as the obvious functional possibility when the body's adaptation to the new extreme factors of environment.

We can notice that the phagocytic activity of neutrophil leukocytes of the students who have been living in the Almaty city for three years, that is, the index is decreased to the initial level and it is the satisfactory response of the cardiovascular system adaptation.

In contrast with the results of local students who live in the Almaty city, the results of the students who are from the other parts of the republic of the Kazakhstan were magnificently affected by the adverse ecological and climatic factors.

Conclusion

As a result of a complex study to the ecological – physiological, statistical and dynamical. The cardio system and blood's regional and constitutional features of the local students who live in Almaty city and the students who are from the other parts of the republic of the Kazakhstan were identified. According to the results above we have made the following statements:

1. The blood and cardio system's indices of the students who are from the south and west parts of the republic of the Kazakhstan weremore sensitive than the indices of the local students who live in Almaty city, so their organisms faced to adapt.

2. The results show that the physiological indices of the students who were from the other parts of the republic of Kazakhstan functional condition have a close connect of the initial year, but in the local students there was no any change.

3. Three years' time do not enough to totally adapt the new climatic – geographical environment and adverse ecological factors, three years' time do not enough.

4. The complex study of cardio system and hematology show that the physiological function activity of the students who are from the other parts of the republic of Kazakhstan are not as stable as the local students physiological function activity.

5. The physical tolerance indices of the local students who live in Almaty city are much higher than the students who are from the other parts of the republic of Kazakhstan. Especially indices of the students who are from the south and west parts of the republic of Kazakhstan increased after the physical workloads.

6. It is shown that the adverse ecological factors of environment have harmful effect to the human cardio and blood indices. The characters and extent of the effect was depending on the ecological condition. In contrast with the local students, the foreign students who are from the other parts of the republic of the Kazakhstan affected more.

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Study on the effect of the Kazakh Traditional Medicine Kezimuk granules to the immunologic function of cyclophosphamide induced immunosuppressed mice

Abstract: The paper reports the study of the effect of the Kazakh mmedicine Kezimuk granules on himoral immunity, cell-medicated immunity and immunological function of cyclophosphamide immunosupressed mice. 60 SPF Kunming mice, half male and half female, were randomly divided into 6 groups. Each group contains 10 mice: Respectively the normal group (saline solution) comparing to the control group (Yupingfeng granules) and the model group (saline solution) comparing to high, medium and low dose Kezimuk granule groups. The high, medium and low dose ,2.72g/kg, 1.36g/kg, and 0.68g/kg, of Kezimuk were equivalent to 4, 9, and 18 times of it and the recommended dose was calculated through the basis of relative surface of mice, referring to humans. Doses were daily intaken by intragastric administration for 2 weeks. After drug administration from the seventh day to ninth day ,for 3 days, each group, except the normal control group, received intra-peritoneal injections,pyrophosphate,at a dose of 50mg/kg to induce the immunocompromise. The thymus index, spleen index, serum IL-2 content and the body weight of the mice, were measured to observe the effect of each dose of Kezimuk granules on pyrophosphate to induce immunosuppression of the cellular immunity and humoral immunity in the mice. Comparing to the model group Kezimuk granules significantly increase the thymus index and spleen coefficient and remarkably increase the index of B Lymphocyte proliferation, T Lymphocyte proliferation and the serum IL-2 content as well as body weight of immunocompromised mice ($P<0.05$); the results for the middle and high dose of Kezimuk granule groups were significantly higher than the model group ($P<0.05$). Kezimuk granule might have a protective effect on immunocompromised mice and improve immunologic function.
Key words: Kezimuk granules, pyrophosphate, cellular immunity; humoral immunity.

Introduction

Kezimuk granule is a kind of Kazakh traditional medicine. The formula consists of common bilberry or blue whortleberry (*Vaccinium myrtillus L.*), helichrysum arenarium, equisetum and other herbs. It recorded and clinically confirmed that Kezimuk granule has therapeutical effects on urinary tract infections, cystitis, pyelonephritis, nephrotic syndrome, hypertrophy of the prostate and impeded urination. It can also be used as a general tonic. Preliminarily researches proveed that this prescription plays positive roles on anti-inflammatory effect, as well as on increasing urine volume (1). This fundamental research is to further the study to the effects of the medicine on immunologic function.

Chinese herbal medicine includes many various components to effect immune system, such as polysaccharides, saponins, alkaloids, volatile substances and organic acid to improved immunity, to intensify macrophage activity and to enhance liver detoxification function (2; 3). Also it makes immune cells resist bacteria, fungi and viruses to help human body get against infection as well as to eradicate invasive bacteria and viruses (4; 5). Increased number of immune cells correspondingly induce resistance and activate of macrophages, NK cells and other immune effector cells and secrete various cytokines and other important media. This Kazakh traditional medicine markedly has been effective in enhancing the immune effect of the body, accelerating macrophage proliferation, increasing peritoneal macrophages,

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boosting phagocytosis, as well as improving the killing activity of NK cells and also has been evidently helpful to achieve the objective of inhibiting and get rid of pathogenic microorganisms.

Materials and methods

Test medicine Kezimuk granule is formulated from 3 medicinal herbs: *Vaccinium myrtillus L*, 115g, *Helichrysum arenarium*, 384g, and *Equisetum hyemale L*. 192g. The prescription materials in the formula were provided by the Autonomous Region Kazakh Medicine Institute. Kezimuk granules are dark brown course particles prepared at Xinjiang Jiasite Pharmaceutical Co. Ltd., in strict accordance with the preparation process formulated in the preliminary study; batch number: 20130308. When used it was prepared according to the requirements of the experiment using double distilled water.

Animals: 60 Kunming mice, 8-12 weeks of age, 18-22 g body weight, half male and half female, purchased from the experimental animal center of Xinjiang Medical University. Experimental animal production license number: SYXK(新) 2011-0001.

Reagents: injectable pyrophosphate (CAT. No. C8650); DMSO (MP Biomedicals, LLC); Yupingfeng granules (Guangdong Global Pharmaceutical Co. Ltd., Product batch number: 130735); Mouse serum IL-2 Kit (Joyee Biotechnics Co., Ltd); RPMI1640 culture solution (Thermo Fisher Biochemical Products (Beijing) Co., Ltd.); MTT (MP Biomedicals, LLC); saline solution (Xi'an Jingxi Shuanghe Pharmaceutical Co. Ltd.); serum (Gibco Brand LOT 41G5532K); Concanavalin A (MP Biomedicals, LLC); PBS buffer (Thermo Fisher Biochemical Products (Beijing) Co., Ltd.) (6-12).

Instruments: precision electronic balance (Sartorius scientific instruments (Beijing) Co., Ltd.); Multiskan Spectrum (Benchmark Plus); Syringe (Jiangsu Shenli Medical Products Co., Ltd.); Petri dish (Corning Incorporated); 96 well plates (Corning Incorporated); Centrifuge; Clean bench; CO_2 incubator (RevcoUSA); Microscope (Olympus Corporation, Japan); UV VIS spectrophotometer (Shanghai Lengguang Technology Co., Ltd.).

Methods of research: Establishing the immunocompromised mice model: 60 SPF Kunming mice, half male and half female, were randomly divided into 6 groups. Each group contains 10 mice Respectively the normal group (saline solution) comparing to the control group (Yupingfeng granules) and the model group (saline solution) comparing to high, medium and low dose Kezimuk granule groups. The high, medium and low dosage of 2.72g/kg, 1.36g/kg and 0.68g/kg of Kezimuk was equivalent to 4, 9 and 18 times of it and the recommended dosage of animal model was calculated through the basis of its relative surface, according to the corresponding dosage (13). Doses were daily intaken by intragastric administration for 2 weeks. After drug administration from the seventh day to ninth day, for 3 consecutive days, each group, except the normal control group, received intra-peritoneal injections,pyrophosphate,at a dose of 50mg/kg to induce the immunocompromise.

Test indexes: Determination of the immune organ weight and the body weight of the mice: the day after the last administration of the medicine, the body weights of the mice were accurately weighed with an electronic balance, and after blood was taken by the eye removal technique, the weight of the thymus and spleen were measured under aseptic conditions and the spleen and thymus index calculated according to the formula:

$$\text{Spleen index} = \text{spleen weight (mg)}/\text{body weight (g)} \times 100\%$$

$$\text{Thymus index} = \text{thymus weight (mg)}/\text{body weight (g)} \times 100\%$$

Thymus and spleen lymphocyte proliferation: on the 14th day of treatment, the mice were put to death. The thymus and spleen were removed under sterile conditions and placed in a Petri dish containing PBS buffer with the fat and connective tissue removed. RPMI1640 culture medium was added and the inner core of the thymus and spleen were gently crushed using a sterile syringe. The single cells went through a nylon mesh (200 mesh) into the centrifuge tube, the cells were rinsed with RPMI1640 culture medium, then the separation medium containing lymphocytes was transfered to 15ml centrifuge tubes, centrifuged at 1000rpm for 5 min and the supernatant removed. After mixture of red cell lysate (200ul) and a constant volume of RPMI1640 culture medium, they were centrifuged and its supernatant must be removed. Then add (1ml) PBS buffer and centrifuged them twice with removing its supernatant. Then (1ml) 10%RPMI1640 was added, it was divided into two groups of (500ul) and centrifuged also remove its supernatant. 10% RPMI1640 culture medium (2ml) was added to one group and ConA solution (2ml) was added to the other group. Then they were incubated at 37^{0}C and 5% CO_2 for 24h in an incubation box in 96 well plates. 4 hours before the end of the incubation period they were taken out and 20ul MTT[14](5mg/

ml) was added per well and incubation was continued for 4 hours. After this 4 hours the supernatant was sucked out and discarded, 150ul of DMSO was added per well. They were mixed at low speed vibration in an oscillator, and then the optical density value was determined in a Microplate Reader at a wavelength of 490nm. The value of optical density was calculated according to the following formula:

The optical density (ABS) difference = optical density for wells with ConA – optical density for wells without ConA.

The determination of serum IL – 2 content in the mice: after treatment for each group of mice having finished, blood was taken via eye removal technique and the serum was obtained after centrifugation. Analysis was carried out according to the ELISA kit user guide. Standard diluent (1ml) was added to the ELISA kit standard product and left to stand for 10~15 minutes during which time suction was applied several times to ensure it was completely dissolved. In this way a concentration gradient of (1000pg/ul, 500pg/ul, 250pg/ul, 125pg/ul, 62.5pg/ul, 31.25pg/ul,0) was achieved. A 96 well plate preparation was used with 100ul/ well of the standard product in the replicate wells; serum samples (50ul/ well) and sample analysis buffer (50ul/ well). After incubation at room temperature for 120min, the plates were washed 5 times and on the final wash were placed on thick absorbent paper and patted dry.

Biotinylated antibody working fluid (100ul/well) was added, and then after incubation at room temperature for 1hr, the plates were washed 5 times and on the final wash were placed on thick absorbent paper and patted dry. HRP enzyme conjugate working fluid (100ul/well) was added, and then after incubation at room temperature protected from light for 20min, the plates were washed 5 times and on the final wash were placed on thick absorbent paper and patted dry. Chromogenic reagent TMB (100ul/well) was added, and then incubated at room temperature protected from light for 20min. Finally the suspension liquid was added (50ul/ well) and the OD450 values measured immediately after mixing, to detect the content of IL-2 in serum of each group.

Results and discussion

The effect of different doses of Kezimuk granules on the body weight was measured by electronic balance. This table below shows the difference between the initial weight and the final weight of the mice. See Table 1.

Table 1 – The effect of Kezimuk granules at different doses on the bodyweight of immunocompromised mice

Group	Dose, g/kg	Animal No.	Body Weight Gain
Normal group	NS	10	16.74 ± 5.08
Model group	NS	10	11.11 ± 5.07▲*
Yupingfeng granules	2.25	10	10.65 ± 4.64▲
Kezimuk granule low dose group	0.68	10	15.06 ± 3.61
Kezimuk granule med dose group	1.35	10	18.45 ± 3.57▲
Kezimuk granule high dose group	2.70	10	15.07 ± 4.24▲

Note: compared with the model group, ▲P<0.05; compared with the normal group, *P<0.05.

Analysis: Table 1 shows that the Kezimuk granule high and medium dose groups along with the Yupingfeng granule group were significantly different (▲P<0.05) from the model group. The Kezimuk granule low dose group along with the model group were significantly different (* P<0.05) from the normal group. This shows that Kezimuk granules have a significant effect on the weight gain of mice.

The effect of different doses of Kezimuk granules on the spleen index of immunocompromised mice was determined by weighing its spleens under aseptic conditions and by calculating the spleen index according to the formula. See Table 2.

Table 2 – The effect of Kezimuk granules at different doses on the spleen/body weight ratio of immunocompromised mice.

Group	Dose, g/kg	Animal No.	Spleen Index, mg/g
Normal group	NS	10	0.009 ± 0.001
Model group	NS	10	0.005 ± 0.003*▲
Yupingfeng granules	2.25	10	0.007± 0.002
low dose group	0.68	10	0.013 ± 0.002
med dose group	1.35	10	0.012 ± 0.004▲
high dose group	2.70	10	0.013 ± 0.003▲

Note: compared with the model group, ▲P<0.05; compared with the normal group, *P<0.05.

Analysis: Table 2 shows that all three Kezimuk granule dosage groups along with the Yupingfeng granule group were significantly different (▲P<0.05) from the model group. The model group was significantly different (*P<0.05) from the normal group. The spleen index of the model group was lower than that of the normal group. Compared with model group the Yupingfeng group and Kezimuk groups indicated improved spleen index for immunocompromised mice.

The effect of different doses of Kezimuk granules on the spleen lymphocyte (B) proliferation ability of immunocompromised mice. The preparation of the spleen lymphocyte suspension was conducted according to the steps in 2.2.2, and the absorbance of each well was determined in a microplate reader at a wavelength of 490n. See Table 3.

Table 3 – The effect of different doses of Kezimuk granules on the spleen lymphocyte proliferation ability in immunocompromised mice

Group	Dose, g/kg	Animal No.	Spleen lymphocyte proliferation ability,A
Normal group	NS	10	2.45 ± 0.12
Model group	NS	10	1.44 ± 0.12▲*
Yupingfeng granules	2.25	10	2.20 ± 0.41
low dose group	0.68	10	2.16 ± 0.24▲
med dose group	1.35	10	2.20 ± 0.15
high dose group	2.70	10	2.30 ± 0.40▲

Note: compared with the model group, ▲P<0.05; compared with the normal group, *P<0.05.

Analysis: Table 3 shows that all three Kezimuk dosage groups and the Yupingfeng granule group were significantly different (▲P<0.05) from the model group. The model group was significantly different (*P<0.05) from the normal group. The spleen lymphocyte proliferation in the model group was significantly lower than in the normal group (P<0.05). Compared with the model group, Kezimuk granules showed a significant improvement in the spleen lymphocyte proliferation ability in immunosuppressed mice, and Yupingfeng particles also showed a significant improvement in spleen lymphocyte proliferation ability (P<0.05).

The effect of different doses of Kezimuk granules on the thymus cell (T) proliferation ability of immunocompromised mice. The preparation of the thymus cell suspension was conducted according to the steps in 2.2.2, and the absorbance of each well was determined in a microplate reader at a wavelength of 490n. See Table 4.

Table 4 – The effect of different doses of Kezimuk granules on the thymus cell proliferation ability in immunocompromised mice

Group	Dose, g/kg	Animal No.	Thymus cell proliferation ability, A
Normal group	NS	10	2.47 ± 0.26
Model group	NS	10	1.32 ± 0.16▲*
Yupingfeng granules	2.25	10	2.72 ± 0.27
low dose group	0.68	10	2.22 ± 0.41
med dose group	1.35	10	2.01 ± 0.40▲
high dose group	2.70	10	2.30 ± 0.31▲

Note: compared with the model group, ▲P<0.05; compared with the normal group, *P<0.05.

Analysis: Table 4 shows that all three Kezimuk dosage groups and the Yupingfeng granule group were significantly different (▲P<0.05) from the model group. The Model group and the Yupingfeng granule group were significantly different (*P<0.05) from the normal group. The thymus cell proliferation in the model group was significantly lower than in the normal group (P<0.05). Compared with the model group, Kezimuk granules showed a significant improvement in the thymus cell proliferation ability of immunosuppressed mice, and Yupingfeng particles also showed a significant improvement in thymus cell proliferation ability (P <0.05).

The effect of different doses of Kezimuk granules on the Serum IL-2 in immunocompromised mice. Serum was acquired by taking blood from the eye, which was then stood for 30 minutes and centrifuged according to the steps in 2.2.3, and the optical density was determined in a microplate reader at a wavelength of 450nm.

Table 5 – The effect of different doses of Kezimuk granules on serum IL – 2 in immunocompromised mice

Group	Dose, g/kg	Animal No.	Serum IL-2
Normal group	NS	10	4.17 ± 0.69
Model group	NS	10	2.94 ± 0.21▲*
Yupingfeng granules	2.25	10	3.81 ± 0.27
low dose group	0.68	10	3.98 ± 0.18
med dose group	1.35	10	4.00 ± 0.13▲
high dose group	2.70	10	3.72 ± 0.13▲

Note: compared with the model group, ▲P<0.05; compared with the normal group, *P<0.05.

Analysis: Table 5 showed that all three Kezimuk dosage groups and the Yupingfeng granule group were significantly different (▲P<0.05) from the model group. The model group was significantly different (*P<0.05) from the normal group. Compared with the model group, Kezimuk granules showed a significant improvement in the serum IL – 2 proliferation ability in immunosuppressed mice, and Yupingfeng particles also showed a significant improvement in serum IL – 2 proliferation ability (P <0.05).

Discussion

This study showed that Chinese medicine plays an important role in regulating body's immune system by rectifying immune suppression, and because of its unique asset, people accept and use it generally.[15] The functions of disease prevention and health care of traditional Chinese Medicine is mostly achieved through immunoregulation. This special effect of compound traditional Chinese medicine in

improving immunity system is mainly reflected in promoting the development of immune organs, increasing the weight of immune organs, enhancing immune response, and increasing the concentration of immune cells, thereby improving the ability of resistance to disease. T lymphocytes are important components of cellular immunity as they, along with other cells and cytokines, interact together to achieve immune functions such as recognition, activation and killing. Yupingfeng powder is a well known part in the "Fuzheng Gubiao" treatment principle in traditional Chinese medicine, originating from the ancient medical medical works; "Shi Yi De Xiao Fang". It's comprised of 3 medicinal herbs: milkvetch root (Astragalus membranaceus (Fisch.) Bunge.), Atractylodes macrocephala Koidz., and divaricate saposhniovia root, (Saposhnikovia divaricata (Trucz.) Schischk.). Of these milkvetch root and Atractylodes macrocephala combine to fill important roles in traditional Chinese medicine treatment principles.

Cyclophosphamide is commonly used alkylating agent for the treatment of malignant tumors. With broad-spectrum anti-cancer effect, and being one of the drugs which has been commonly used for chemotherapy, surgery, radiotherapy and adjuvant chemotherapy, and its main side effect is immunosuppression. Because it takes longer to the working period of healthy foods and needs larger doses which would lead to excessive animal immune suppression beyond the regulating ability of the healthy foods concealing its effectiveness. In this research our team took low various doses to establish a more stable immune suppression model. Compared to the control group, the pyrophosphate group showed significant differences indicating the success of the model.

The thymus and the spleen are vital central as well as peripheral immune organs. The spleen generates lymphocytes, and also the place where settles and the proliferates lymphocytes, which makes spleen a the basic organ for producing specific immunity. The thymus can produce a large number of thymus cells, and is a backup and supplement of T lymphocytes. Increasing weight of immune organs to a certain extent can reflect the body's immune ability. The T lymphocytes, which carry out a cellular immune function, develop and mature in the thymus, reside in the spleen, and proliferate during an immune response. The spleen and thymus index level is a response to the T lymphocyte proliferation ability and the cell numbers[16-18] and is one of the parameters that directly reflects the immune function level. The thymus and spleen are, respectively, the central as well as the peripheral immune organs; the thymus makes T cells mature, and the spleen is where the lymphocytes mature and B cells reside. The thymus coefficient and spleen coefficient can directly reflect the function of the immune (19; 20).

In this study Kezimuk granules significantly improved the control model`s spleen index. The concanavalin A (ConA) induced lymphocyte transformation in the experiment carried out by the MTT method and the results of the mice serum IL-2 values show that the increase in immune organs of normal mice is related to the spleen and thymus weight. This is due to response of immune organs, in turn to enhance immune function in the mice expressed in the enhanced lymphocyte transformation and elevated values of serum IL-2 value. The immune regulation network is very complex, and it still needs further research in order to explain the mechanism and function of Kezimuk granules in improving the body's immunity, and to provide reference for more scientific use of this distinctive resource of Xinjiang traditional medicine.

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Synthesis of New, Potentially Biologically Active Derivatives of 1,3,4-Oxadiazole-2-yl carbothioates

Abstract. It is well known that the derivatives of nitrogenous heterocycles have a wide range of biological activity (antituberculosis, antimicrobial, antitumor, antimalarial, anti-inflammatory, etc.), and oxadiazole derivatives also play same important role. In the current work, in order to synthesize the initial synthons we were carried out the esterification of benzoic (**1**) and isonicotinic (**2**) acids were obtained the corresponding ethers (**3**, **4**). Further, interaction with hydrazine hydrate, the corresponding acid hydrazides were synthesized (**5**, **6**). As a result of the cyclization reaction with carbon disulfide were obtained substituted 1,3,4-oxadiazole-2-thiols (**7**, **8**). Finally compounds **7**, **8** undergo the alkylation reaction lead to yield two new compounds *S*-5-phenyl-1,3,4-oxadiazol-2-yl *O*-propyl carbonothioate (**9**) and *S*-5- (pyridin-4-yl) -1,3,4-oxadiazol-2-yl carbonothioate (**10**). The structures of synthesized compounds were confirmed by NMR spectra. The physico-chemical characteristic data of synthesized compounds were in full agreement with corresponded compounds in reference. The synthesized new compounds have an interest for further investigation of biological activities.
Key words: biologicaly active derivatives, oxadiazole derivatives, esterification of benzoic and isonicotinic acids.

Introduction

Chemistry of nitrogen heterocycles belongs to a broad section of organic chemistry. Nitrogen heterocycles – pyridine, oxadiazole, triazole, thiadiazole and their derivatives – are highly reactive and biologically active at relatively low toxicity, and can functionally transformed to obtain analogs of bioactive compounds.

At present, the search for chemical compounds possessing biological activity, conduct according to certain scientific principles and quantitative approaches that allow predicting the structure of compounds, essentially lead and their purposeful synthesis. There are several trends in the development of scientific research in this field, one of them is the introduction of pharmacophore fragments into the molecule, increase the activity of the preparation, for example: the addition of phenyl, pyridine and dichlorophenyl radicals increases the water solubility, and the introduction of the alkyl group increases the lipophilicity, the complex and ether groups increase the pharmacological activity and the sulfur atom reduces toxicity.

In organic chemistry, the synthesis of cyclic oxygen-containing compounds attach great importance in view of their valuable biological properties. Oxadiazoles and their derivatives are an integral part of many natural biologically active compounds, which have a wide spectrum of biological activity [1-5]. Continuing research on the synthesis of target bioactive compounds including nitrogen heterocycles, we carried out synthesis of two new oxadiazole derivatives.

Experimental

***General experimental procedures*:** Thin layer chromatography of the synthesized compounds were carried out on Silufol UV-254 plates, were exhibited by a UV lamp. The melting points of the new synthesized compounds were tested on a Buchi Melting Point B-540, Germany.

The NMR spectra of the new synthesized compounds were recorded on a Bruker WM 250 spectrometer and a Bruker DRX 500 spectrometer operating at 250.500 MHz in a DMSO solution.

***The general procedure for the synthesis of ethyl esters of cyclic carboxylic acids (3, 4)*:** A mixture

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(0.1 mol) of cyclic carboxylic acid, 54.6 g of absolute ethanol, 7.36 g of conc. H_2SO_4 with stirring is heated for 3 hours at a temperature of 80-850C. At the end of the reaction, ethanol is distilled off. Extracted with diethyl ether, the final product accelerated in an oil pump vacuum.

Ethyl benzoate (3) was obtained in 12 g (80%) yield, and isonicotinic acid ethyl ester (4) was obtained with 13.7 g (90.7%). All physico-chemical parameters were corresponded to the reference data.

***The general procedure for the synthesis of hydrazides (5, 6)*:** A mixture of (0.15 mol) cyclic carboxylic acid ethyl ester and (0.2 mol) hydrazine hydrate heated in a water bath for 6 hours. The resulting crystals washed with cold ethanol.

Benzoic acid hydrazide (**5**) was obtained in a yield of 17.2 g (84.3%).The isonicotinic acid hydrazide (**6**) was obtained in 10.9 g (86.5%) yield. All the physical and chemical parameters are also consistent with the reference data.

***The general procedure for the synthesis of substituted 1,3,4-oxadiazol-2-thiol (7, 8)*:** To hydrazide (0.01 mol) in ethanol at 0^oC is added 0.76 g of carbon disulphide and 0.46 g of potassium hydroxide, the reaction mixture is heated until the evolution of the carbon disulfide ceased (about 12 hours).

Excess ethanol is distilled off, and the residue is dissolved with water and acidified with HCl (10%) till pH = 5. The precipitate is filtered off, dried and recrystallized from C_2H_5OH. 5-Phenyl-1,3,4-oxadiazole-2-thiol (**7**) was obtained in 1.53 g (85.4%) yield, and 5-(pyridin-4-yl) -1,3,4-oxadiazol-2-thiol (**8**) was obtained in a yield of 1.47 g (82.1%).

While identifying the synthesized compounds, the physico-chemical data fully corresponded to the reference data. A fairly high yield of compounds allowed further modification of the compounds.

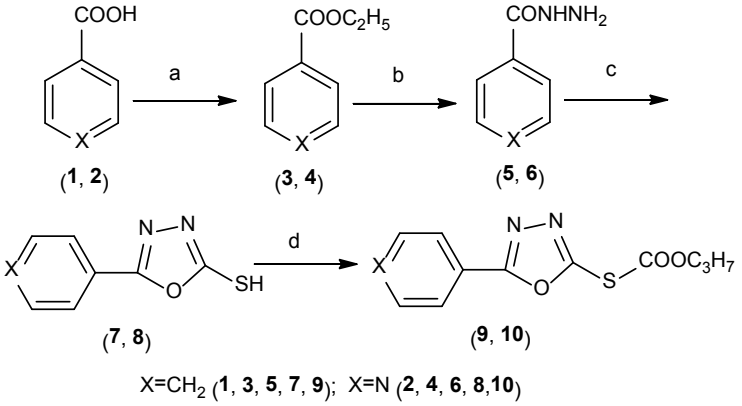
***The general procedure for the synthesis of substituted 1,3,4-oxadiazol-2-yl O-propyl carbonothioates (9, 10)*:** To a solution (2 mmol) of substituted 1,3,4-oxadiazole-2-thiol and (2 mmol) NaOH in 50 ml of acetonitrile is added dropwise (2 mmol) of propyl carbonobromidate. The mixture is heated, the reaction is monitored by TLC. After completion of the reaction, the acetonitrile is distilled off under vacuum. The resulting crystals are recrystallized from a suitable solvent.

S-5-phenyl-1,3,4-oxadiazol-2-yl *O*-propyl carbonothioate (**9**) was obtained with T = 87-88 ° C (recrystallized from ethyl acetate), and yielded 0.47 g (90%).

O-propyl S-5- (pyridin-4-yl) -1, 3, 4-oxadiazol-2-yl carbonothioate (**10**) was obtained with $T_{mel.}$ = 287-288 ° C (recrystallized from acetonitrile), and yielded 0.46 g (87%).

Results and discussion

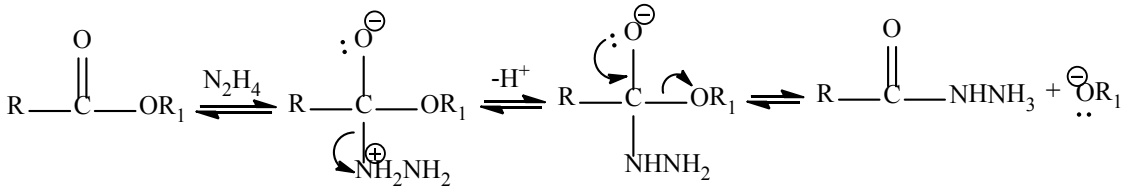
Based on biological activities of oxadiazole derivatives as well as continuation of purposeful synthesis, the structure of the synthesized compounds have been introduced various pharmacophore groups [6, 7]. The synthesized two new compounds *S*-5-phenyl-1,3,4-oxadiazol-2-yl *O*-propyl carbonothioate (**9**) and *O*-propyl *S*-5- (pyridin-4-yl) -1,3,4-oxadiazol-2-yl carbonothioate (**10**) have not been reported before. Intermediate compounds in the synthesis of the final compounds are esters of cyclic carboxylic acids (**3**, **4**) their hydrazide (**5**, **6**) and substituted oxadiazoline (**7**, **8**), and the reaction procedure please see the Scheme 1.



Scheme 1 – Synthesis of compounds (**9**) and (**10**). Reagents and solvents:
(a) C_2H_5OH, H_2SO_4, 80-85^{0}C, 3 hours; (b) N_2H_4*H_2O, 90^{0}C, 6 hours; (c) CS_2, KOH, C_2H_5OH, 90^{0}C, 12 hours; (d) $BrCOOC_3H_7$, NaOH, MeCN, 80-85^{0}C, 8-12 hours.

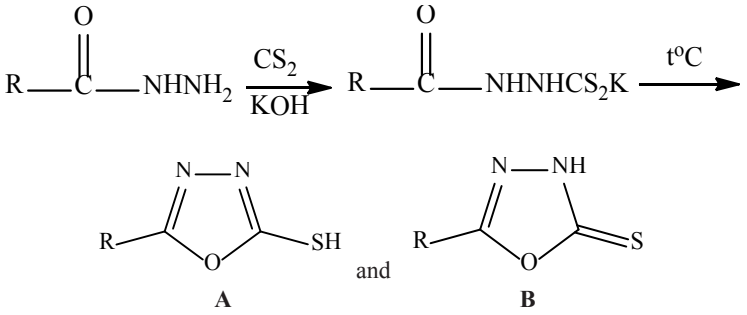
The starting synthons in the synthesis of potentially biologically active compounds are the ethyl esters of benzoic acid (**3**) and isonicotinic acid (**4**). These esters were synthesized by esterification of benzoic and isonicotinic acid by the interaction with ethanol in sulfuric acid.

From the research there is evidence that the reactions, obtaining the hydrazides are extremely numerous. One of the main ways of obtaining hydrazides is the interaction of esters of carboxylic acids with hydrazine hydrate. Hydrazide is nucleophilic agents with good reactivity, which can easily interact with electrophile.



Thus, in the reaction of ethyl ester of benzoic acid (**3**) and ethyl ester of isonicotinic acid (**4**) with aqueous hydrazine hydrate synthesized benzohydrazide (**5**) and hydrazide of isonicotinic acid (**6**), respectively. Physicochemical constants of the synthesized compounds were in complete agreement with the reference data, and the reaction with high yield of compounds can be used as a synthon in further syntheses study.

At next step, we carried out the reaction of cyclization of hydrazides (**5**, **6**) with carbon disulfide in presence of alkali. The reaction of cyclization were obtained 5-phenyl-1,3,4-oxadiazole-2-thiol (**7**) and 5- (pyridine-4-yl) – 1,3,4-oxadiazole-2-thiol (**8**). The intermediate compound can be potassium salt of 3-aroyldithiocarbazate. Below shown a diagram of cyclization:

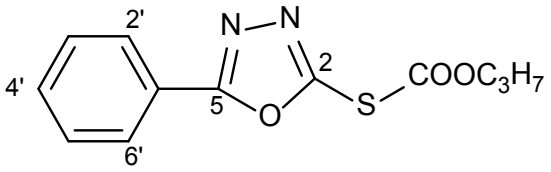


As a result of the cyclization process, it is possible to form two isomeric products, namely 1,3,4-oxadiazole-2-thiol (A) and 2-mercapto-1,3,4-oxadiazole (B) derivatives or in a thionic form, respectively. In the alkaline medium, there is predominantly a thiol form (A), and in acidic and neutral media, the thionic form (B). The physico-chemical parameters of the thions obtained and fully confirmed the structure of the compounds, which allow us to make further modification to them.

With the aim of producing compounds containing a large number of pharmacophore groups, we synthesized *S*-5-phenyl-1,3,4-oxadiazol-2-yl *O*-propyl carbonothioate (**9**) and *O*-propyl *S*-5- (pyridin-4-yl)-1,3,4-oxadiazol-2-yl carbonothioate (**10**) by reacting substituted 1,3,4-oxadiazol-2-thiols (**7**, **8**) with propyl carbonobromidate in acetonitrile under heating during the 8-12 hour. The course of the reaction controlled by thin layer chromatography.

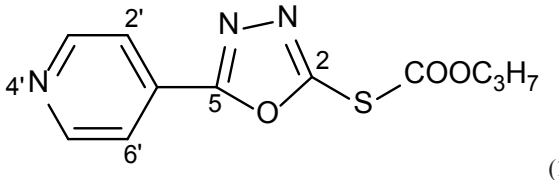
In this study, we were synthesized two new compounds, the derivatives of 1,3,4-oxadiazol-2-yl O-propyl carbonothioate (**9**, **10**) that previously not been described in the literature. Structures of *S*-5-phenyl-1,3,4-oxadiazol-2-yl *O*-propyl carbonothioate (**9**) and *O*-propyl *S*-5- (pyridin-4-yl) -1,3,4-oxadiazol-2-yl carbonothioate (**10**) were exactly proved by the basis of the spectra of ^{1}H and ^{13}C NMR. The NMR data are shown in Table 1.

Table 1 – ^{1}H and ^{13}C NMR data of new substituted oxadiazole carbonothioates (**9**, **10**)



(**9**)

^{1}H NMR (400 MHz, DMSO)			^{13}C NMR (101 MHz, DMSO)	
Protons	δ_H	*J*, Hz	Carbon	δ_C
2H, H = 2`, 6`	7.93(d)	7.8	1C, -COO	174.2
1H, H = 4`	7.70(t)	7.3	2C, oxad	157.6, 147.4
2H, H = 3`, 5`	7.62(dd)	13.6, 6.8	6C, Ph	133.1, 129.5, 129.4,
2H, H = OCH_2	4.38 (t)	6.5		126.7, 126.1, 121.6
2H, H = CH_2	1.81- 1.71(m)		1C, -O-CH_2	70.3
3H, H = CH_3	1.00(t)	7.4	Alk. CH_2, CH_3	21.4, 10.1



(**10**)

^{1}H NMR (400 MHz, DMSO)			^{13}C NMR (101 MHz, DMSO)	
Protons	δ_H	*J*, Hz	Carbon	δ_C
2H, H = 2`, 6`	8.81(d)	5.8	1C, -COO	178.17
2H, H = 3`, 5`	7.81(d)	6.0	2C, oxad	159.23
2H, H = OCH_2	4.20-4.15 (t)	14.2, 6.8	5C, Ph	151.26
2H, H = CH_2	1.80 (dt)	7.4		130.21,
3H, H = CH_3	1.01			120.43, 120.05
			1C, -O-CH_2	75.41
			Alk. CH_2, CH_3	22.82, 13.01

Conclusion

In summary, we have synthesized 10 compounds by using different reactions methods and in which two compounds (*S*-5-phenyl-1,3,4-oxadiazol-2-yl *O*-propyl carbonothioate (**9**) and *S*-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl carbonothioate (**10**) are novel compounds. Meanwhile, they are potentially biologically active compounds. The research work is continued and biological activities of two new compounds (**9**, **10**) are being tested.

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The obtaining of composite materials based on carboxymethylcellulose and polyvinyl alcohol

Abstract. In this work, hydrogels based on carboxymethylcellulose (CMC) and polyvinyl alcohol (PVA) by the method of radiation crosslinking were obtained. The presence of absorption bands of hydroxyl, simple ether and carbonyl groups in the hydrogel compositions by FTIR spectroscopy method was revealed. Also, the presence of both components CMC and PVA in hydrogel composits was proved. The kinetics of swelling of composite materials based on CMC/PVA with different volumetric ratios was studied. The influence of absorbed dose on the degree of swelling and gel contents was determined. Also, in this work the morphology of hydrogels by scanning electron microscopy (SEM) were studied. Also, optimum conditions the possibility of use as a drug carrier have been defined. Technological scheme of the obtained composite materials based on CMC/PVA was development.

Key words: carboxymethylcellulose, polyvinyl alcohol, hydrogel, radiation crosslinking, composite material.

Introduction

Currently, polymeric materials based on the natural polysaccharides and synthetic polymers find more and more broad application in the most various areas of the industry, such as pharmaceutical industry, medicine, agriculture. In this regard, the increasing value is gained by development of new composite materials on the basis of natural polysaccharides and synthetic polymers.

Poly(vinyl alcohol) (PVA) has a wide commercial application due to its unique chemical and physical properties. It is a nontoxic, highly crystalline, and water-soluble polymer and has good film forming and high hydrophilic properties. Poly(vinyl alcohol) could be considered as a good host material due to good thermo-stability, chemical resistance and film forming ability. Due to their simple structure and unique properties , polymers based on PVA have found applications in different industries including textile, paper, adhesives, food, biomedical and pharmaceutical in particular [1].

Carboxymethyl cellulose (CMC) is a biopolymer derived from cellulose. It is a copolymer of β -D-glucose and β -D-glucopyranose 2-O-(carboxymethyl)-monosodium salt which are connected via β -1,4-glycosidic bonds. CMC is widely used in pharmaceutical, cosmetic, and food applications. It is a nontoxic, biocompatible polymer, which resistant to the surrounding environment [2].

Materials and methods

Materials

In our work, we used carboxymethylcellulose (CMC) powder, purchased from «Altey» (Russia) used without purification. PVA (number average molecular weight 70 $\times 10^4$ g/mol) was purchased from Merck KGaA (Germany) used without purification.

Preparation of solutions

PVA solutions with concentration of 10 wt% were prepared by dissolving dry polymer in the deionized water at 80°C with constant stirring for 5 h. Then these solutions were stirred at room temperature overnight. Aqueous CMC solutions (10wt%) was prepared by dissolving CMC powder in the deionized water and mixed at 60°C and stirred to obtain clear solution for 6 h.

Preparation of Radiation Crosslinked Carboxyethylcellulose/Polyvinyl alcohol Hydrogels

To obtain hydrogels 10 wt % of polyvinyl alcohol and 10 wt % carboxymethylcellulose solutions mixed in the following proportions $\varphi_{CMC:}\varphi_{PVA}$ =70:30, 50:50, 30:70. Then 20 ml of each prepared CMC/PVA solutions was poured into a Petri dish. Afterward, all samples were irradiated by Electron beam (E-beam) irradiation at the semi-industrial electron accelerator ELV-4 (1,3 MeV) at the Institute of Nuclear Physics (INP), (Almaty region, Alatau village).The absorption dose of radiation were 40, 80 and 120 kGy.

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Swelling Degree and Gel fraction of Carboxyethylcellulose/Polyvinyl alcohol Hydrogels

The swelling degree of CMC/PVA hydrogels was determined by gravimetric methods. Samples of composite materials (0,1 g.) was placed in 10 ml distilled water, then every 15 min was measured by the steady mass of the sample on an analytical weight. The degree of swelling was calculated using the following formula:

$$\alpha = \frac{m_t - m_0}{m_0}$$

where, m_0 – initial mass of sample, g; m_t is the mass of the swollen sample in t time, g.

The gel fraction of hydrogels was measured by extraction in hot distilled water at 100°C for 48 h and dried at 70°C for 48 h until they reached constant weight. The gel content was defined by equation below:

$$Gel = \frac{W_d}{W_0} x100$$

where, W_d is the dried gel weight after extraction, and W_0 is the initial weight of polymer.

The structure of the obtained composite materials was carried out by IR spectrometric analysis**.** FTIR spectroscopy of pure PVA, pure CMC and CMC/PVA hydrogels were recorded using Carry 660FTIR spectrophotometer (Agilent, USA). The samples were finaly with potassium bromide (KBr) to make pellets under hydraulic pressure of 600kg/cm^2 and scanned between 4000 and 400cm^{-1}.

The morphology of the freeze dried samples of the CMC/PVA hydrogels were examined with Tabletop Scanning Electron Microscope (Hitachi, Japan) at acceleration voltage of 10 kV. All samples were freeze dried (Thermo LL300) and the dried hydrogels were gold sputter coated before analyzed by SEM.

*The study of the kinetics of drug release from hydrogel.*was estimated by UV spectrophotometry instrument «Shimadzu UV / VIS-2401 PC» (Japan). To study the kinetics of drug sorption by hydrogel 1g of sample were placed to 1 hour in Lidocaine solution. Every hour sorption of drug by measuring of changes of the optical density of lidocaine solution at a wavelength λ=266 nm determined. Measurement was performed on quartz cell with a 1 mm thick. The calibration curve was constructed for Lidocaine f (c) = D – dependence of the optical density of the solutions D concentration c (%).

To study the release of lidocaine hydrochloride from hydrogel adsorbed hydrogels for 24 hours were placed in a isotonic solution and measured the optical density every hour. Concentration of absorbed and desorbed lidocaine by a calibration curve was determined.

The amount of drug released after time t was determined by the calibration line according to the (3) formula:

$$W = \frac{C}{C_0} \cdot 100\% \qquad (3)$$

where, W is the amount of released drug, C is an Lidocaine concentration in the surrounding solution at time t, C_0 is a concentration of drug in the feed solution.

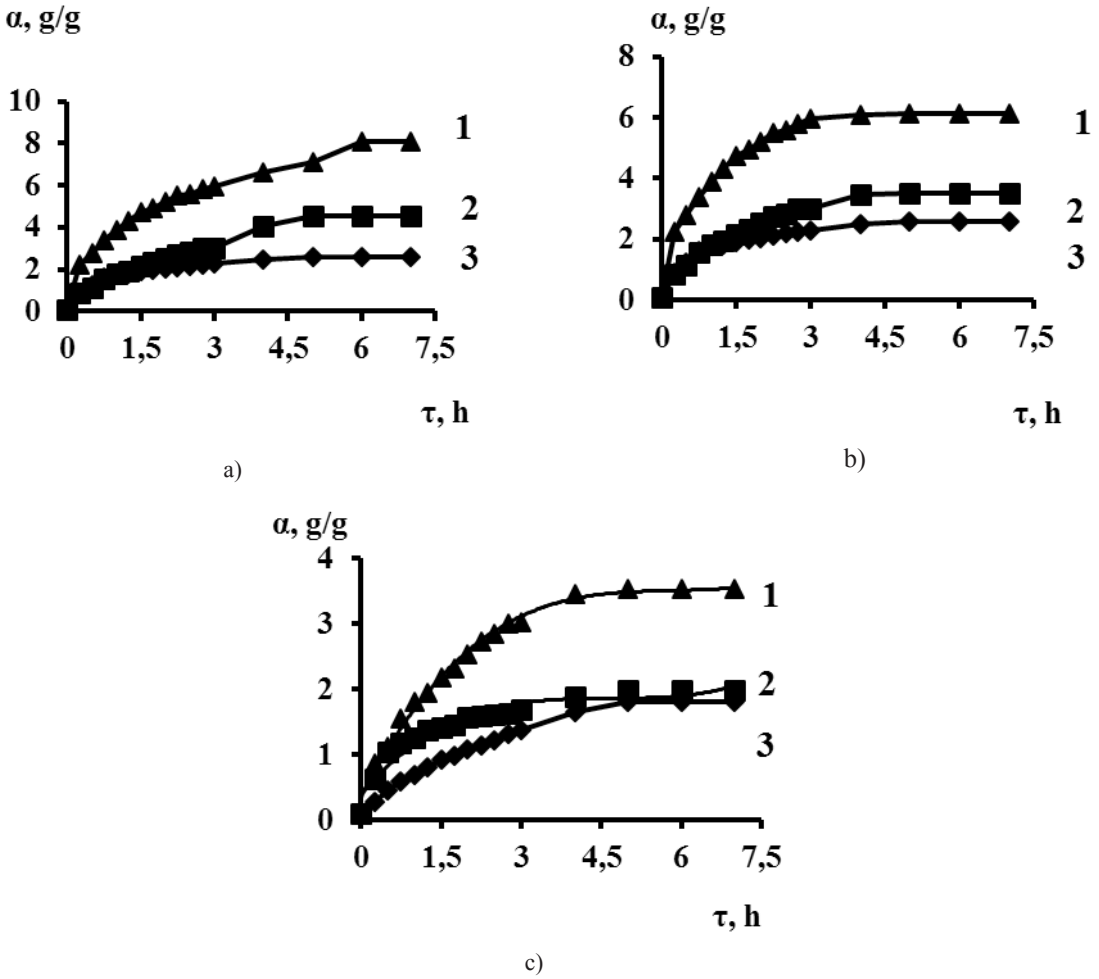
Results and Discussion

Swelling Behaviour of Polyvinyl alcohol/Carboxymethylcellulose Hydrogels

To learn the ability to swell and to prove that the carboxymethylcellulose and polyvinyl alcohol were crosslinked, at different doses of irradiation (40 kGy, 80 kGy, 120 kGy) kinetics of swelling of hydrogels based on PVA-CMC was investigated. It can be seen that at lower doses of irradiation the degree of swelling increases (Figure 1). The maximum equilibrium degree of swelling of $\varphi_{CMC:}\varphi_{PVA}$= 30:70 hydrogels at 40 kGy was 8,1 g/g and at 120 kGy was 2,6 g/g. Additionally, as can be seen from figure 1 swelling capabilities of hydrogel compositions are increased by increasing the PVA content. This proves that the hydrophilicity of PVA in comparison with cellulose ether is higher and can be explained by the fact, that increasing content of PVA in the hydrogel will result to enhancing the crosslinking by intramolecular hydrogen bonding between the PVA chains in the hydrogel network structure, which resulting to increase swelling.

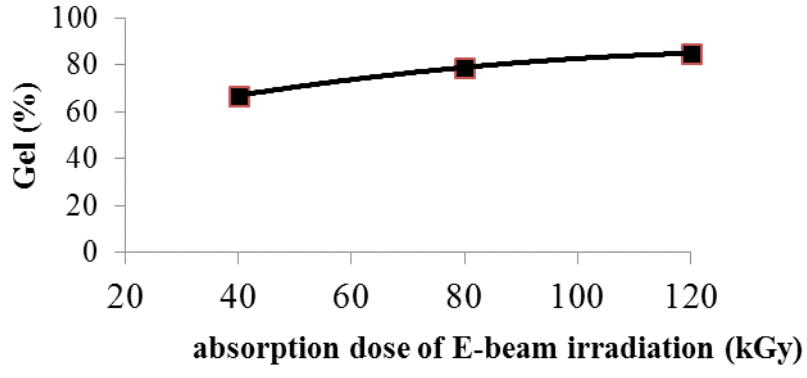
Gel Fraction of Crosslinked PVA/CMC hydrogels

Gel fraction is defined as the amount of insoluble polymer in any solvent. Gel contents of PVA/CMC hydrogels at various absorption dose of radiation (kGy) is presented in figure 2. In this figure, it was clearly seen that the gel contents of the crosslinked CMC/PVA hydrogels with volume ratio $\varphi_{CMC:}\varphi_{PVA}$= 30:70 increased with an increase of absorption dose of radiation.



φ_{CMC}:φ_{PVA}= 30:70 (a); φ_{CMC}:φ_{PVA} = 50:50(b); φ_{CMC}:φ_{PVA} = 70:30 (c)
absorption dose of E-beam irradiation = 40 (1); 80 (2); 120 (3) kGy.

Figure 1 – The kinetics of swelling of hydrogels based on CMC/PVA by radiation crosslinking



φ_{CMC}:φ_{PVA}= 30:70

Figure 2 – Gel fraction of crosslinked CMC/PVA hydrogels
at various absorption dose of E-beam irradiation (kGy) .

The high gel fraction of hydrogels; caused by enhanced irradiation dose as a result of higher degree of crosslinking onto polymer network which cause higher gel content. The gel fraction of PVA/CMC samples at radiation dose of 40 kGy was 78% and at radiation dose of 120 kGy was 87%.

Fourier Transform Infrared Spectroscopy Studies

The IR spectra of pure PVA, CMC and crosslinked CMC/PVA hydrogel are shown in Figure 3. The PVA spectra shows characteristic broad band at 3284cm^{-1} corresponding to the O–H stretching vibration of the hydroxyl group of the PVA. The sharp band at 1715cm^{-1} corresponds to the C= O stretching of the acetate group of PVA.

The backbone aliphatic C–H stretching vibrations give sharp bands at 2942cm^{-1}. The IR spectrum of CMC shows the absorption bands due to C–H stretching (2894cm^{-1}) and that due to C–O stretching of the ether group of the carboxymethylation of cellulose or the ether linkage [1,4-β-d-glucoside] of cellulose at 1051cm^{-1}. The absorption bands in the spectrum *correspond* with the *data given* in the [3] literature.

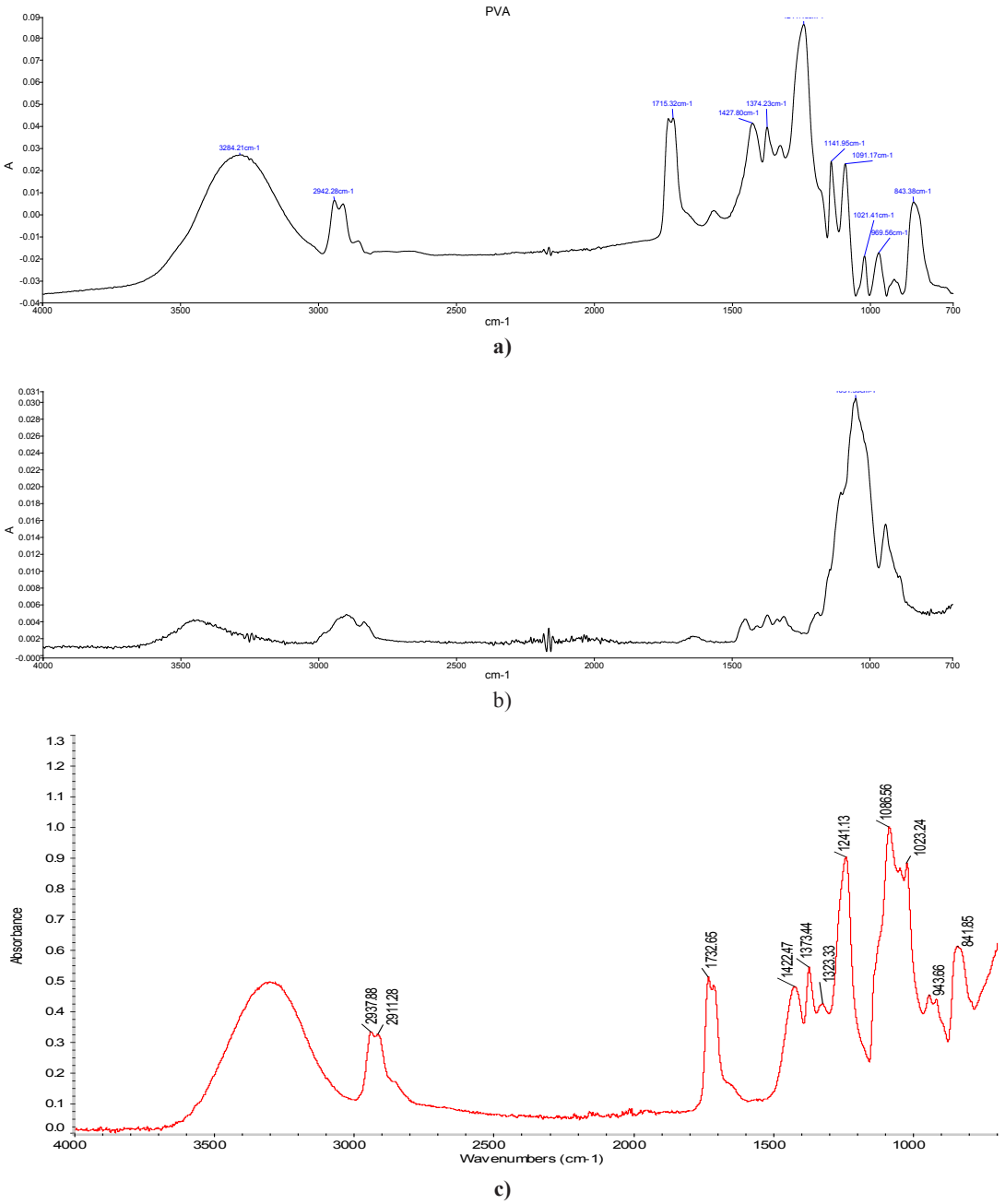


Figure 3 – FTIR spectra of PVA (a); CMC (b) and composite material based on CMC/PVA (c)

SEM studies

In general, the scanning electron microscopy (SEM) shows microstructure morphologies of hydrogels. The pore morphology of hydrogels can be related to their water uptake capacity. The hydrogels with denser and tighter structure will have a smaller pore size. The surface morphology of CMC/PVA hydrogels was detected by SEM. In the Figure 4 presents photomicrographs of hydrogels based on CMC/PVA. A homogenous structure with smooth surface can be observed in the PVA/CMC hydrogel. This picture confirmed that the CMC/PVA hydrogel has a porous structure

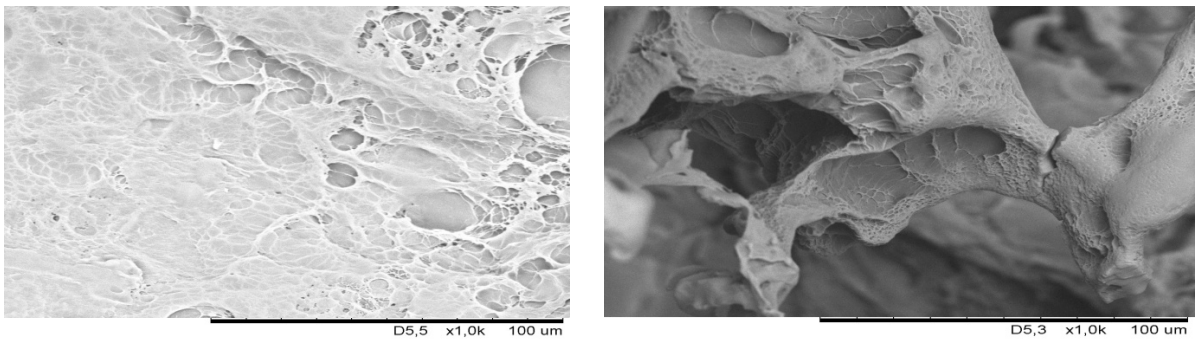


Figure 4 – SEM micrographs of hydrogel based on CMC/PVA

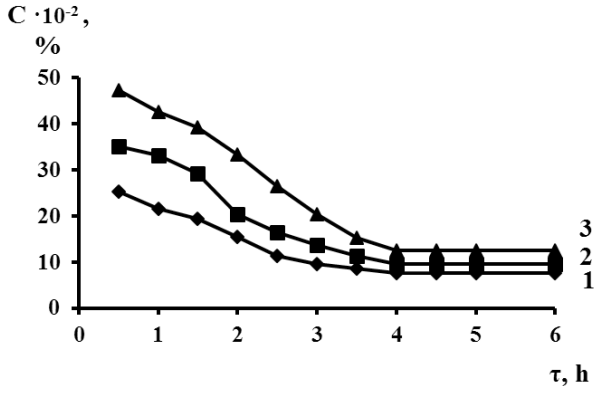
Studies of interactions of hydrogels based on CMC-PVA with medical substance

Nowadays, at designing and modification of new and well-known drugs, the improvement of their therapeutic effects is an important and actual problem. The immobilization of the drug into a biocompatible and biodegradable polymer matrix is the one way of solving this problem, because it has advantages over usual dosage forms, including reformed efficacy, diminished toxicity, cost effective therapeutic treatment. Hydrogels can transport drugs in a controlled manner due to the open porous structure [4].

Polyvinyl alcohol and cellulose ethers are widely used in controlled release studies because of its hydrophilic and nontoxicity.

In this study the possibility of using the hydrogel based on carboxymethylcellulose and polyvinyl alcohol as a drug carrier was considered. For this purpose, the kinetics of absorption and release of drug from hydrogel were studied. As a drug the local anesthetic – lidocaine hydrochloride was used. The absorption of lidocaine to the hydrogel was investigated by UV spectroscopy at a wavelength of 266 nm. It can be seen that the rate of the drug absorption by hydrogel increases with enhanced PVA content. As a result, the hydrogel with volume ratio $\varphi_{CMC:}\varphi_{PVA}$= 30:70 showed a high absorption ability of the drug substance. It can be concluded that the composite material with volume ratio $\varphi_{CMC:}\varphi_{PVA}$= 30:70 shows good absorption ability.

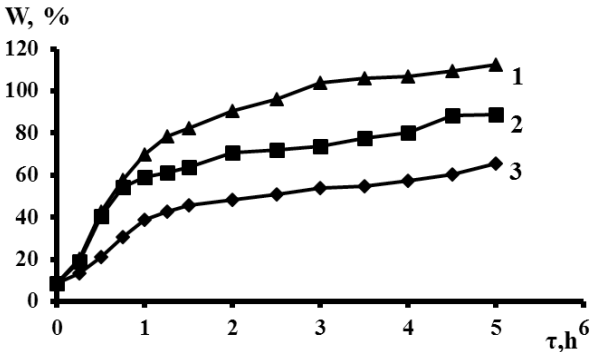
Besides this, to provide the prolonged effect the release of drug from the hydrogel should be slow. Thus, the kinetics of drug release from the hydrogel to an isotonic solution (0.9% NaCl) was studied to determine the release time. It has been noticed that the drug release during the process is intensive due to the influence of low-molecular ions. As a result, it was found that from the hydrogel with the volume ratio $\varphi_{CMC:}\varphi_{PVA}$= 70:30 the drug release goes quickly, while at volume ratio $\varphi_{CMC:}\varphi_{PVA}$= 30:70 release of the drug is slow.



$\varphi_{CMC:}\varphi_{PVA}$= 30:70 (1); $\varphi_{CMC:}\varphi_{PVA}$= 50:50(2); $\varphi_{CMC:}\varphi_{PVA}$= 70:30 (3)

Figure 5 – The kinetics of sorption of Lidocaine by hydrogel based on CMC/PVA at 40 kGy

As a conclusion, basing on the obtained results of adsorption-desorption process, the composite materials at volume ratio $\varphi_{CMC:}\varphi_{PVA}$= 70:30 are perspective in use as the matrix of lidocaine carrier.



$\varphi_{CMC:}\varphi_{PVA}$= 30:70(1); $\varphi_{CMC:}\varphi_{PVA}$= 50:50(2); $\varphi_{CMC:}\varphi_{PVA}$= 70:30 (3).

Figure 6 – The kinetics of desorption of Lidocaine from hydrogel based on CMC/PVA at 40 kGy in isotonic solution

Technological part

To obtaining the hydrogel based on carboxymethylcellulose and polyvinyl alcohol technological schemes at figure 7 can be used.

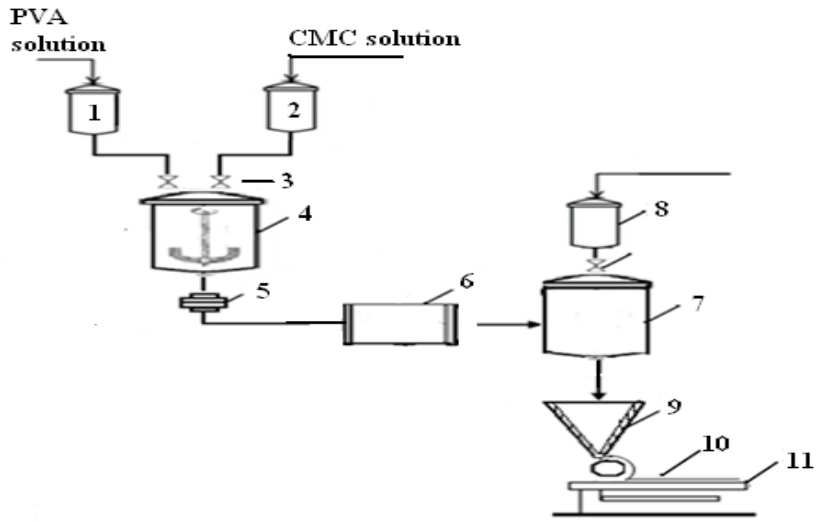
Process of hydrogel dressing receiving consists from the following stages:

1. Preparation of initial polymer solutions (polyvinyl alcohol and carboxymethylcellulose);
2. Mixing
3. Radiation exposure
4. Impregnation of hydrogel by medicinal substance
5. Molding of hydrogel
6. Reception of finished products

For production of hydrogel 10 wt.% of carboxymethylcellulose and polyvinyl alcohol solution are used. Substances are used without preliminary cleaning.

According to this scheme solutions of PVA and solutions of CMC through dosing devices mixing in the reactor (4) until homogenous blend. Further, blends of CMC/PVA are supplied to the ELV-4 electron accelerator for radiation at absorption dose of 40 kGy for obtaining hydrogels. After the completion of radiation hydrogel is supplied to the mixer (7) for mixing it with the drug flowing from storage bunker of drug (8). Then the hydrogel with the drug substance is delivered for cutting and packaging.

For this method the smaller dose of radiation is used that promotes more economic production of hydrogel bandages.



1 – bunker of PVA solution; 2 – bunker of CMC solution; 3 – dosing devices; 4 – reactor for the mixing of initial components;5 – filter; 6 – electron accelerator ELV-4; 7 – mixer for introducing of the drug into the hydrogel; 8 – storage tank of drug; 9 – фильера; 10 – cutting; 11 – packaging.

Figure 7 – Principal technological scheme of obtaining hydrogel films based on carboxymethylcellulose (CMC) and polyvinyl alcohol (PVA) containing drug

Conclusion

CMC/PVA solutions were irradiated by E-beam irradiation in the following proportions $\varphi_{CMC:}\varphi_{PVA}$ =70:30, 50:50, 30:70 at various doses of 40, 80 and 120 kGy. Presence of absorption bands of hydroxyl, simple ether and carbonyl groups in the hydrogel compositions by IR spectroscopy method was revealed. The kinetics of swelling of composite materials based on CMC/PVA with different volumetric ratios was studied. The gel fraction increases with increasing irradiation dose, while the swelling of CMC/PVA hydrogel increase with decreasing irradiation doses and increase with increasing CMC content. The maximum degree of swelling hydrogel was 8,1 g/g at the sample $\varphi_{CMC:}\varphi_{PVA}$= 30:70 hydrogels. The morphology of the hydrogels has a porous structure by the method of scanning electron microscopy was determined. Basing on the obtained results of adsorption-desorption process, the composite materials at volume ratio $\varphi_{CMC:}\varphi_{PVA}$= 70:30 are perspective in use as the matrix of lidocaine carrier. Principal technological scheme of obtaining of composite material based on CMC/PVA was developed.

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Development of hydrogel ointments based on carbopol with α-lipoic acid

Abstract: This article presents the results of hydrogel ointments development based on carbopol polymer with α-lipoic acid for their application as transdermal therapeutic delivery systems for antibacterial treatment of diabetic foot syndrome. Complex formation of bioavailable α-lipoic acid with hydroxypropyl-β-cyclodextrin was studied using differential scanning calorimetry and scanning electron microscopy. Optimal rheological characteristics of obtained ointments have been estimated. Antimicrobial activity in relation to *Staphylococcus aureus and Escherichia coli* have been observed for ointments containing α-lipoic acid. Transdermal drug delivery properties of obtained hydrogel ointments were investigated using cellulose membrane as a model system and a pig skin.
Key words: hydrogel ointment, alpha-lipoic acid, carbopol, hydroxypropyl-β-cyclodextrin, diabetic foot syndrome.

Introduction

Modern pharmaceutical technology as one of the basic tasks considers the creation of dosage forms based on biopolymers with drugs, with high therapeutic activity, providing controlled release of drugs and their specific delivery to the site of the pathological process. One of the most serious diseases at present is the diabetic foot syndrome [1]. This disease connects with leg problems due to elevated blood sugar levels. During this disease the sensitivity of nerves becomes broken – neuropathy, and the blood flow in the vessels of the legs worsens [2]. As a result, the risk of wounds injury and infection increases. Most of antibacterial ointments presented in pharmacies contain antibiotics that are not desirable to be used often, or are produced on fat bases (animal and vegetable fats, petrolatum, etc.), which create a film that does not allow air to enter the lesion focus. Also, to select the best antibiotic, it is desirable to do bacteriological research of tissues. Often patients with the diabetic foot syndrome have impaired renal function. During diabetic foot syndrome after destruction of the protective layer of the skin, deep tissues undergo bacterial colonization. In such a situation, a standard therapeutic dose of antibiotics can harm a patient's health [3]. Nowadays a method of complications treatment of type 1, 2 diabetes with α-lipoic acid (ALA) which is one of the most powerful antioxidants with antimicrobial properties, is known. Therefore, studies on the development of hydrogel ointments with α-lipoic acid are an actual trend.

ALA decomposes gradually at room temperature and easily polymerizes at temperatures higher than its melting point and insoluble in water [4-5]. Thus, the stabilization of ALA is of great interest for industrial applications. In order to stabilize ALA by complexation, the scientists used cyclodextrins (CD) [6-7]. These cyclic oligosaccharides consist of six (α-CD), seven (β-CD) or eight (γ-CD) α-1,4-linked glucopyranose units, with a hydrophilic hydroxyl group on their outer surface and a hydrophobic cavity at the center [8]. CDs are able to form complexes with various ionic and lipophilic substances, taking the whole molecule or part of it into its cavity. Such formation of a molecular complex affects many physicochemical properties of guest molecules, such as water solubility, stability or bioavailability [9-10]. Thus, a complex of inclusion of ALA and CD, which dissolves in water was obtained in this work. What makes it possible to use it for the preparation of hydrogel ointments based on carbopol.

Carbopol is one of the most common thickeners for aqueous phases. Carbopol polymers are acrylic acid crosslinked with polyalkenyl ethers or divinyl glycol. Carbomer molecules are closely packed spiral molecules. After dispersing in water, the molecules begin to swell and partially "unwind". The most common way for complete "unwinding" of molecules is to convert the acid carbopol to a salt. These polymers

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are anionic polymers that need to be neutralized for gelling. Organic amines, such as triethylamine or alkali, can be used to neutralize these polymers in liquids [11].

The advantages of using carbopol gels as a therapeutic transdermal agents : good rheological properties, good alternative to oily ointment preparations, anionic hydrogels with good buffering capacity, which helps to maintain the desired pH [12], high viscosity already at low concentrations, a wide range of concentrations and characteristic behavior of the flow, compatibility with many active ingredients, bioadhesive properties, good thermal stability, excellent organoleptic characteristics, good tolerability [13].

Materials and methods

Carbopol (Kadpol 980) was purchased from «Shree Chemicals» (India) and was used as received.α-lipoic acid was purchased from «AXXO GmbH» (Hamburg) and was used without purification. Hydroxypropyl-β-cyclodextrin supplied by «AXXO GmbH» (Hamburg) and was used without purification. Polyethylene glycol (M_n=400) produced by «ChemMed» company (Moscow), was used without purification. Glicerin produced by «ChemMed» company (Moscow) and was used as received. Sodium hydroxide was purchased from «Skat» company (Kazakhstan). Sodium Phosphate and Potassium Phosphate were purchased from «Skat» company (Kazakhstan) and were used without purification. Cellulose membrane (M_n=12-14000 Dalton) was purchased from the «Medicell International Ltd» (London).

Hydrogel ointment preparation.

Hydroxypropyl-β-cyclodextrin was used to dissolve α-lipoic acid. The sample of ALA and HP-β-CD in a molar ratio of 1: 1 was rubbed for 1.5 hours in a mortar. Thus, a water-soluble inclusion complex was obtained. Then, the necessary concentration of the ALA solution in water was prepared at a temperature of 38^0 C in a water bath for 1 hour, the resulting solution was filtered off. To this solution was added a sample of carbopol, made alkaline and thoroughly mixed. At the end, polyethylene glycol and glycerin were added as a humectant and texture improvement of the ointment.

Methods of analysis

Differential Scanning Calorimetry.

The analysis was carried outon the NETZSCH STA 449F3 Jupiter (NETZSCH-Gerätebau GmbH, Germany) in a stream of nitrogen at a heating rate of 10 ^{0} C / min. 1.1-1.9 mg of the test substance (pure α-lipoic acid, hydroxypyr-β-cyclodextrin, ALK-GP-β-CD complex) were placed in the aluminum crucible preliminarily suspended with the lid and pressed. The prepared crucible and the test crucible were placed in a measuring chamber. The thermograms were received in the temperature range 30-100 0C.

Differential-thermal analysis.

The analysis was carried out on the NETZSCH STA 449F3 Jupiter (NETZSCH-Gerätebau GmbH, Germany) in a stream of nitrogen at a heating rate of 100C / min. It were used crucibles from Al2O3. The thermograms of pure gel base on carbopol, α-lipoic acid substance, carbol -based gel with α-lipoic acid were obtained in the temperature range 20-300 ^{0} C.

Scanning electron microscopy.

The analysis was carried out at DGP "National nanotechnology laboratory of open type" KazNU. Al-Farabi on a Quanta 3D 200i Dual system, FEI scanning electron microscope at a voltage of 15 kV with an increase of 2000, 5000 per sample.

Analysis of the rheological parameters of the hydrogel ointments.

The rheological profile of the samples was studied on Anton Paar Modular Compact Rheometer 102 (Anton Paar GmbH, Austria). The measuring geometry of the plate / plate was used. The measurements were carried out at a temperature of 25 ° C. The device made it possible to measure the tangential shear stress at a shear rate of 1.5 to 1312 s-1. When constructing the rheogram, the shear rate was increased from 1 to 450 1 / s (the upper curve), and then decreases from 450 to 1 1 / s (the lower curve).

Drug Diffusion and Skin Penetration Study.

In vitro study of the release of ALA and penetration through the membrane and porcine skin was carried out using the Franz Diffusion Cell. The amount of released ALA was recorded with the help of the UV spectrophotometer Analytyc Jena Specord 200. The spectra of ALA solutions were recorded in the wavelength interval 190-400 nm. The wavelength for α-lipoic acid was 333 nm, which coincided with the reference data according to the state pharmacopeia of the Republic of Kazakhstan. The cellulose membrane and the skin of the porcine ears were held for one hour in a phosphate buffer solution pH 6.86, because the pH of the body cells lies in this range. Then it was placed between the donor and acceptor parts of the Franz Diffusion Cell.

Antimicrobial studies of α-lipoic acid.

The studies were performed on nutrient agar using Gram-positive bacteria – Staphylococcus aureus IMV 3316 and Gram-negative bacteria – Escherichia coli IMV 877 as test microorganisms.

Results and their discussion

The development of medicinal forms, such as hydrogel ointments, which increase bioavailability, the effectiveness of the action of medicinal preparations and cause prolonged action, has acquired in recent decades an undeniable scientific and practical value. In this work, hydrogel ointments based on carbopol with different content of α-lipoic acid were obtained. ALA is a powerful antioxidant, it is used in the treatment of diabetes mellitus, it also has antimicrobial properties, which makes it possible to use it for the production of hydrogel ointments with antimicrobial activity in antibiotic therapy of diabetic foot syndrome. Since ALA does not dissolve in water, the first step was to find the optimal method for increasing the bioavailability of ALA, compatible with the composition of the ointment.

Complex formation of α-lipoic acid with hydroxypropyl-β-cyclodextrin

Complex formation of ALA and HP-β-CD have been assessed by DSC method. The results of the DSC show the disappearance of the peak of active pharmaceutical substances as a result of complex formation. The differential scanning calorimetry method allows analyzing substances in different aggregate states, and provides comprehensive information on the phase transitions of various compounds, which can be used for conformation of complexation because DSC method determines the energy changes in the test substance. DSC thermograms were obtained in a temperature range of 30-100 ° C on a combined TGA-DSC calorimeter NETZSCH STA 449F3 Jupiter in a stream of nitrogen at a heating rate of 10 ° C / min.

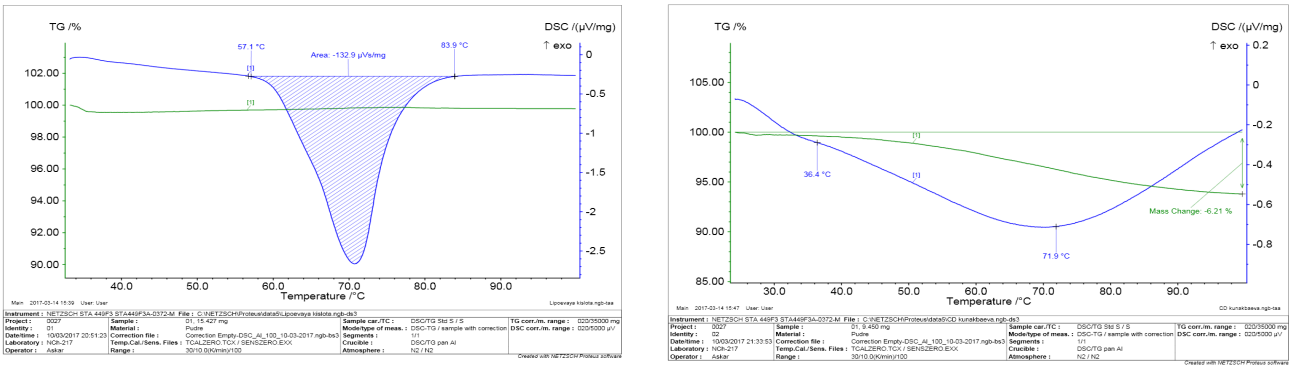


Figure 1 – TGA-DSC pure substance of α-lipoic acid (left) and pure substance of hydroxypropyl-β-cyclodextrin (right)

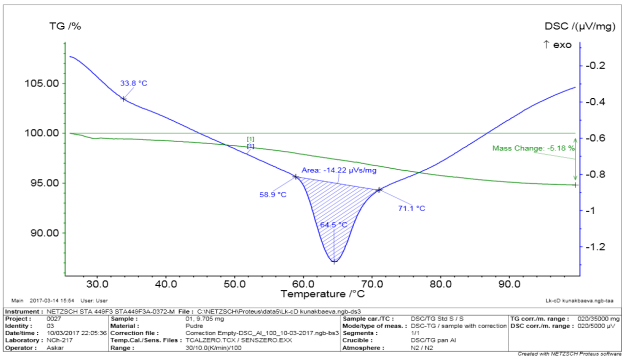


Figure 2 – TGA-DSC analysis of the inclusion complex of GP-β-CD and ALA obtained by grinding

On the ALA thermogram (figure 1, left), a narrow endothermic peak is observed at a temperature of 70.90 ° C, which characterizes the melting point of ALA. On the thermogram HP-β-CD (figure 1, right), a wide endothermic peak is observed with a maximum at 71.90C, which indicates intensive evaporation of the sorbed water inevitably present in hygroscopic substances. On the thermogram of a mixture of ALA and GP-β-CD (figure 2), the melting temperature of ALA is shifted to a region of lower temperatures (on 6.4 ° C), compared to the initial ALA, which is probably due to the interaction of ALA with the surface of GP- CD. The thermogram contains a small wide endothermic peak characterizing the evaporation of water sorbed by GP-β-CD from the environment. The interaction of ALA with the outer surface of GP-β-CD can be explained by the fact that GP-βCD is capable of forming a «cellular» (grid-like) structure due to hydrogen bonds between -OH groups. When the melting temperature of ALA (70.9 ° C) is reached, the ALA melt penetrates into the cells of this grid and, thus, stabilizes. Therefore, we can conclude that the presence of the small endotermic peak (figure 2)

is probably due to the fact that it is possible, when the inclusion complex is formed, that unused crystals of GP-β-CD and ALA are preserved.

To confirm the formation of inclusion complex, the scanning electron microscopy method is also have been used. The investigation is based on point scanning of the surface of the investigated sample by a focused electron beam. Photomicrographs obtained with scanning electron microscopy using the Quanta 3D 200i Dual system, FEI at a voltage of 15 kV with an increase of 2000, 5000 for each sample, show that the shape and particles of the resulting inclusion complex of α-lipoic acid and hydroxypropyl-β-cyclodextrin are significantly different. Images of the substance GP-β-CD (Figure 3) show that the particles are large enough, porous, spherical. The ALA particles (Fig. 4) have rather smooth plate-shaped planar surfaces. A micrograph of the obtained ALK-GP-β-CD complex (Fig. 5) shows the change in the shape of the particles as a result of the mechanical treatment of the mixture of starting materials. The particles have an amorphous structure, which confirms the formation of the inclusion complex, i.e. the ALA molecule enteres the GC-β-CD cavity. But unreacted crystals of GP-β-CD are also preserved.

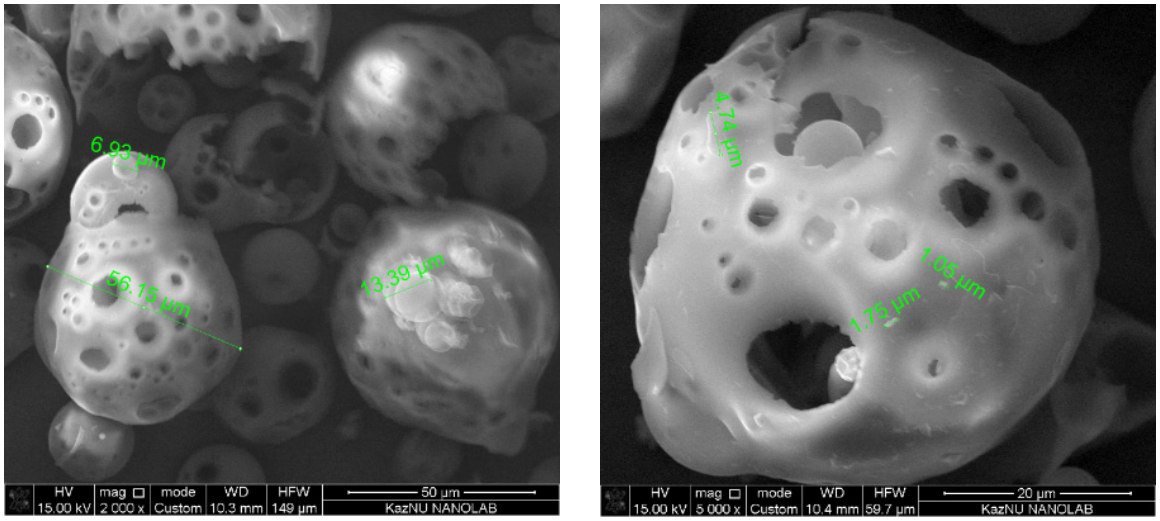


Figure 3 – Scanning electron microscopy of the substance hydroxypropyl-β-cyclodextrin at magnification 2000 (left) and 5000 (right)

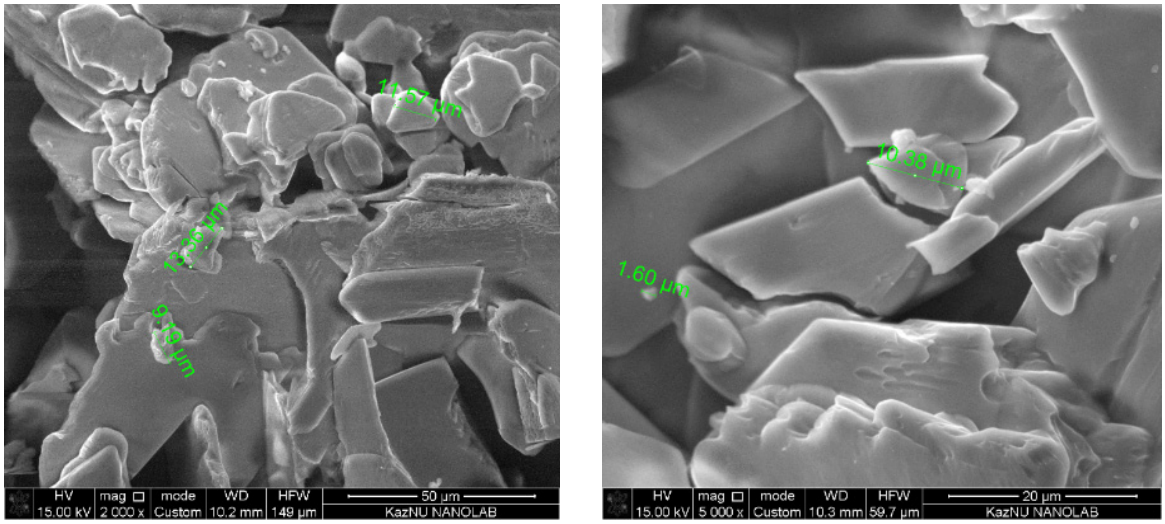


Figure 4 – Raster electron microscopy of α-lipoic acid substance at magnification 2000 (left) and 5000 (right)

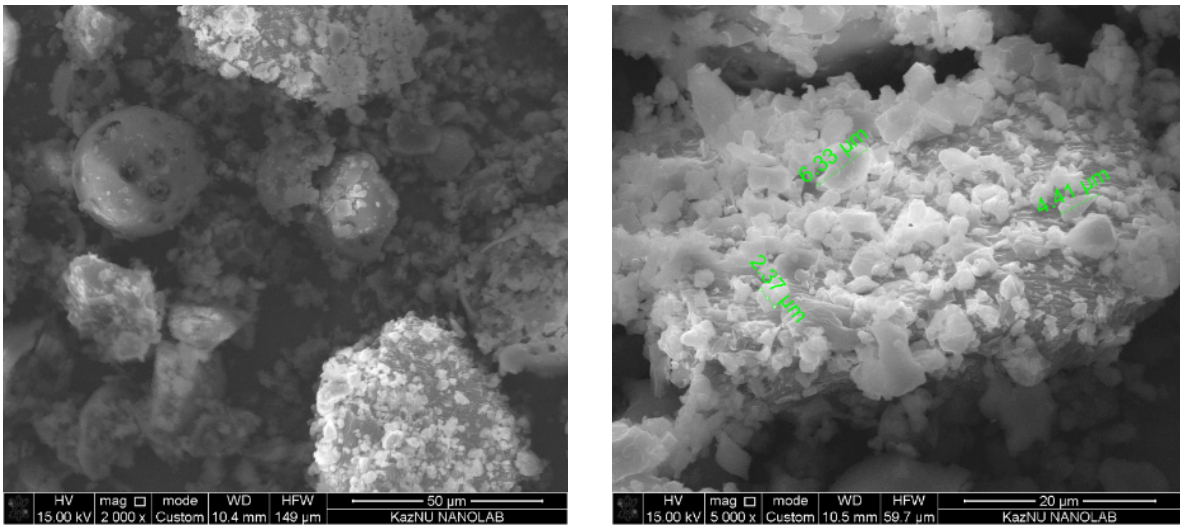


Figure 5 – Raster electron microscopy of the ALA-HP-β-CD complex obtained by grinding at magnification 2000 (left) and 5000 (right)

The solubility test of ALA and HP-β-CD complexes was carried out in accordance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan I, vol. 1, 2.9.3, using a device with a blade. The dissolution medium is water, the volume of the dissolution medium is 500 ml, the temperature of the dissolution medium is 37 ± 0.5 ° C, the blade rotation speed is 100 rpm, the dissolution time is 5, 10, 15, 20, 30, 45 min. The determination was made by the method of absorption spectrophotometry in the ultraviolet and visible regions (SP RK, т. 1, 2.2.25) at a wavelength of 333 nm. The wavelength is determined by scanning a standard solution of α-lipoic acid in the wavelength range from 300 to 365 nm. According to the results obtained, the inclusion complex of ALA and HP-β-CD in a molar ratio of 1: 1 has a maximum solubility of 89% compared to other ratios (Figure 6).

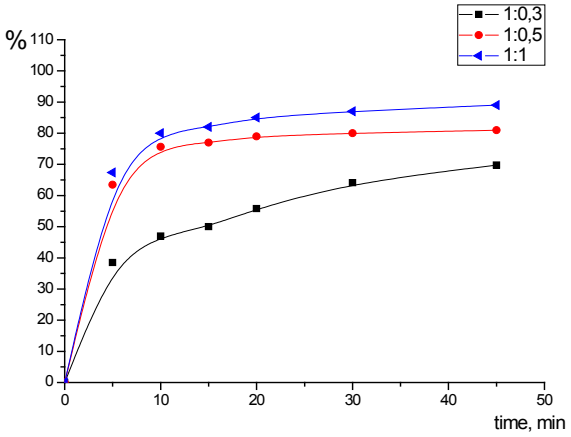


Figure 6 – Dissolution of the inclusion complex of HP-β-CD and ALA in distilled water (in the different molar ratio)

Investigation of the mechanism of interaction of lipoic acid and structurant (polymer) was carried out by differential-thermal analysis. To study the mechanism of interaction of drug substance a in lipoic acid gels based on carbopol, a linear heating of a substance of lipoic acid, pure gel bases and gels from lipoic acid in the temperature range from 20 ° C to 300 ° C in Al2O3 crucibles was carried out. The curves of differential thermal analysis (DTA) of the substance of lipoic acid, hydrogel base and gel of lipoic acid without auxiliary substances are presented at the figure. 7.

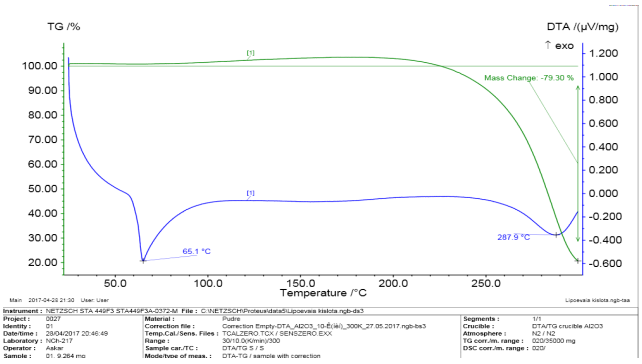


Figure 7 – Differential-thermal analysis curves (DTA) of the substance of lipoic acid

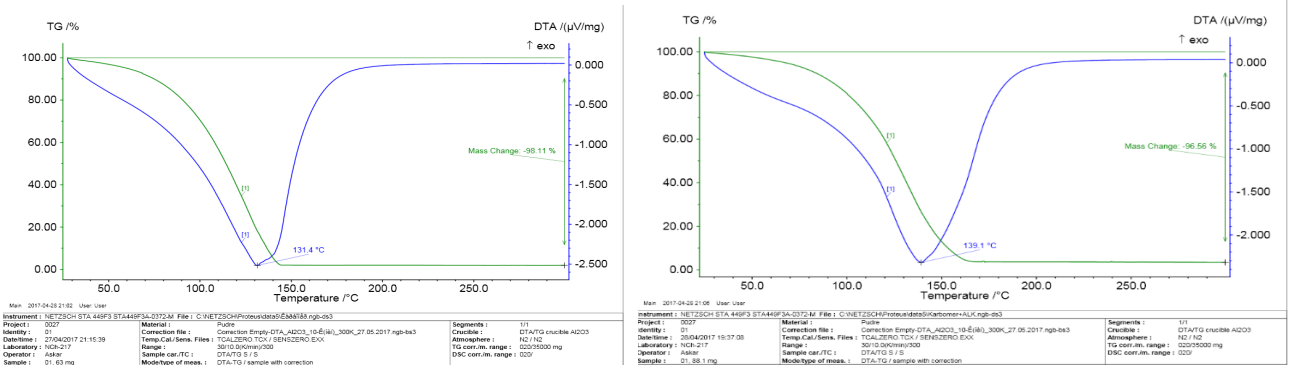


Figure 8 – Differential-thermal analysis curves (DTA) of the carbopol-based gel (left) and the carbopol gel with ALA (right)

According to DTA data presented at the figure 7, an endothermic peak is observed at 65.1 ° C, explained by the melting point of the substance, which agrees with the melting temperature of lipoic acid of 61 ° C. The endothermic peak at a temperature of 287.9 ° C indicates the decomposition of ALA with continuous heating, which is confirmed by the TGA curve dropping from 250 ° C. The 2nd curve of DTA shown at the figure 8 (on the left) shows that when the gel base obtained from carbopol is heated, an endothermic effect with has the maximum at a temperature of 131.40C, which may be related to the melting of the polymer and the desorption of water. The TGA curve of a pure gel base shows that, even at 142 ° C, only 2% of the total mass remains from the gel. The data of curve 3 of DTA (figure 8, right) characterizing the processes of heating the gel with a drug substance, have similarities with the processes of heating the gel base. Thus, endothermic processes are observed in the interval t = 20-190 ° C, which have only one maxima at T= 139.10 ° C, which can also be associated with processes of desorption of water, melting of the polymer. But the endothermic processes in the gel containing ALA shift by 7.7 ° C to the right than in the pure gel base, which is probably due to the action of ALA on the carbopol gel. Also, according to the TGA curve, 4% of the total gel weight remains from the gel at 162 ° C, and only 2% of the total gel weight remains at the pure base at 143 ° C, which is related to the content of ALA in the obtained ointment.

It is known that most of the ointments and gels under the influence of mechanical forces behave like elastic bodies that have reversible deformation. The process of applying the gel ointment and the efforts expended on the distribution of the gel on the skin are similar to the process occurring during the gel shift in the rheometer and the shear stress, which characterizes the material's resistance to shear deformations.

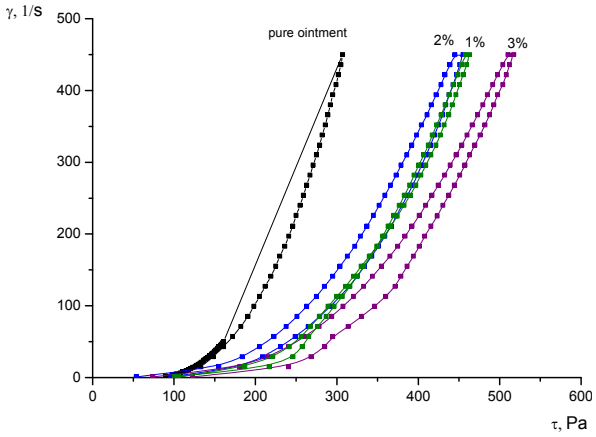


Figure 9 – Rheogram "Hysteresis loop" of hydrogel ointments based on carbopol with different concentrations of α-lipoic acid

To study the thixotropic properties of the studied samples of hydrogel ointments, the curves, obtained as a result of the deformation of these samples in the coordinates «shear rate-shear stress», were constructed. By the thixotropy is meant the property of a disperse system to change its structure under the influence of mechanical influences and to restore the former structure after the cessation of this influence. All hydrophilic ointments have pronounced thixotropic properties, which are expressed in the fact that with mixing the structure breaks down and the consistency changes at a constant temperature, it becomes almost liquid. If you remove the mechanical force, the strength of the structure is restored, but almost never reaches the original level. The obtained curves of the samples of hydrogel ointments (the so-called «flow rheograms») have a nonlinear character and are described by two lines in the «ascending and descending», forming the so-called «hysteresis loop».

The «upward» curve characterizes the destruction of the system and differs from the «descending» curve in that it characterizes the restoration of the system that preserves the residual deformation after a strong weakening of the structure under the influence of the previously applied stress. The presence of upward and downward curves (figure 9), forming hysteresis loops, indicates that the samples under study have thixotropic properties, which means that they are characterized by good spreadability and good extrusion ability from tubes.

The obtained results and the comparative analysis showed that the rheological characteristics shear stress are highest in 3% lipoic acid gel, and exceed both 1 and 2% lipoic acid gel. It was found, that the introduction of lipoic acid into the gel base in an amount of 1% of the weight of the gel, increased the value of the effective viscosity and the processes of structure formation in the system. All investigated samples of gels belong to thixotropic systems and. The rheological characteristics of the obtained ointments lie in the rheological optimum for hydrophilic ointments, which is characterized by a yield strength of 45-160 Pa.

The figure 10 shows the effect of temperature on the dynamic viscosity of hydrogel ointments obtained with ALA. It is seen, that with an increase in temperature from +3 to +260C, the noticeable decrease in viscosity is observed, which become constant at a temperature of 15-26 ° C. Hydrogel ointments on carbopol in the temperature range -4 – + 280^{0}C showed the stability of dynamic viscosity.

Investigation of α-lipoic acid release from hydrogel ointments and their transdermal penetration

The main barrier to penetration into the body of various substances is the skin, which is a multilayered complex system. Transdermal delivery of ALA is carried out by penetrating them through the upper layer of the skin – the dermis. Under the dermis there is a hypoderm consisting of adipose tissue, in which large blood vessels are located, as well as the bases of the hair follicles and sweat glands. On the basis of information on the structure of the skin, it can be concluded that transdermal delivery will be effective provided that the ALA, overcoming the epidermis, will enter into sufficient quantity in the dermis where the capillaries of the blood vessels are located.

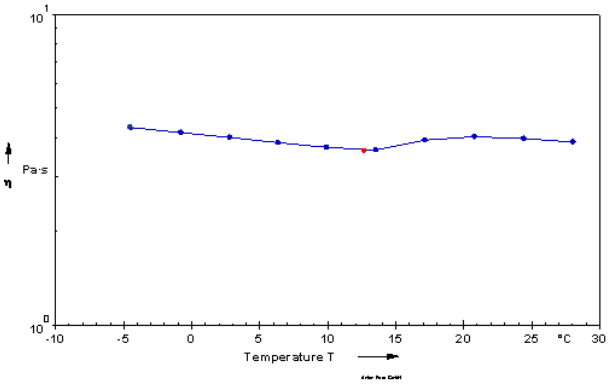


Figure 10 – Effect of temperature on the viscosity of a hydrogel ointment based on carbopol with 1% α-lipoic acid

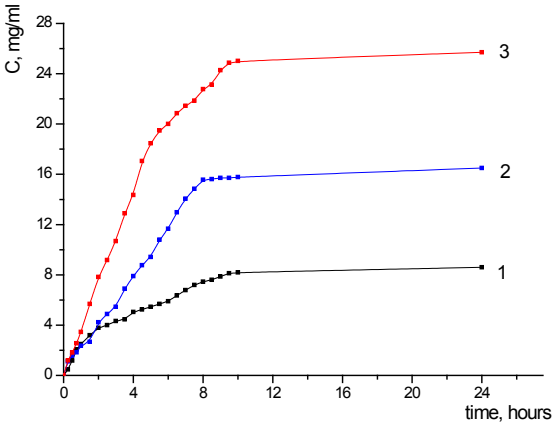
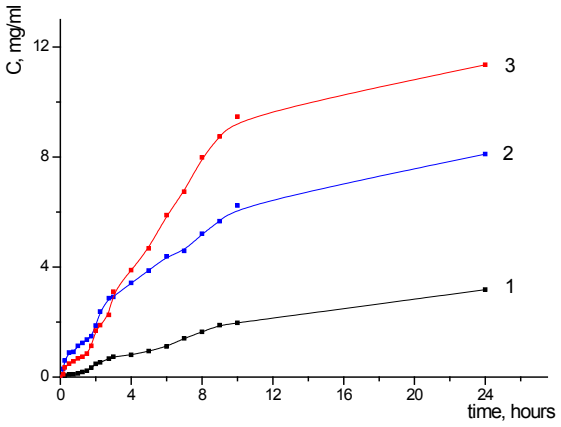


Figure 11 – Permeability of α-lipoic acid from the hydrogel ointment based on carbopol through a membrane



(1) – 0,9% ointment of ALA, (2) – 1,8% ointment of ALA, (3) – 2,7% ointment of ALA

Figure 12 – Permeability of α-lipoic acid from the hydrogel ointment based on carbopol through pig skin

In vitro study of the release of ALA from the carbopol-based gels obtained with α-lipoic acid was performed using the Franz Diffusion Cell. Prior to operation, the membrane was kept in a phosphate buffer solution at pH 6.86. Pre-thawed pig ears, prepared and also kept in a buffer solution for 1 hour. According to the obtained results , which shown at figure 11-12, the gel base due to carbopol provides a prolonged effect. Thus, during the first 10 hours in all ointments, a prolonged release from the ointment base and penetration of ALA through the membrane are observed (Figure 11). After 10 hours of release of ALA from the ointment, the prolongation slows down and gradually the values reach the plateau. With the increase in the content of ALA in the composition of the ointment, the amount of released ALA increases. The maximum concentration of released ALA in 2.7% of the ALA ointment, which was 25.7 mg / ml. When studying the permeability of the obtained hydrogel ointments with ALA through the pig skin skin (figure 12), with an increase in the concentration of ALA in the composition of the ointment, the increase in the amount of penetrated substance through the skin was also observed. Compared with the membrane, the amount of penetrated ALA is less, since it is related to the structure of the skin. The cells of the skin layers prevent direct penetration of the ALA, which makes it difficult to move. So, 3.18 mg / ml of ALA penetrated from 0.9% of the ointment, which is less by 5.42 mg / ml compared to penetration through the membrane. Despite this, a prolonged effect of the ointment with ALA is observed within 24 hours.

Table 1 – Antimicrobial activity of the samples

Number of a sample	Chemical composition of samples	Diameter of growth inhibition zone of test microorganisms, mm	
		Staphylococcus aureus IMV 3316	*Escherichia coli* IMV 877
№1	A pure gel based on carbopol	0	0
№2	ALA solution	27	23
№3	Ointment based on carbopol with ALA	16	15

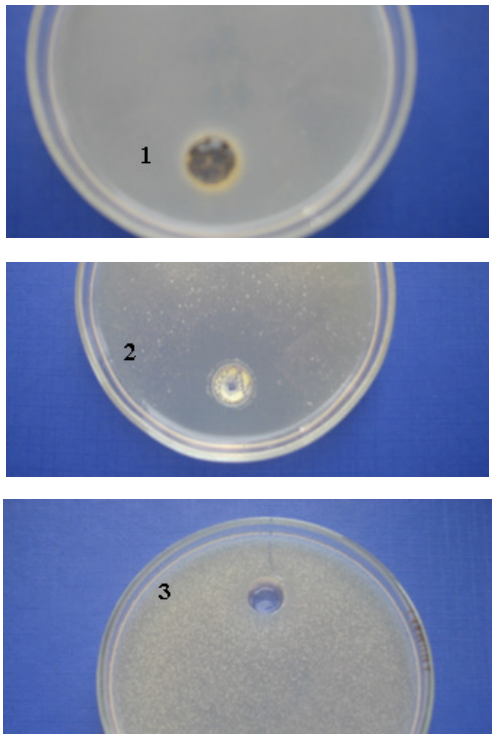


Figure 13 – Antimicrobial activity to Staphylococcus aureus IMV 3316

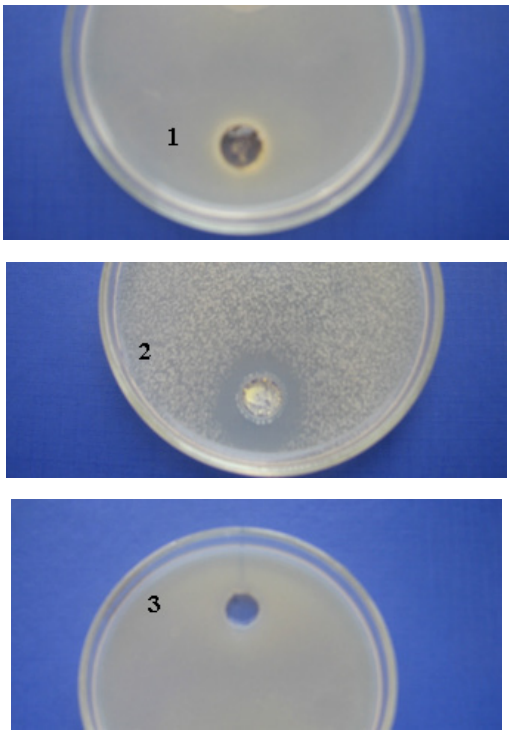


Figure 14 – Antimicribial activity to Escherichia coli IMV 877

The antimicrobial study of obtained hudtogel ointments with ALAwas made with respect to clinical opportunistic gram-positive and gram-negative pathogens of infections. The results are shown in the table 1. The presence of antimicrobial activity in samples № 2-3 with respect to clinical opportunistic gram-positive and gram-negative bacteria (Staphylococcus aureus, Escherichia coli) was established. No antibacterial activity was detected in the sample № 1-gel based on carbopol (figure 13-14).

Conclusion

Thus, the formation of the inclusion complex of α-lipoic acid with hydroxypropyl-β-cyclodextrin was studied, which provides an improvement in the solubility of ALA in water. Hydrogel ointments based on a slightly cross-linked polymer of acrylic acid-carbopol with α-lipoic acid have been developed. The rheological characteristics of the obtained hydrogel forms were studied. According to the obtained rheograms, all hydrogel ointments have pronounced thixotropic properties. The transdermal activity of the obtained hydrogel ointments with a different concentration of α-lipoic acid was studied, which showed that ALA ointments have a prolonged action, which ensures a slow release of the drug substance over a long period of time. The ointments with α-lipoic acid have antimicrobial activity in relation to Staphylococcus aureus and Escherichia coli. The transdermal characteristics were investigated using cellulose membrane and pig skin.

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Preparation of sulfur pigments based on the interaction of sulfur with aniline

Abstract. The article presents the results of obtaining and investigating the various ratios of the components of aniline hydrochloride-S-CaO (Al_2O_3) 1: 0.4: 0.6; 1: 1: 0.6; 1: 2: 10; 1:10:10 – up to ten times the binding of sulfur and oxides to aniline, having a different color range from dark green to light brown, depending on the composition of the components. In sulfurous composites with various molar ratios of sulfur components, which plays the role of an inorganic filler, it binds almost completely, while remaining chemically inert.
Key words: aniline, polyaniline, sulfur, aniline dyes, styrene, ammonium persulfate, acrylic acid, pigments.

Introduction

Polyaniline is synthesized from the inexpensive aniline monomer through a simple oxidative process in aqueous media, meaning the overall process is scalable and relatively inexpensive. The impurities produced are easily washed away with water and alcohols, and the resulting material takes on a brilliant green color, making it a visually appealing material to investigate. One of the most interesting properties of polyaniline is the dependence of its resistivity on the local pH environment, where a small amount of acid or base can produce a resistivity change of 10 orders of magnitude or greater. While polyaniline in its undoped state displays resistivity in the insulating regime, doping with different acids has led to highly conductive materials with recent reports of essentially metallic polyaniline. The combination of highly tunable electronic properties with a facility for nanostructure formation through relatively simple synthetic methods ensures that polyaniline will continue to be of great interest to the materials science community [1].

Idealized formulae of polyaniline at different oxidation and protonation states:

L = leucoemeraldine (closed valence; shell reduced form; benzenoid structure); E = emeraldine (radical cation intermediate form; combination of quinoid and benzenoid structures); P = pernigraniline form (quinoid structure); LH_{8x}, $EH_{8x}{}^{1}$, $EH_{8x}{}^{2}$ are the respective protonated forms:

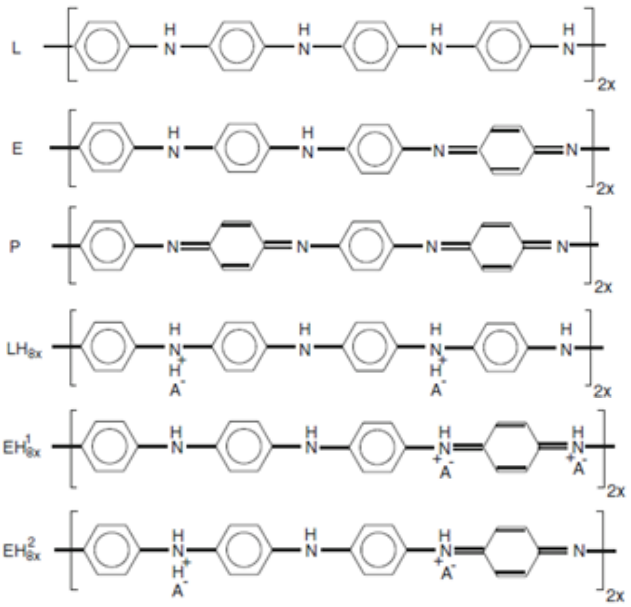
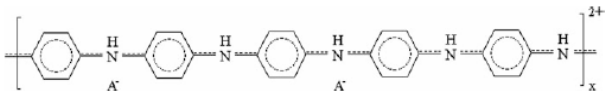


Illustration of delocalization (polaron lattice) of the emeraldine state:



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Another application of these high surface area materials is to act as functional scaffolds for inorganic nanoparticles. The facile and reversible oxidation and reduction of polyaniline allows the polymer

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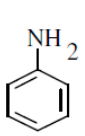
to behave as either an oxidizing agent or a reducing agent when combined with different materials. Treatment of polyaniline nanofibers with metal salts allows polyaniline to act as a reducing agent that donates electrons to the oxidized metal ion, causing deposition of metallic particles on top and within the polyaniline nanofiber network. The resulting inorganicorganic nanocomposite materials maintain the high surface area and dispersibility of the original polyaniline nanofibers and thus can be deposited and used as sensor materials or other electronically active materials. Gold-decorated polyaniline nanofibers have been explored for use in bistable memory devices.

Polyaniline nanofiber composites with metal nanoparticles can also be used in heterogeneous catalysis. Reduction of palladium (II) salts by polyaniline produces very small particles of palladium (0), the catalyst used for carbon-carbon bond formation between aromatic molecules in Suzuki coupling reactions. Since polyaniline nanofibers form a dispersion and not a solution, centrifugation can be used to pull the dense fibers out of the dispersion in order to recover the product of coupling reactions without timeconsuming extraction steps [1].

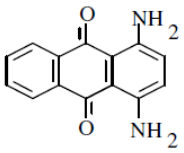
The technology has long been known for dyeing with aniline as an oxidizable dye, but the problem is to restrict the oxidation to the stage of emeraldine salt formation.

Aniline may be considered as an analog of dispersed aminoanthraquinone dyes, and correspondingly at the sorption stage one can use dyeing modes employed for ordinary dispersed dyes:

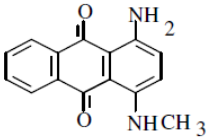
Aniline



Dispersed violet K



Dispersed violet 4C



A two-stage process can be used in dyeing fibers, for example, of polyamide, with the formation of an electrically conducting dye: polyaniline (sorption of the aniline by the fiber in the first stage as a dispersed dye and subsequent oxidation in the second stage), or in a single stage with parallel sorption and oxidation [3].

Materials and methods

Obtaining of core pigments. A sulfur alloy with calcium oxide was preliminarily prepared. For the production of alloys, the ground powders of sulfur and calcium oxide (2:3, 5:3, 1:10, 1:1) were placed in a porcelain crucible covered with a watch glass and heated to 115°C for 15 min. The resulting alloys were cooled and ground in a mortar. A sulfur alloy with calcium oxide in various molar ratios was added to a pre-prepared solution of aniline hydrochloride. Then, with constant stirring, a solution of ammonium persulfate ($n(C_6H_5NH_2)$: $n((NH_4)_2S_2O_8)$ = 1:1.25) was added. The reaction was carried out for two hours at room temperature and constant stirring. The precipitate was then filtered off, washed with three portions of 0.2M HCl and acetone and air dried.

Ratio of reagents:

a) aniline hydrochloride (0,005 moles, 0,648 g), ammonium persulfate (0,00625 moles, 1,425 g), sulfur (0,002 moles, 0,064 g), calcium oxide (0,003 moles, 0,168 g). The molar ratio is 1: 1,25 :0,4:0,6.

b) aniline hydrochloride (0,005 moles, 0,648 g), ammonium persulfate (0,00625 moles, 1,425 g), sulfur (0,005 moles, 0,16 g), calcium oxide (0,003 moles, 0,168 g). The molar ratio is 1:1,25:1:0,6.

c) aniline hydrochloride (0,01 moles, 1,295 g), ammonium persulfate (0,0125 moles, 2,85 g), sulfur (0,02 moles, 0,64 g), calcium oxide (0,1 moles, 5,6 g). The molar ratio is 1:1,25:2:10.

d) aniline hydrochloride (0,01 moles, 1,295 g), ammonium persulfate (0,0125 moles, 2,85 g), sulfur (0,1 moles, 3,2 g), calcium oxide (0,1 moles, 5,6 g). The molar ratio is 1:1,25:10:10.

e) aniline hydrochloride (0,005 moles, 0,648 g), ammonium persulfate (0,00625 moles, 1,425 g), sulfur (0,002 moles, 0,064 g), aluminum oxide (0,003 moles, 0,306 g). The molar ratio is 1: 1,25 :0,4:0,6.

f) aniline hydrochloride (0,005 moles, 0,648 g), ammonium persulfate (0,00625 moles, 1,425 g), sulfur (0,005 moles, 0,16 g), aluminum oxide (0,003 moles, 0,306 g). The molar ratio is 1:1,25:1:0,6.

g) aniline hydrochloride (0,01 moles, 1,295 g), ammonium persulfate (0,0125 moles, 2,85 g), sulfur (0,02 moles, 0,64 g), aluminum oxide (0,1 moles, 10,2 g). The molar ratio is 1:1,25:2:10.

l) aniline hydrochloride (0,01 moles, 1,295 g), ammonium persulfate (0,0125 moles, 2,85 g), sulfur (0,1 moles, 3,2 g), aluminum oxide (0,1 moles, 10,2 g). The molar ratio is 1:1,25:10:10.

Quantitative determination of sulfur in organic substance. A sample of the material is weighed, placed in a round-bottomed flask, 3 drops of 4% NaOH solution are added and 10 ml of 30% hydrogen peroxide is added. The flask is covered with a watch glass, heated to a boil and boiled for 20 min-

utes. Then add another 10 ml of 30% hydrogen peroxide and continue to boil for another 30 minutes. Then add 30-40 ml of distilled water and continue to boil until about half the content of the flask is reduced. The solution is then filtered. An excess of 0.1 M $BaCl_2$ solution was added to the filtered solution. A white precipitate is formed. It is filtered on a pleated filter, dried and weighed. The sulfur content in the material is calculated by the formula:

$$W(S) = m(BaSO_4) * 32{,}06*100/ 233{,}4 * m(sample);$$

sample weight is 0.1 g.

Results and discussion

When applying a layer of polyaniline to sulfur alloys with calcium oxide, color changes in powders were observed with a decrease in the molar content of PANI. Photographs of powders are given below.

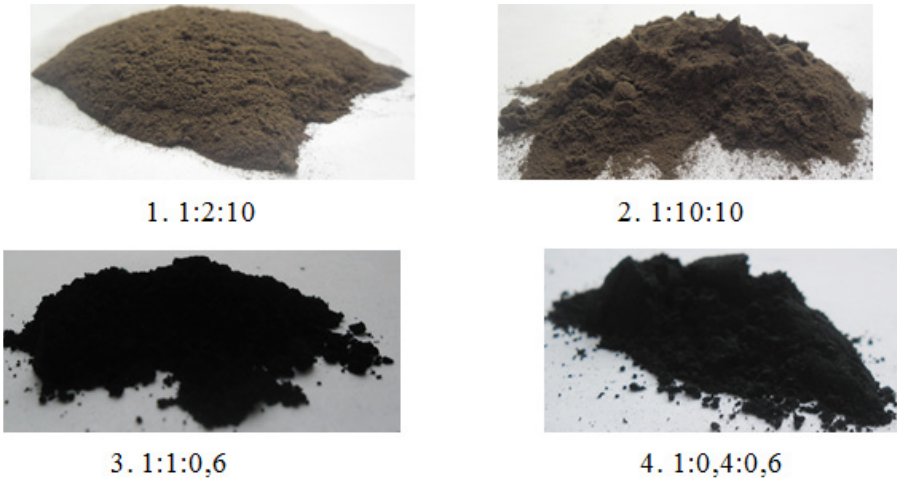


Figure 1 – PANI-S-CaO composites with different molar ratios

For the detection of by-products formed by the addition of sulfur alloys with calcium oxide, an analysis of the UV spectra of aqueous filtrates was carried out. On their basis, it can be said that when a calcium oxide is added to the composites, the hypochromic shift is noticeable.

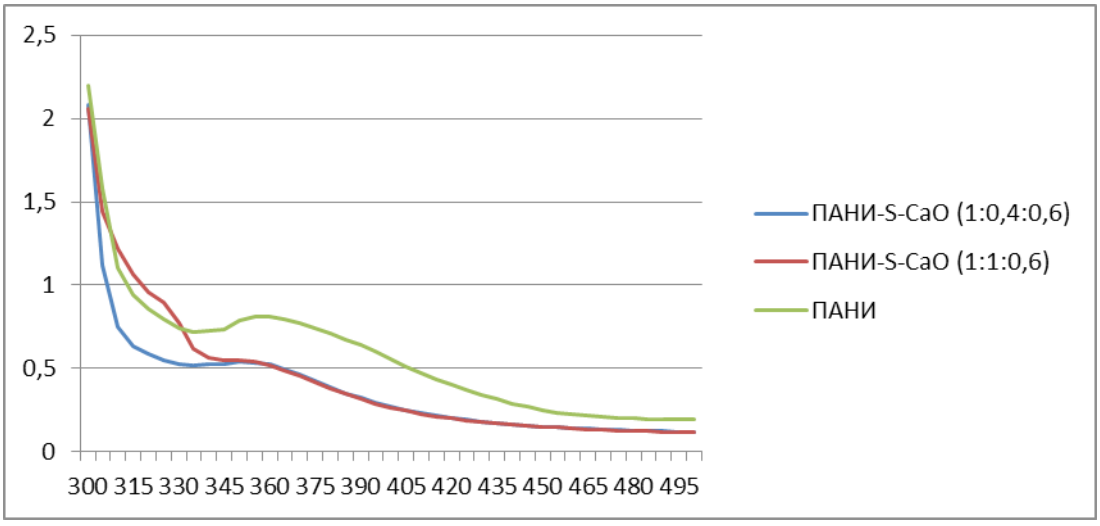


Figure 2 – UV spectra of PANI filtrate and PANI-S-CaO composites with different molar ratio

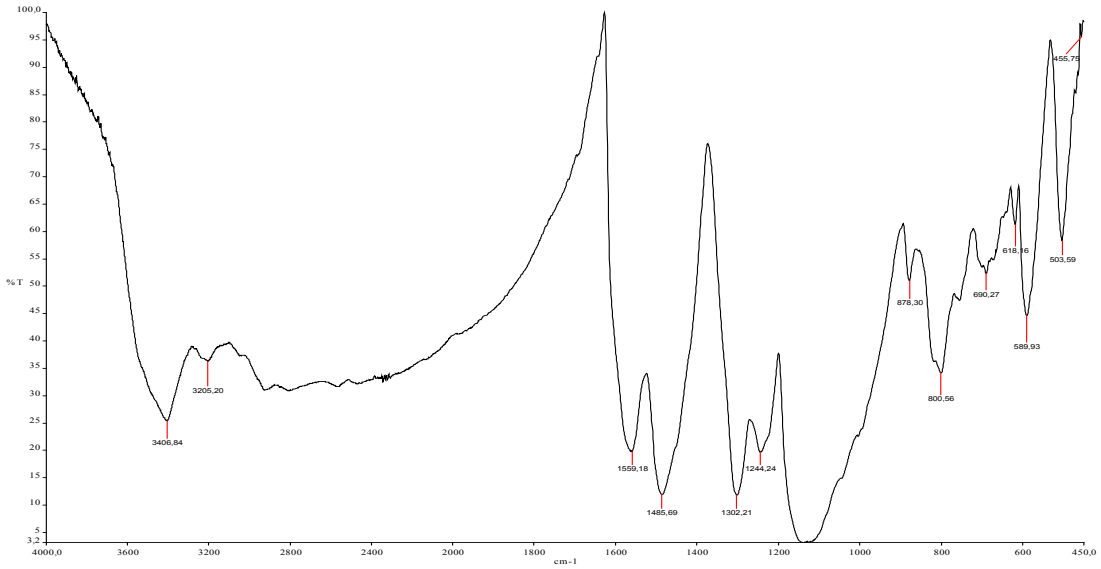


Figure 3 – IR spectrum of PANI-S-CaO composite (1: 1: 0.6)

Three peaks are observed in the PANI-S-CaO (1: 1: 0.6) spectrum, which are absent on the spectra of PANI and its composite with aluminum oxide: 3205, 618 and 456 cm^{-1}. The first of the peaks is characteristic of the bound -OH group in intracomplex compounds, the second for -C-S-, the third for -S- S-. What is said about the binding of sulfur with polyaniline. On the basis of this spectrum, it can be concluded that the organosulfur compound is formed.

After applying polyaniline to powders of sulfur-alumina alloys, the resulting composites had physical properties characteristic of PANI.

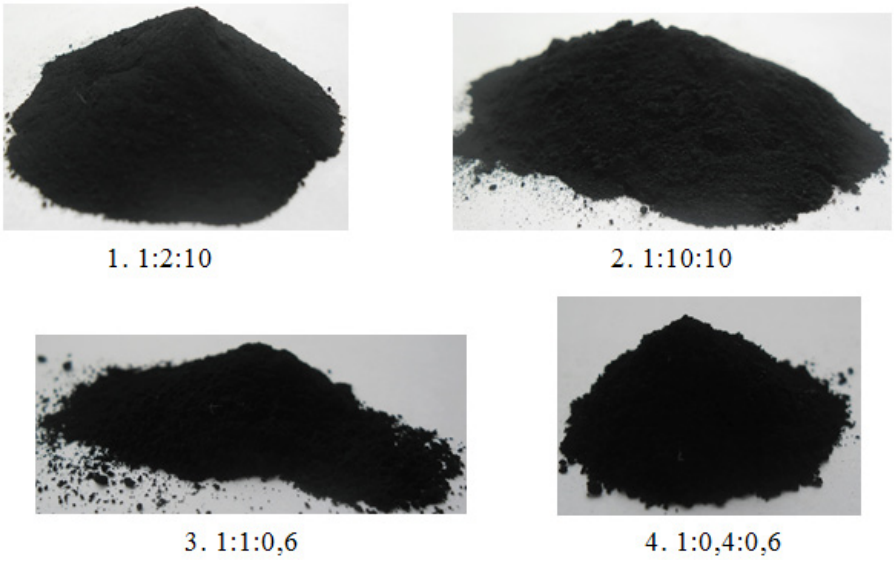


Figure 4 – PANI-S-Al_2O_3 composites with different molar ratios

To detect the by-products formed by the addition of sulfur alloys with alumina, an analysis of the UV spectra of aqueous filtrate was carried out. On their basis, it can be said that no side reactions are observed when alumina is added to the composites.

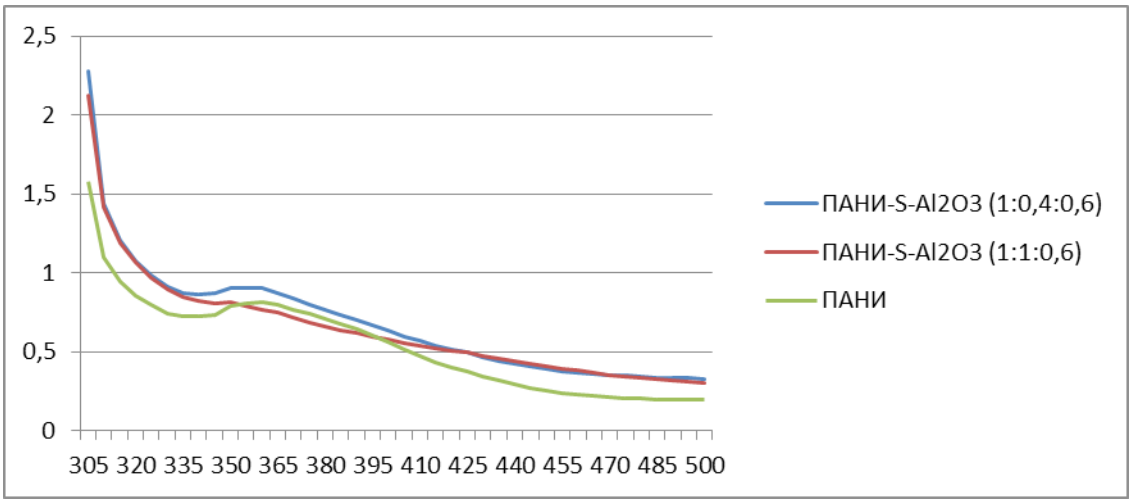


Figure 5 – UV spectra of PANI filtrate and PANI-S-Al_2O_3 composites with different molar ratio

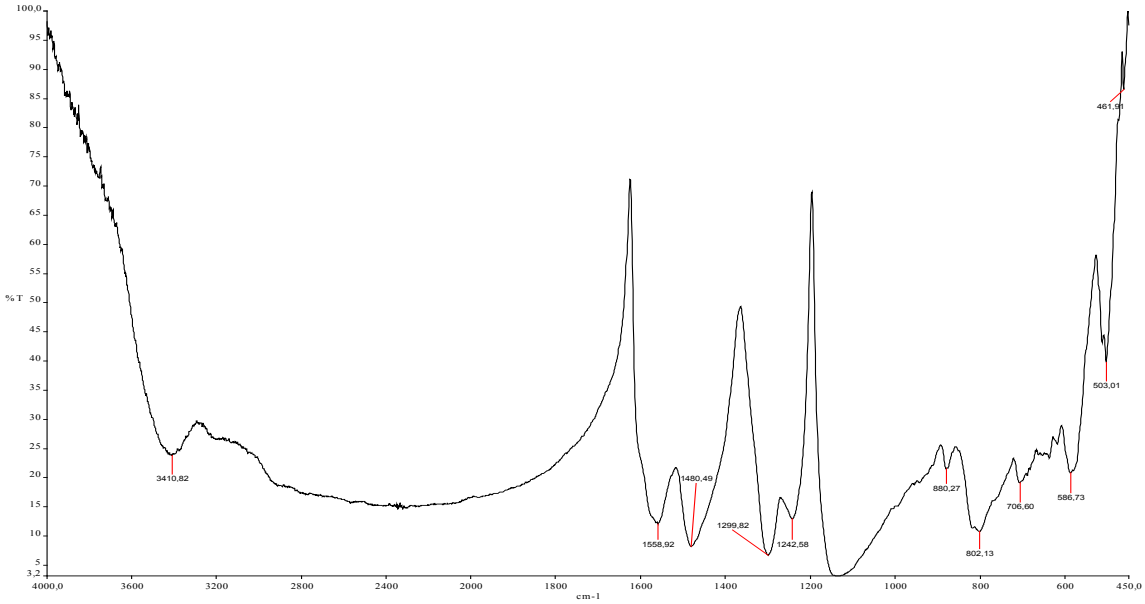


Figure 6 – IR spectrum of PANI-S-Al_2O_3 composite (1: 1: 0.6)

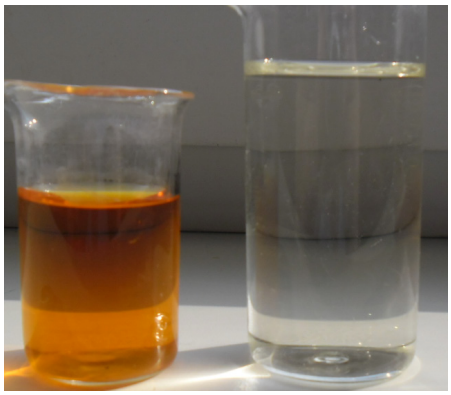


Figure 7 – Solution of methylorange before and after addition of adsorbent – PANI-S-Al_2O_3 composite (5: 2: 3)

The IR spectrum of the PANI-S-Al_2O_3 composite (1: 1: 0.6) is similar to the spectrum of polyaniline.

The property of polyaniline composites with oxides of various metals, such as the adsorption of dyes from wastewater, has been well studied. Thus, 8 mg of the composite adsorbs methylorange from a solution with a concentration of 0.06 g / l.

Conclusions

The possibility of obtaining composites based on the interaction of aniline with sulfur and ammonium persulfate for the production of pigments. Pigments of different qualitative composition with different component ratios were obtained. The dependence of the color change of the resulting composites on the quantitative composition was found.

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Catalysts for neutralization of waste gases of the vehicles and industry

Abstract: The article is about synthesis of catalysts on block metal carriers for neutralization of waste gases of the vehicles and the industry. Alumina or alumina with the addition of a zeolite or Ce^{4+}, Ti^{4+}, Zr^{4+}, La^{3+}, Fe^{3+} were used for preparation of the washcoats. The compounds of Mn, Ni, Co, Fe and the platinum group metals were applied as the active phase. The synthesized catalysts were tested in the process of complete oxidation of NO_x, CO, C_3H_8 and $NO_x+C_3H_6+O_2$ reaction. It was revealed that activity of Pd-Mo of catalysts increased at addition in composition of the carrier of cations Ce^{4+} и Zr^{4+}. The activity of palladium catalyst in oxidation reactions CO was equal to 90-100% and was independent of the concentration of metal, in complete oxidation of C_3H_8 at 473-623 K it was 78-90%, for Pt -containing catalyst – 95-100%. It was revealed that in case of the catalysts with base metals introducing of Ti^{4+} significantly increased the degree of reduction of nitrogen oxides with propylene.
Key words: catalyst, neutralization, waste gases, ecology

Introduction

The problem of cleaning of motor transport exhaust gases and the industrial wastes-one of the most urgent issue of the modern world. The exhaust gases of motor transport contain in their composition the hydrocarbons, carbon monoxide, nitrogen oxides, carcinogenic substances which are toxic substances and represent a considerable threat for health of the population [1-4]. Increase in number of means of motor transport – one of the reasons of deterioration in a condition of ecology in the cities and large settlements. Today decrease in harmful emissions of motor transport and the industry to the international standards is possible only by catalytic methods [5-7]. As the catalysts of CO and hydrocarbons oxidation and decomposition of nitrogen oxides are used mainly noble metals on carriers having a high catalytic activity and heat resistance to poisons. Creation of effective and stable catalysts of neutralization of waste gases of the industry and exhaust gases of motor transport and research of physical and chemical bases of improvement of the catalytic systems – an actual issue in the field of an ecological catalysis. One of the promising directions in this area – the partial replacement of noble metal on the oxide component [8, 9]. The work purpose – synthesis of catalysts on block metal carriers and research of their effectiveness in the reactions of purification of toxic industrial and vehicle emissions.

Experimental

For preparation of the block carrier the heat-resistant foil 50 μ thick exposed to goffering is used, on a strip of a smooth foil the strip of a corrugated foil is imposed, then both strips of a foil are displaced in the cylindrical block and are fixed by welding. The following stage in preparation of catalysts – the washcoat (a secondary carrier) is applied on the prepared block metal carriers with the honey comb structure of channels. In Figure 1 the laboratory samples of neutralizers are shown.

Figure 1 – the laboratory samples of neutralizers on metal blocks

 Printed in Kazakhstan

As a washcoat with the addition of zeolite or alumina modified by additives Ce^{4+}, Ti^{4+}, Zr^{4+}, La^{3+}, Fe^{3+} were used. For the preparation of solutions of the active components of the catalysts oxides of Mn, Ni, Co, Fe, prepared from acetates and formiates were applied. The previously weighed blocks were immersed in the required solution, then were shaken slightly from the excess solution between channels of the blocks, then were dried at 423 K for 2 hours in an oven, after which they were calcined in an electric furnace at 873 K for 2 h. The metal salts are decomposed to form metal oxides on the surface of the carrier the block. The blocks were weighed again and by a difference of masses before and after deposition the concentration of the deposited metal was determined. In case of use of catalysts on the basis of platinum metals in the reactions of oxidation of CO, hydrocarbons and nitrogen oxides decomposition platinum metals were transferred to a colloidal state. It was carried out by impregnation of catalysts by previously prepared solutions of polymers with the added solutions of active metals with the subsequent thermal decomposition. In the work platinum nano-sized particles were prepared by reduction with hydrogen in an aqueous solution containing chloroplatinic acid and citric acid. As the stabilizer of colloid platinum particles isopropyl alcohol is used. The obtained colloidal platinum metal by such methods was coated on carrier block. The metal sols supported on the block carriers were dried in an oven for 4 h at the temperature of 423 K. The catalysts were heated in a muffle furnace at 773 K for 2 h. Also in the synthesis of catalysts acetates of Pt and Pd and their π-complexes as active components of catalysts are used. Solutions of acetate of palladium were prepared by dissolution of Pd in the acetic acid containing 3% of HNO_3. Nitric acid was removed in the course of evaporation before complete finishing of release of nitrogen oxides. П-complexes of Pd and Pt were prepared by reacting of allyl alcohol with the salts of these metals with subsequent drying of the catalysts at 423 K and calcination at T = 773 K for 2 h. For increase of thermal stability catalysts were modified with additives of the second metal and oxides of refractory metals. For test of catalysts the flowing catalytic installation with the tubular reactor of integrated type was used and the reactions were investigated accordingly early developed method [10-13].

The gas mixture was prepared by feeding of hydrocarbons from container and the compressed air from the line into the mixer. The hydrocarbon content of the mixture was about 0.5. %. The oxygen concentration was varied from 2.0 to 10.0 vol.%. The gas mixture was analyzed by GLC and OPTOGAZ gas analyzer before and after the reaction. Crystal 2000M and Chrom 3700 chromatographs with the flame ionization detector are used. Analysis duration – 20-30 min. The activity of the catalysts was determined at temperatures of 423-773 K.

Characteristic of activity of the catalyst was the degree of conversion (α) of initial reagent (hydrocarbon, carbon monoxide, nitric oxide), defined by the formula:

$$\alpha = C_{init.} - C_{fin.} - C_{init.} \cdot 100\ \%,$$

where $C_{init.}$ and $C_{fin.}$ – are the initial and final concentrations of a reagent in volume of a test.

Results and discussions

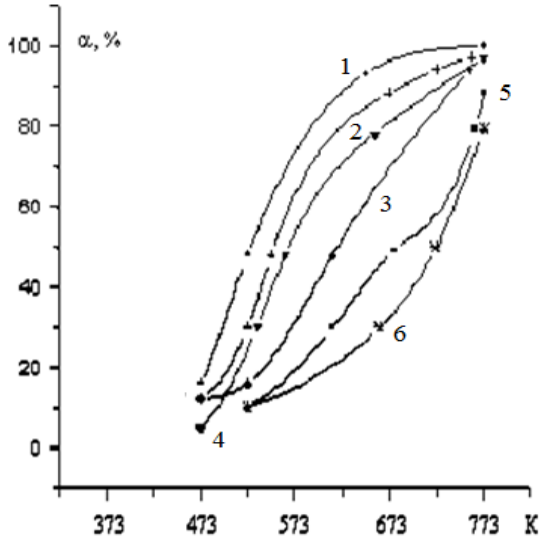
As an example, the results on studying of influence of concentration of Pt and Pd in π-allelic complexes on activity of block catalysts in reaction of oxidation of CO, C3H8 and reduction of NOx at a volume speed of a stream of gases – 35,000 h-1 are given in the Table.

Table – Influence of concentration of π- allylic complexes of Pt and Pd on a degree of conversion of CO, C3H8,NOx

Catalyst	The active metal concentration, %	Conversion degree, % at the different temperatures of research of catalysts, K											
		CO				C_3H_8				NO_x			
		773	623	523	473	773	623	523	473	773	623	523	473
Pt π-allyl	0.01	100	100	89	16	95	85.0	50	0	20	20.7	13.9	0
Pt π-allyl	0.05	100	100	92	50	98	94.0	73	0	20.7	21.1	14.4	0
Pt π-allyl	0.1	100	100	100	87	100	99.0	73	17	34.2	36.7	17.1	1.2
Pd π-allyl	0.1	100	100	100	83	53	22.0	5.0	0	18.4	16.8	8.0	0
Pd π-allyl	0.15	100	100	100	90	59	32.0	18	0	24.0	26.6	16	0.5

Data of the Table show that activity of palladium-containing catalyst in reaction of oxidation of CO doesn't significantly depend on concentration of metal and reaches 90-100% in the studied interval of temperatures. By the way the efficiency of Pd-catalyst in reaction of complete oxidation of C_3H_8 at low temperatures (623-473 K) was much lower, than that of the Pt-catalyst, and was 78-90%. The higher activity of the Pt-catalyst in reaction of NO reduction in comparison with Pd the catalyst was observed at contents of Pt equal to 0.1 % and T=773 K.

Research of Pt and Pd catalysts on thermo stability was carried out by maintaining of the catalyst with an interval of 5 h at T=773 K in a reactionary gas mixture with the contents of 0.5 % of propane-butane with the subsequent analysis of products of reaction. The total duration of researches was 100 h. The most stable appeared the catalysts obtained from acetates Pt, less stable – on basis Pd.



1-0.1% Pt-initial Pt, 2- 0.1% Pt-50 h. of testing, 3-0.1% Pt- 100 h., 4- 0.2% Pd-initial Pd, 5- 0.2% Pd-50 h. of testing , 6- 0.2% Pd-100 h. of testing

Figure 2 – Dependence of stability of catalysts in reaction of 0.5 % of propane-butane in air oxidation

It's known that by supporting of the secondary oxide covering on metal blocks of honey comb structure, as a rule, the carriers with the developed specific surface and porous structure are produced [14-16], and high thermal stability of the secondary oxide covering is provided with introduction to it of the modifying additives. There are the works about inclusion of cerium, zirconium, lanthanum cations into the secondary alumina coating, which stabilize γ-Al_2O_3 phase and provide resistance to poisons or sintering of Pt, Pd, Rh-active components of the catalysts [17, 18].

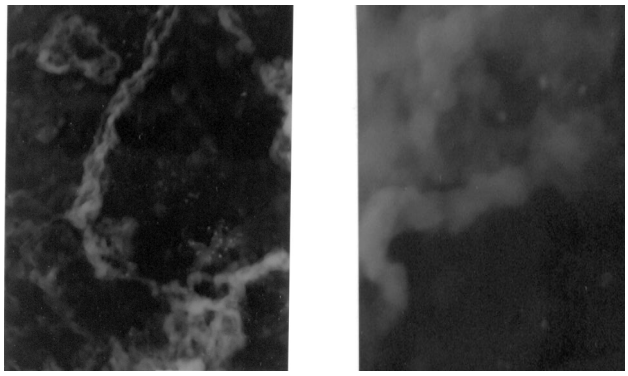
In this work Pd-Mo-catalysts on Al_2O_3 carrier modified by additives of Ce^{4+}, Ti^{4+}, Zr^{4+}, La^{3+}, Fe^{3+} were prepared and investigated in reaction of $NO_x+C_3H_6+O_2$. Activity of Pd-Mo of catalysts increased when modifying the carrier with cations of Ce^{4+}, Zr^{4+}.

Activity of Pt-Cu catalysts on the zeolite-containing carriers NaY, ZSM-5 and their hydrogen forms was studied. High activity of the catalyst is noticed when supporting of the active phase on the carrier in H^+-form. A number of oxidic catalysts with the differing compositions of the secondary carrier (γ-Al_2O_3,γ-Al_2O_3+TiO_2) and of the active agent (Co+Mn, Fe+Mn) promoted and not promoted by platinum is studied. Introduction of titanium dioxide into the secondary carrier considerably increases extent of reduction of nitrogen oxides by means of propylene on both compositions of oxidic catalysts in all interval of the studied temperatures (423-773 K) and was equal to 34% at 523 K on the Co-Mn-catalyst, to 29% -on Fe-Mn, and at 773 K-33% and 55%, respectively. Promotion with platinum improves the reduction ability of cobalt-manganese catalyst on Al2O3 only at temperatures higher than 673 K. On the titanium-containing sample in the presence of platinum degree of NO conversion is decreased.

The samples of catalysts based on platinum group metals were investigated by electron microscope EM-125K with single stage replica method. In a sample with Pt the small congestions of dense particles which don't grow together in units and are dispersed on a carrier surface are observed. The particles sizes are predominantly 10.0 nm, 5.0 nm and less 9.0 nm (fig.3, a). In the sample with Pd there are some isolated dense particles in size 12.0 nm-15.0 nm (fig.3, b). The platinum obtained by reduction in solution has the sizes about 15.0-18.0 nanometers, at the same time, the platinum colloid obtained by reduction with lemon acid had almost monodisperse distribution, the average size of particles was equal to 8.0 nanometers. It is found that the organometallic complexes of Pd and Pt at magnification of 33,000 times represent translucent areas of the clots of polymer filled with dispersed particles of 3.0 nm. At higher magnification (in the 62,000 times) also small rare congestions of more dense particles of 5.0 nm in size are observed.

Investigation of Pt and Pd-containing catalyst by means of XPA showed X-ray scattering, which confirmed the high dispersion of catalysts obtained

by thermal decomposition of organometallic complexes.



a b

Figure 3 – EM – pictures of distribution of noble metals: Pt (a), Pd (b) on the metal carrier

Physical and chemical researches of catalysts on the basis of base metals were carried out by XRD method on x-ray diffractometer DRON-4.0.7 with the copper anode. Samples for research were prepared by mechanical destruction of the catalyst put on a block metal framework. The fallen part of the catalyst was crushed in an agate mortar up to 100 μ and was used for research by method XPA. It was found, that the oxide catalysts represented spinel with cubic lattice NiMnO4 with peaks 2Å, 52Å, 148Å, 203Å. Also there were small intensive peaks of CeO_2 (308Å) and alumina (160Å, 256Å). The conducted research of supports and catalysts by means of XPA showed the formation TiO_2 – anatase structure, peaks 3.52; 1.89; 2,38 Å. The carrier based on V_2O_5-WO_3 – peaks 4.38, 3,4, 2,8 Å, it has an orthorhombic lattice. In the active phase NiO-crystal lattice is not formed. Catalysts based on base metals have been investigated with an electron microscope EM-on device 125M single by stage replica method. On a nickel-vanadium-tungsten sample the congestions of dense particles which don't grow together in units and are dispersed on a carrier surface are observed. The sizes of particles were mainly 15.0-20.0 nanometers.

For samples of Pd-Mo catalysts (fig.4) there are found Al_2O_3 peaks (25.5, 35.0, 37.8, 43.4, 52.9). It was found that a position of a peak of PdO (it should be at aprox. 33.9-34.1) is insignificantly moved to the left, it is apparently due to slight interaction between Mo and Pd. Data XRD not don't confirm formation of solid solution or alloy of Pd-Mo.

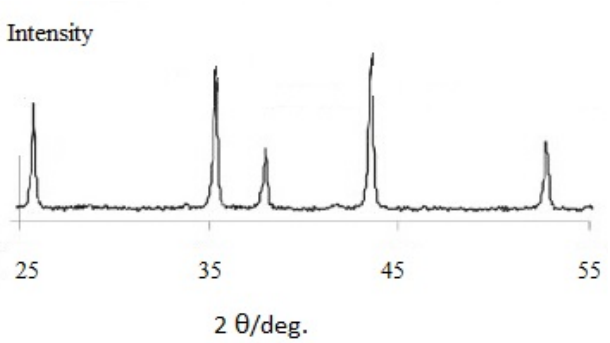


Figure 4 – XRD image of Pd-Mo/Al_2O_3 catalyst (after calcination)

Conclusions

Catalysts for neutralization of toxic gases of the industry and motor transport on metal carriers with the honey comb structure of channels are prepared. For preparation of the secondary carrier were used either aluminum oxide or aluminum oxide with the addition of a zeolite or Ce^{4+}, Ti^{4+}, Zr^{4+}, La^{3+}, Fe^{3+}. For preparation of solutions of the active components of catalysts are applied oxides of Mn, Ni, Co, Fe, obtained from acetates and formiates. Also were synthesized the samples of catalysts based on metals of the platinum group converted into colloidal state. The activity of Pd-Mo catalysts carrier is increased by modifying with cations Ce^{4+}, Zr^{4+}. The activity of the palladium catalyst does not depend on the metal concentration in the CO oxidation reaction and is about 90-100%. However, the effectiveness of the Pd-catalyst in the C3H8 complete oxidation reaction at low temperatures (623-473 K) is significantly lower than of Pt-catalyst, and is 78-90%. For catalysts based on base metals introducing into the secondary carrier of Ti4+ significantly increases the degree of reduction of nitrogen oxides with propylene, at 523 K on a Co-Mn-catalyst – 34%, on Fe-Mn – 29%, at 773 K – 33% and 55%, respectively.

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Fractional composition of compounds of zinc and lead in light chestnut soils

Abstract: The objective of this work was study and assessment of the main regularities of distribution of forms of finding of Zn, Pb in light chestnut soils of the territory of the island "Polkovnichii" (Semey, Kazakhstan) as a basis of assessment of influence of technogenic pollution and development for successful monitoring of a surrounding medium, protection of soils. It was found that average total of Pb content of 1.2 times, of Zn content – 2.4 times lower than their clark contents in soils. The examined soils on the level of the average content of mobile forms of zinc are the medium soils by the presence of this element. Reliable direct correlative dependence on a humus and pH for acid soluble (extracting agent – 2.5% of CH_3COOH) form of lead is revealed. Reliable positive correlative dependence on values pH the forms of Zn strongly bound to organic matter of the soil is found. It was revealed that the migration of forms of finding of lead and zincum on a profile of the soil happens nonuniformly: accumulation of zinc is noted in the top horizons of the soil – humic A_n and the humic accumulative horizon of A_1. It was found that accumulation of lead happens in the illuvial horizon of B_1 of the investigated soils.
Key words: zinc, lead, total content, mobile forms, migration, light chestnut soils

Introduction

An information about the content of toxic ingredients in the soil of a particular region take a very important place at solving majority practical environmental problems of anthropogenic pollution [1-6]. At pollution level assessment as background usually take uncontaminated territories with a similar soil cover for which concentration of heavy metals is known [7, 8]. In this case it is very difficult to observe the principle of analogy because the urban soils – urbanozems strictly speaking under natural conditions have no analogues. In this connection there is an urgent task of finding the local urbanized background that could be used to assess the level of soil contamination.

The aim of this research was study and assessment of the main regularities of distribution of forms of finding of Zn, Pb in light chestnut soils of the territory of the island "Polkovnichii" (Semey, Kazakhstan) as a basis of assessment of influence of technogenic pollution and development for successful monitoring of a surrounding medium, protection of soils.

Experimental

The natural light chestnut soils typical of "Polkovnichii" island were chosen for investigation. The researched territory is located within the city of Semey of the East Kazakhstan region of the Republic of Kazakhstan. The city of Semey is divided by the Irtysh River on left-bank and right-bank. In the middle of the Irtysh River there is an island "Polkovnichii" (fig.)

The samples of all of the genetic horizons of the soil profile were taken for investigations. Determination of macrocomposition of all tests of soils (pH, a humus, CO_2 of carbonates, granulometric composition) was carried out by standard methods [9]. Among the heavy metals as studying objects lead and zinc have been taken. The choice of these elements is explained two reasons: the first – the physiological importance of zinc in living organisms and in particular for plants, and the second – the lack of information on the biogeochemistry of these elements on the island "Polkovnichii" needed to solve a number of scientific and practical issues [1-3, 7]. The content of heavy metals in the explored soils was determined on the KFK-3 device by a photocolorimetric dithizone method by G.Ya.Rin'kis's recipe [9-11]. The reproducibility of the method was equal to ± 4.2%. Selection of fractions of Pb and Zn was carried out by method of parallel extraction. All analytical data were processed by mathematical analysis and mathematical statistics in soil science according to E.A.Dmitriev [12].

Figure – An island"Polkovnichii", Semey 070000, Kazakhstan

Results and discussions

The obtained results showed (Table) that the average total content of lead in the general set of soil samples of the investigated territory in 1.2 time, of zinc – is 2.4 times lower than their clark contents in soils.

In magnitude of the average total content the studied heavy metals are arranged in such a way: Zn>Pb. On value of coefficient of a variation (in %) heavy metals in the researched soils are located in the following decreasing order: Pb (34)>Zn (17).

The content of total forms of heavy metals on a profile of soils is distributed unevenly. The maximal content of lead is characteristic of the illuvial horizon of B_1, minimum – of the humic horizon A. The maximal content of a total form of Zn is characteristic of the humic horizon of A_n and the humic and accumulative horizon of A_1, minimum – of the transitional horizon of BC. The increased content of a total form of Zn in the humic and humic and accumulative horizons is explained by the increased contents in them of the maximum quantity of a humus, and also higher value pH. In turn, the increased content of a total form of lead in the illuvial horizon B_1 is explained by the increased contents in this horizon of the maximum quantity of physical clay (<0.01 mm), possible carbonaceous and alkaline geochemical barriers.

Table – The lead and zinc total content of in the light chestnut soils of "Polkovnichii" island (in mg/ kg)

Metal	K_v,	M±m,	V, %	Clark in a soil [8]	Clark in the lithosphere [8]	Maximum concentration limit in the soil [4]
Pb	4.91 – 12.24	8.32 ± 1.05	34.0	10.00	16.0	100
Zn	14.84-23.67	20.86± 1.37	17.0	50.00	83.0	300

Note: K_v – a range of a variation, M±m – an arithmetic average and its mistake, V – variation coefficient.

As it was shown in numerous researches, an organic matter and its components form various complex compounds with heavy metals [1, 7], and the fine-grained mineral phase is the strong adsorbent of heavy metals [6]. Results of the carried out researches show that with increase in content of physical clay in the soil the content of a bulk form of lead increases, and also with increase in content of a humus in the soil the tendency to increase in content of a bulk form of Zn takes place. Reaction of aqueous soil slurries in the humic and humic and accumulative horizons is neutral, in particular, at values pH, the close to neutral, the maximum of absorption of heavy metals by organic matter and clay minerals is reached [6, 13].

Amplitude of fluctuation of content of elements in the soil is a little various: for lead accumulation in the top horizon of the soil sharply increases with a depth, on the contrary, accumulation in the humic and accumulative horizon which gradually decreases is characteristic of Zn. It indicates that lead in the researched soils is less mobile metal, is capable to form steady compounds with finely dispersed mineral phase of the soil. It should be noted that zinc in these investigated soils relates to metals less mobile in the soil; a key role in this belongs to an organic substance capable of forming stable complexes with zinc, which is very important and has great practical significance for the management of migration and bioavailability of this element in the soil. The research of the work showed that the observed the unequal correlation between the total content of heavy metals and indicators of humus, soil pH and physical clay. A positive reliable correlative dependence of content of a total form of lead only on availability of physical clay is revealed. In the investigated light chestnut

sandy loam soils reliable positive correlative dependence between the total content of Zn and existence of a humus, and also value pH is found. The established level of total contents in soils of the studied urbanized background is much lower than the recommended maximum permissible limits. V.V. Kovalsky established the threshold concentrations of some elements in soils according to their possible pathological influences on farm animals [14]. When compared with these threshold concentrations total zinc content is within the lower threshold boundary. The investigated light chestnut soils of "Polkovnichii" island are characterized by low total zinc content in comparison with soils of different regions [10, 11, 13, 14]. According to gradation to Ya.V. Peyv and G.Ya. Rin'kis [9], by the level of average content of the mobile form of Zn the researched soils belong to the medium soils accordingly presence of this element.

Data on the background content of zinc and lead in the studied soils are very valuable, give the possibility of systematic observations in this natural region and improve the objectivity of the evaluation of the emerging environmental conditions. In recent years farmers to the soil introduce Zn-containing fertilizers therefore carrying out a research of influence of physical and chemical properties of soils of the region on effectiveness of zinc fertilizers has applied value. Thus, as a result of the research it was found that the studied soils are characterized by deficiency of zinc content and by the level of the average content of mobile forms of zinc belong to the medium soils by the presence of this element. This must be considered when using zinc-containing fertilizers into the soil. It is necessary to carry out detailed investigation of the influence of physical and chemical properties of the soil in the region on the effectiveness of zinc fertilizers, and to continue research in this direction.

Conclusions

So, the soils of the island "Polkovnichii" (Kazakhstan) were studied. It was found that the total content of lead fluctuated from 4.91 to 12.24 mg/kg, the average value was equal to 8.32 mg/kg, the coefficient of a variation was equal to 34.0%; the total content of zinc was in limits of 14.84-23.67 mg/kg, the average value was equal to 20.86 mg/kg, the coefficient of a variation was twice less, than for lead and amounts 17%. It was revealed that the average total content of lead in 1.2 times, zinc – 2.4 times lower than their clark contents in soils. The total content of zinc in the soils of the examined area is located within the lower threshold limits. Migration of forms of finding of lead and zincum on a profile of the soil happens nonuniformly: accumulation of zinc is noted in the top horizons of the soil – humic A_n and the humic accumulative horizon of A_1. It indicates the leading role of soil organic matter in accumulation of this element. Lead accumulation occurs in the illuvial horizon B_1 of the investigated soils, due to the increased content of physical clay in this horizon.

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Synthesis of cryogels based on bentonite clay-polyacrylic acid and research their physical-chemical properties

Abstract: In this paper, we present ways of synthesizing sorbents based on bentonite clay-polyacrylic acid used in industrial wastewater and studying their physico-chemical properties. Cryogels were synthesized by radical polymerization based on bentonite clay (BC), brought from the East Kazakhstan region of Tarbagatai region, and polyacrylic acid (PAA), characterized by porosity by the definition of physico-chemical properties. 1 %, 3 % and 5 % of bentonite clay, 0,25 % ammonium persulfate and 0,25 % sodium metabisulphite as initiator, 10 % acrylic acid, 10 % sodium hydroxide were used of synthesis of cryogels based on BC-PAA. The optimal temperature is -30°C and 24 hours. In addition, the physico-chemical properties of obtained cryogels based on BC-PAA are investigated, and their density is about 1,5531 g/cm^3, yield 95 %. This shows the economic efficiency of cryogels. Morphological structure of composite cryogels were carried out on a Leica DM 6000M optical microscope, Ntegra THERMA atomic force microscope and Quanta 3D 200i Dual system scanning electron microscope, which resulted in porosity, chemically crosslinked, homogeneous and negatively charged composite cryogel. Sorption properties of cryogels in relation to ions of heavy metals are considered.

Key words: bentonite clay, polyacrylic acid, composite material, sorbent, heavy metal ions.

Introduction

The results of saving natural resources and anthropogenic conditions, as well as environmental problems of maintaining a balance in the environment, are increasing day by day. One of such environmental problems is the problem of pollution of industrial wastewater, non-compliance with the established requirements of water quality.

One such composite material that meets these requirements is cryogels. Cryogels have several special properties, the main of which is porosity. This property makes it possible to apply them in all branches of science and technology.

In the development of the science of chemistry for the purification of industrial wastewater sorption methods differed. Recently, for this purpose, according to the correspondence of organic and inorganic polymers, the importance of the use of composite materials in which the mechanical, physico-chemical and sorption properties have improved is growing.

The following are the causes, for which clay and polyacrylate were chosen:

1) polyacrylic acid does not belong to the colonies of the polyanions, that is, it is an ion with an negative spell. Bentonite clay conjoin from particles with detrimental spells, that is, two two-component gel are identical sponges. If they were illegal, then, in the result of the electro-tactical action, a saline complex would appear, and then an unequal connection. Components of displacement with the same name can connect with one another with non-circular forces, such as water links, hydrophobic activities. Such a system is characterized by the following features: the collected crucifixes are single-handed and protect the important of their creatures – a whirling cope.

2) cryogel has active centers (-COOH, -OH) and hydrophobic groups that support the binding of metal ions. In connection with these objects, it is possible to suggest that the materials on their basis can bind ions of heavy metals.

For these reasons, in this work, the possibility of obtaining cryogels based on polyacrylic acid and bentonite clay at different times and in different ratios of the original components was investigated. The influence of various external factors was considered:

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the amount of bentonite clay (1, 3, 5 mol.), temperature, concentration of the medium.

It can be argued that the reason for introducing bentonite clay into the PAA cryogel is to increase the coping resistance of cryogels. Therefore, bentonite clay is a good sorbent, it copes heavy metal ions up to 100 %. Therefore, due to the growth of PAA cryogels and the high coping efficiency of BC, it is possible to receive a sorbent with a high sorbent cope.

Materials and methods

In this work cryogels were synthesized based on bentonite clay, brought from the East Kazakhstan region of Tarbagatai region and polyacrylic acid.

For studying interaction of bentonite clay with organic polymer for the beginning have prepared and have purified bentonite clay. Clay represents itself mix of particles of various sizes and contains, except clay, numerous impurity of other minerals. The used bentonite clay of the field Manyrak (East Kazakhstan region) of pink color was purified with the help of Salo method. Fat repeated washing by the distilled water.

Three phases are determined by results of X-ray-phase analysis (DRON-4-07) in composition of the studied bentonite clay: α-quartz SiO_2, montmorillonite $Al[OH]_2\{Si_4O_{10}\}\cdot mH_2O$ and amorphous phase. In this structure montmorillonite prevails.

The most optimal condition was chosen by changing and observing several times the condition of obtaining and composition of the cryogel. Synthetic cryogels were synthesized based on 10% polyacrylic acid (PAA) by the method of polymerization. As a result, the crosslinking agent used N, N'-methylene-bis-acylamide (MBAA), initiate-ammonium sulfate (APC) and sodium methabisulfite (SMBS), used sodium hydrate and water. At the time of bentonite clay content of 1 %, 3 % and 5 %, 10 % PAA, 10 % hydrochloride of total mass, 1 % N, N'-methylene-bis-acylamide, 0,25 % ammonium peroxide and 0,25 % sodium methabisulfite from the scale of the monomer. The cryogels PAA and BC-PAA were obtained with a radial polymerization at a temperature of -30 °C for 24 hours [1].

Results and discussion

The physicochemical properties of synthesized cryogels based on BC-PAA have been studied. For studying of the density, kinetics of swelling and the morphology of the gelled gels an optical microscope, scanning electronic microscope, an atom-force microscope was used.

Composition and the physico-chemical characteristics of the produced composite gels are shown in Table 1.

Table 1 – Physicochemical characteristics of composite cryogels

Structure (weight. %)	G, %	S, %	j, %	ρ, г/см^3
Gel PAA	57,7	42	6,52	1,2070
Cryogel PAA	90,3	10	3,21	1,5974
Cryogel BC-PAA (1:10)	90,8	9	3,14	1,5781
Cryogel BC-PAA (3:10)	91,7	8	3	1,5677
Cryogel BC-PAA (5:10)	95,9	4	2,27	1,5531
G – yield of cryogels, %; S – the output of the sol fraction, %; j – degree of cross-linking, %; CA = 1 %.				

As can be seen from the table, for all comedy cryogels there is one common sign: when the clay is increased in size, the yield of cryogels increases, and the yield, the role of the fraction and the degree of reduction decrease [2]. The output of the fraction of the solution is peppered to the output of the clay, that is, it decreases. Sol fraction shows quantity not polymerized monomers in clarification time cryogels. With the decrease of this value, the output of clay increases, so that it may be suppressed that polycrystalline substances enter into polimerization. The reason for the decrease in the degree of crosslinking from the clay, as the clay increases in the composite, is that the quantity of the polimeral agent in according decreases, so the degree of crosslinking decreases.

The density of cryogels based on BC-PAA was measured on the basis of the Radwag AS 220/X. The component of the synthesized cryogels, that is, when the quantity of BC increases in size, and increases in size, in proportion, the density decreases. The reason for this is the coexistence of BC, in which the mol-

ecules of monomers are introduced, forming single, well-built composite cryogels [3].

When studying the properties of the collected cryogels, you must first pay attention to the disintegration and the morphological structure of the clay in composite. The literal review shows that the process of formation of emotions occurs on many intermediate stages. At the first stage, the tactoid appears, that is, the polymer disposes of the agglomerates of the organoclay. In the second stage, there is a transition of the polymer to the interstitial space of the organoclay, in the course of which they are exposed to 2-3 nm. On the third stage, the disintegration of the clay layers of the organoclay. On the next stage there is a crumbling with a thin layer. But, in the formation of polymeric nanocomposites, higher structures may be higher than this, which is linked to the degree of clay pumping. Because of the higher quantities and the low degree of clay in the polymeric material, there may be agglomerates of minerals.

On the results of the studies of cryogels with the help of an optical and an atomic-force microscope, it is possible to see that the upper layer of the structure of the body is slightly spoiled. This proves that the particles of BC are introduced into the polymeric grids, and a one-way interconnected gel is formed (Figure 1).

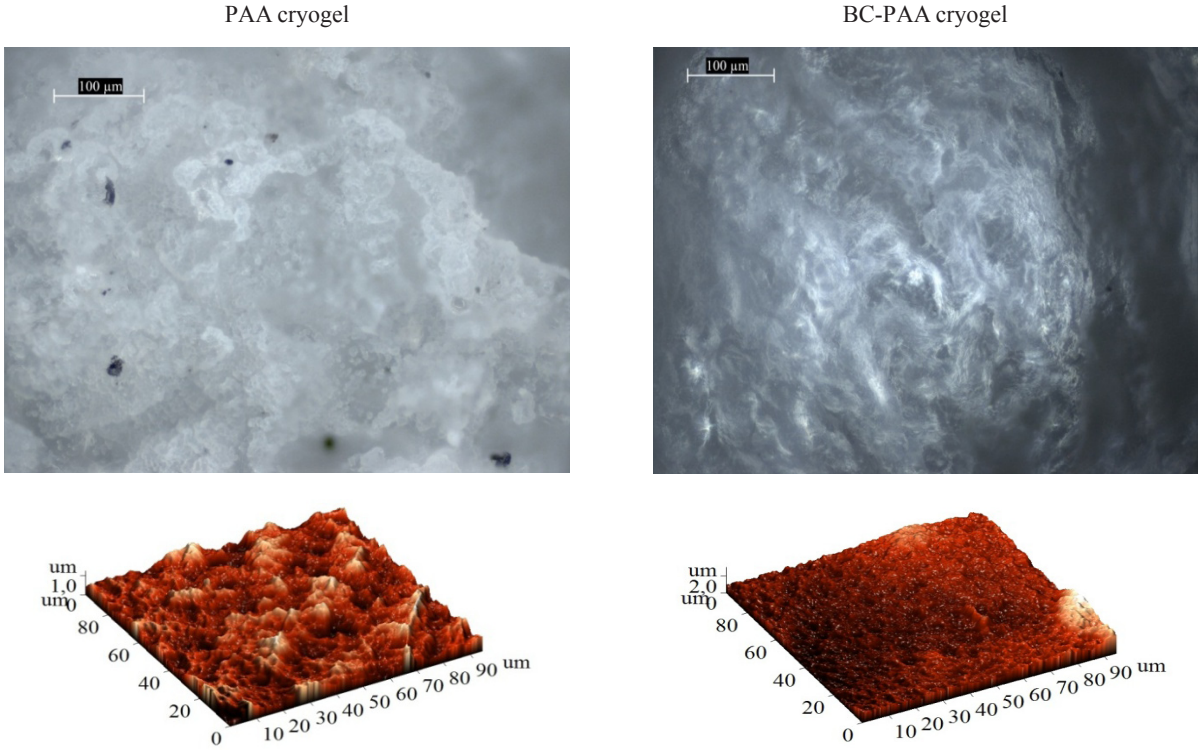


Figure 1 – Figures of cryogels obtained by optical microscope and an atomic-force microscope (AFM)

Interest to the microstructure of the gel is associated with the possibility of using them in the catalysis at the rate of the source, on electronic microequipment. In physical gels, polymer chains, when linked, form microstructures such as aggregates, micelles, multiplets, crystallites [4]. The size of these microstructures, as indicated by the avtop, is in the range of 1-100 nm [5]. The formation of crystalline gels is indicated in the examples in the complexes sodium yellow-alginate, PAA gels. In the opinion of detectors, the smallest dimensions of these crystals are 1 nm, in the range 4,6-7,1 nm, and they fulfill the role of cross-linked sites of networks from polymer networks. For the further establishment of the morphological structure of BC-PAA cryogels with the method of scanning electron microscopy, studies were carried out (Fig. 2) [6]. From the received information, it is possible to observe that the cryogels are composed of microstructural units with a size of 5-10 nm.

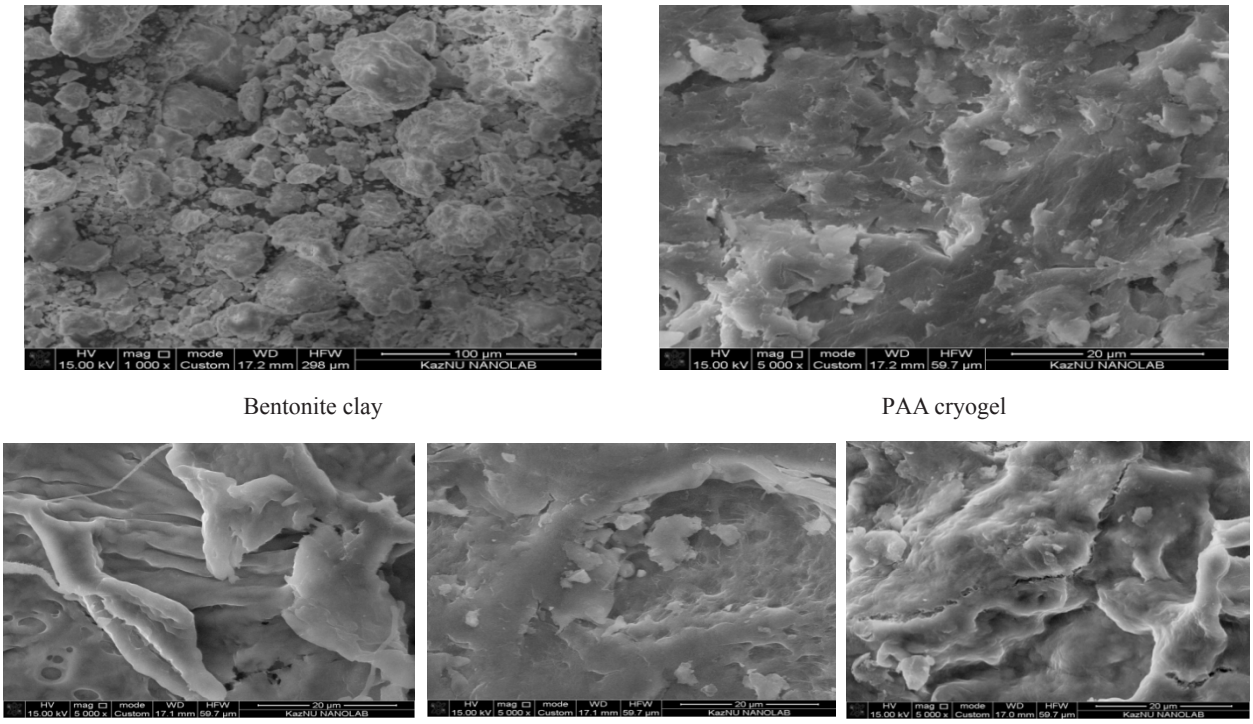
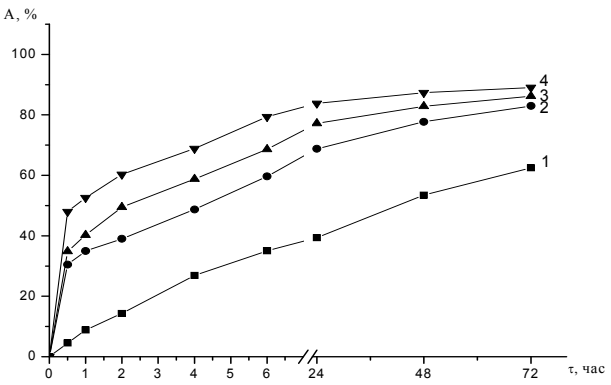


Figure 2 – Figures SEM of the BC-PAA cryogel

Sorption is one of universal methods in purification of the dissolved organic substances, an also of ions of heavy metals. In the same purposes, artificial and natural sorbents are much used. Therefore that, sorbents belong to effective, easily available materials in sorption of ions of heavy metals. Researches of sorption of ions of heavy metals by cryogels for an assessment of their sorption ability are conducted.

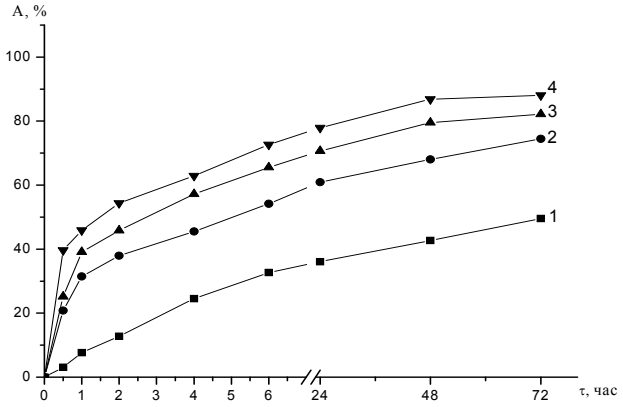
Results of sorption of cryogels based on BC-PAA in solutions of ions of metals are given in figures 3-4. By results of a research, sorption of cryogels BC-PAA on 3 days was in limits ~ 80-90%.

First, if cryogels based on bentonite clay and polyacrylic acid compare with PAA cryogel, it is visible that sorption values high (figures 3-4). It can be explained with presence of bentonite clay at structure of cryogel. High sorption ability of bentonite clay are investigated in many works. Increase in sorption value with increase in content of bentonite clay is connected with electrostatic interaction.



Crygel BC-PAA; NaOH = 10%; CA = 1,0%;
Initiator = 0,5%; C_{Pb2+} = 10 mkg/ml; τ = 72 h
PAA – 1; [BC:PAA] = [1:10] – 2; [3:10] – 3; [5:10] – 4.

Figure 3 – Sorption kinetics of cryogels in lead solution



Crygel BC-PAA; NaOH = 10%; CA = 1,0%;
Initiator = 0,5%; C_{Cd2+} = 10 mkg/ml; τ = 72 h
PAA – 1; [BC:PAA] = [1:10] – 2; [3:10] – 3; [5:10] – 4.

Figure 4 – Sorption kinetics of cryogels in cadmium solution

Conclusion

Composite cryogels have been synthesized based on bentonite clay-polyacrylic acid at different time and temperature by radical polymerization. Among these was selected the most optimal – the received cryogel at a temperature of -30 °C for 24 hours.

The physicochemical properties of the produced cryogels based on BC-PAA are studied, their density is approximately 1,5531 g/cm^3, the yield is 95 %. It shows that, cryogels – economic favorable. Morphological structures composite cryogels are investigated and in result of what existence of a time and education, uniform and mutually compatible, negatively loaded composite cryogels have been revealed. Physico-chemical studies have shown that the components of the formation form a complex in terms of the water connections stabilized by hydrophobic interactions. Values of sorption of BC-PAA composite cryogels from PAA cryogel is considerable in difference high (~90 %).

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