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CARACTERÍSTICAS DAS RAÇAS AUTÓCTONES DA RÚSSIA E DO CAZAQUISTÃO DE ATRAVÉS DO TESTE DE MICRONÚCLEOS**CHARACTERISTICS OF AUTOCHTHONOUS BREEDS OF RUSSIA AND KAZAKHSTAN BY MICRONUCLEAR TEST****ХАРАКТЕРИСТИКА АВТОХТОННЫХ ПОРОД РОССИИ И КАЗАХСТАНА ПО МИКРОЯДЕРНОМУ ТЕСТУ**

MARZANOV, Nurbiy S.¹; MARZANOVA, Saida N.²; BAITLESSOV, Yerbulat U.³; BOZYMOVA, Aigul K.³; DAVLETOVA, Ainur M.⁴;

¹Laboratory for Molecular Bases of Selection, Ernst Federal Science Center for Animal Husbandry, Podolsk, Russian Federation

²Department of Immunology and Biotechnology, Skryabin Moscow State Academy of Veterinary Medicine and Biotechnology, Moscow, Russian Federation

³Department of Ecology and Biotechnology, West-Kazakhstan Innovative-Technological University, Uralsk, Republic of Kazakhstan

⁴Department of Higher School of Livestock Production Technology, Zhangir Khan West Kazakhstan Agrarian-Technical University, Uralsk, Republic of Kazakhstan

* Correspondence author
e-mail: nmarzanov@yandex.ru

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RESUMO

O artigo é dedicado aos resultados do teste de micronúcleos em duas raças autóctones bem conhecidas da Federação Russa e da República do Cazaquistão: Romanov e Edilbay. Nas ovelhas Romanov, as maiores taxas de ocorrência de micronúcleos foram encontradas em cordeiros ($8,7 \pm 0,9$), depois em ovelhas produtivas ($5,6 \pm 0,03$ ‰) e ovinos ($4,8 \pm 0,3$ ‰). A raça Edilbay apresenta diferenças nos resultados da análise de micronúcleos nos eritrócitos, os quais são mais pronunciados nos dois tipos da mesma raça: "Birlik" e "Suyunduk". A coudelaria "Birlik" está localizada nos Urais, na região oeste do Cazaquistão. Ovelhas Edilbay da raça Suyunduk são criadas na coudelaria "Makash", na região (óblast) de Atyrauz, na República do Cazaquistão, localizada próximo ao local de teste nuclear de Azgir. A diferença no teste de micronúcleos deve-se ao fato de que as ovelhas Suyunduk serem expostas a fatores de estresse ambiental devido à proximidade de locais com alto nível de contaminação por radionuclídeos. O efeito constante da seleção natural promove a reprodução de animais com maior resistência a fatores de estresse ambiental. Isso explica a baixa ocorrência de células com anormalidades citogenéticas em ovelhas do tipo "Suyunduk" Edilbay. Esse tipo tem maior potencial de adaptação a condições desfavoráveis de reprodução do que as ovelhas da raça Edilbay Birlik. Um alto nível de indicadores de teste de micronúcleos nas ovelhas Romanov está associado às características do sexo e faixas etárias, bem como à carga ambiental das condições climáticas na Rússia Central. A classificação dos micronúcleos é proposta. Acredita-se que os micronúcleos grandes e médios sejam formados sob a influência de vários mutagênicos no corpo, causando uma grande divisão de cromossomos individuais na metáfase e na anáfase. Pequenos micronúcleos indicam uma diminuição na viabilidade celular potencial.

Palavras-chave: ovelhas, eritrócitos, estresse ambiental, distúrbios citogenéticos.

ABSTRACT

The article is devoted to the results of the micronucleus test in two well-known autochthonous sheep breeds of the Russian Federation and the Republic of Kazakhstan: Romanov and Edilbay. In Romanov sheep, the highest rates of micronucleus occurrence were found in lambs (8.7 ± 0.9 ‰), than in producing rams (5.6 ± 0.03 ‰) and ewes (4.8 ± 0.3 ‰). The Edilbay breed has the differences in micronucleus test results in erythrocytes are most pronounced between two intrabreed types: "Birlik" and "Suyunduk". The breeding plant "Birlik" is located in the Ural,

the West Kazakhstan region. "Suyunduk" type of Edilbay breed sheep are raised in the breeding plant "Makash" in the Atyrauz region of the Republic of Kazakhstan, located near the Azgir nuclear test site. The difference in the micronucleus test is due to the fact that sheep of the "Suyunduk" type are exposed to environmental stress factors due to the proximity of places with high levels of radionuclide contamination. The constant action of natural selection contributes to the reproduction of animals with increased resistance to environmental stress factors. This explains the low occurrence of cells with cytogenetic abnormalities in sheep of the "Suyunduk" Edilbay breed sheep type. This type has a higher potential for adaptation to adverse breeding conditions than the Birlik type of Edilbay breed sheep. The high level of micronucleus test indicators in sheep of the Romanov breed is associated with the peculiarities of gender and age groups, and also the environmental stress of climatic conditions in Central Russia. A classification of micronucleus is proposed. It is believed that large and medium-sized micronucleus are formed by the action of various mutagens on the body, causing a large in the division of individual chromosomes in metaphase and anaphase. Small micronucleus indicate a decrease in potential cell viability.

Keywords: *sheep, red blood cells, environmental stress, cytogenetic abnormalities.*

АННОТАЦИЯ

Статья посвящена результатам микроядерного теста у двух известных автохтонных пород овец Российской Федерации и Республики Казахстан: романовской и эдильбаевской. У овец романовской породы самые высокие показатели встречаемости микроядра были обнаружены у ягнят ($8,7 \pm 0,9$), затем у баранов-производителей ($5,6 \pm 0,03$ %) и овцематок ($4,8 \pm 0,3$ %). Эдильбаевская порода имеет различия в результатах анализа микроядра в эритроцитах, которые наиболее выражены у двух внутривидовых типов: «Бирлик» и «Суюндук». Племенной завод «Бирлик» расположен на Урале, в Западно-Казахстанской области. Овец эдильбаевской породы типа «Суюндук» разводят на племенном заводе «Макаш» в Атырауской области Республики Казахстан, расположенном недалеко от Азгирского ядерного полигона. Разница в микроядерном тесте связана с тем, что овцы типа «Суюндук» подвержены факторам стресса окружающей среды из-за близости к местам с высоким уровнем радионуклидного загрязнения. Это способствует размножению животных с повышенной устойчивостью к факторам стресса окружающей среды, что объясняет низкую встречаемость клеток с цитогенетическими аномалиями у овец типа «Суюндук» эдильбаевской породы. Этот тип обладает более высоким потенциалом для адаптации к неблагоприятным условиям размножения, чем овцы типа «Бирлик». Высокий уровень показателей микроядерного теста у овец романовской породы связан с особенностями половой и возрастной групп, а также с экологической нагрузкой климатических условий в Центральной России. Предложена классификация микроядра. Считается, что крупные и средние микроядра образуются под действием различных мутагенов на организм, вызывая большое деление отдельных хромосом в метафазе и анафазе. Мелкие микроядра указывают на снижение потенциальной жизнеспособности клеток.

Ключевые слова: *овцы, эритроциты, экологический стресс, цитогенетические нарушения.*

1. INTRODUCTION

Bioindication, as an integral part of biomonitoring, has developed as a methodology for scientific research, and this is especially important since today, consequence of scientific and technological progress have become an objective reality (Gorovtsov *et al.*, 2017; Jiyenbekov *et al.*, 2018; Minakova *et al.*, 2018; Hodov *et al.*, 2019). The use of methods based on morphogenetic indicators of developmental stability and cytogenetic homeostasis are a promising direction since they meet practically all the requirements of biomonitoring (Udroiu, 2007; Udroiu and Sgura, 2017; Bakhtiar *et al.*, 2017; Cox *et al.*, 2019; Belhadj Slimen *et al.*, 2019).

Morphogenetic indicators characterize the level of cytogenetic stability of an individual organism and can form its phenotype under the conditions of a specific environment (Zakharov,

and Trofimov, 2017; Kolosov *et al.*, 2017; Mukhametzharova *et al.*, 2018). Cytogenetic homeostasis can be studied using a micronucleus test, the essence of which is to calculate the number and volume of cells with micronuclei (Shapiro *et al.*, 2003; Astafieva, 2017; Gülden Yilmaz and Gül, 2017; Monte *et al.*, 2019; Sabbioni *et al.*, 2018; Maass *et al.*, 2018; Wu *et al.*, 2019).

The micronucleus test is one of the methods for determining substances that exhibit genotoxic effects on the body (Hristova *et al.*, 2017; McAllister *et al.*, 2017; Luijten *et al.*, 2018; Lu *et al.*, 2018). Literary data indicate that the micronucleus test is not inferior, and sometimes even exceeds the chromosomal aberration tests in terms of informativeness and efficiency (Ilinskikh *et al.*, 2011; Sabbioni *et al.*, 2019). The micronucleus test developed by W. Schmid (Schmid, 1975). It is based on using in vivo bone marrow cells of higher vertebrates, fish, and

amphibians as biomarkers (Albarella *et al.*, 2019; Žaja *et al.*, 2019). However, in recent years, it has been replenished with a number of new techniques (analysis of micronuclei obtained by a block of cytokinesis, analysis of rodent erythrocytes micronuclei, and other similar techniques) that cover animal and plant cells, as well as various types of smear staining (Schmid, 1975; Fenech, 2000; Ilinskikh *et al.*, 2011; Astafieva *et al.*, 2015; Hayashi, 2016; Wilde *et al.*, 2017; Afusat *et al.*, 2017; Arieli, 2017; Dossybayev *et al.*, 2019).

2. MATERIALS AND METHODS

Peripheral blood samples for research were taken from 69 sheep of two breeds: Romanov and Edilbay. Sheep (n=35) belonged to the Romanov breed were taken from breeding plants Yaroslavl region. The Edilbay breed was represented by two interbreed types: "Birlik" (n = 16) and "Suyunduk" (n = 18) from the breeding plant "Volgograd–Edilbay" Volgograd region. This farm purchased sheep of the "Birlik" type from the "Birlik" breeding enterprise of the West Kazakhstan region, and "Suyunduk" from the "Makash" of the Atyrauz region the Republic of Kazakhstan. Blood from the jugular vein was taken in tubes with a 0.5% EDTA solution. Smears were made on glass slides, thoroughly washed and degreased using a mixture of Nikiforov (a mixture of equal volumes of ethyl alcohol and anhydrous sulfuric ether; It is used for fixing blood products, bacterial preparations and organ prints).

To count erythrocytes with micronucleus, a drop of peripheral blood was diluted with the physiological solution (1:1), and smears were prepared on the glasses, which were fixed with methyl alcohol and dried at room temperature. Smears were stained using the Pappenheim method using Mai-Grunwald and Giems solutions (Romejs, 1953; Sarkisov and Perov, 1996; Tabecka-Lonczynska *et al.*, 2019; Younas *et al.*, 2013).

Red blood cells with micronuclei were counted for each test animal in 37 workers. In each field, approximately 80 erythrocytes were found, which is 3000 cells. For the convenience of analysis, the total figure was divided by three. The results are presented per 1000 cells. The obtained data were expressed in ppm (‰) – the ratio of the number of erythrocytes with micronucleuses to the total number of cells counted. Cell analysis was carried out using a Motic binocular microscope with a built-in digital camera (DMBA300), an increase of 1000 (Astafieva *et al.*, 2015; Astafieva,

2017).

The statistical significance of differences in the frequencies of cytogenetic anomalies between breeds and groups of animals was evaluated by Student's t-test. A difference of $P < 0.05$ and more were considered statistically significant (Glanc, 1999).

3. RESULTS AND DISCUSSION:

The results of the micronucleus test on the Romanov breed of sheep are presented in Table 1. It was found that the highest rates of occurrence of micronucleuses were detected in lambs (8.7 ± 0.9), then in rams (5.6 ± 0.03 ‰), and in third place were ewes (4.8 ± 0.3) (Table 1).

There is a significant difference in micronucleus dough between rams and ewes ($P < 0.01$) and between adult groups of animals and young animals ($P < 0.01$), which may be due to the rigid selection of producers for the tribe and age since blood was taken from old sheep. We believe this is due to the relative immaturity of the cellular component of the immune system in lambs, which is responsible for the elimination of red blood cells with micronucleus. Our results for young animals confirm the data on the increased level of micronucleus in comparison with adults. Two interbreed types of Edilbay breed "Birlik" and "Suyunduk" were studied in comparison with the obtained materials on Romanov breed (Table 2).

The differences in the results of the micronucleus test in erythrocytes are most pronounced between different interbreeding types. In the Edilbay sheep breed "Birlik" type, the frequency of occurrence of erythrocytes with micronucleus (4.6 ± 0.3) was higher compared to "Suyunduk" sheep type (3.5 ± 0.2). According to the presented data, it is clear that the frequency of occurrence of erythrocytes with micronucleus in individuals of the Romanov breed (5.8 ± 1.18 ‰) is even higher than that of interbreeding types of the Edilbay breed. The reason for the differentiation of the "Suyunduk" and "Birlik" sheep breed types may be that the breeding plants were located in different environmental conditions. The breeding plant "Birlik" is located in the Ural region, in the West Kazakhstan region.

The "Suyunduk" type of Edilbay sheep breed were descended from the breeding plant „Makash" in the Atyrauz region of the Republic Kazakhstan. The breeding plant "Makash" is located next to the well-known Azgir nuclear test site of the Volgograd region Russian Federation. The observed micronucleus differentiation is due

to the fact that sheep of the "Suyunduk" Edilbay sheep breed type were subjected to constant environmental stress factors due to the proximity of their breeding sites to the region with high levels of radionuclide contamination (Astafieva *et al.*, 2015; Astafieva, 2017).

The chronic action of natural selection contributes to the reproduction of animals with increased resistance to environmental stress factors. This probably explains the relatively low frequency of occurrence of cells with cytogenetic abnormalities in animals of "Suyunduk" Edilbay sheep breed type. The "Suyunduk" type of Edilbay sheep breed has a higher potential for adaptation to unfavorable breeding conditions than the "Birlik" type, which is the result of the action of natural selection in generations. As for the high level of performance in Romanov sheep along with the characteristics of gender and age groups, the environmental stress of the climatic conditions of Central Russia is possible. Thus, the micronucleus test is one of the methods for determining the influence of substances that exhibit genotoxic effects on the body. Microkernels are fragments of the nucleus in eukaryotic cells that do not contain the complete genome necessary for its survival (Figures 1 and 2).

The size of micronucleus depends on the reasons underlying their formation. It is believed that large and medium micronucleus are formed by the action of various mutagens on the body, which causes a large in the division of individual chromosomes in metaphase and anaphase. At the same time, small micronucleuses indicates a decrease in the potential viability of cells (Figure 3).

4. CONCLUSIONS:

As a result of the study, in accordance with the sizes of micronucleus and the forms of their occurrence, a classification consisting of 5 points was proposed. When determining the size of a micronucleus, they proceeded from the average size of the normoblast core, averaging 1.5 microns. The classification includes the presence of five types of occurrence of micronucleus in the cell:

1. The first type is one or several large micronuclei in erythrocytes, up to 1/4 the size of an average normoblast nucleus.
2. The second type is one micronucleus in the erythrocyte, ranging in size from 1/15 to 1/10 of the average size of the normoblast core.
3. The third type is one small micronucleus

in the erythrocyte, 1/40 of the average size of the normoblast nucleus.

4. The fourth type – micronucleus, which are several small formations, in an amount from 2 to 10 pieces, the size of a microkernel of the 2nd and 3rd types, i.e., from 1/40 to 1 / 15– 1/10 of the average size of the nucleus of a normoblast.

5. The fifth type is when both large and small micronucleuses are detected simultaneously in the studied red blood cells.

In the studied blood smears of sheep of two breeds, the Romanov and Edilbay, mainly large micronucleus were found. Most often, large micronucleus were one in a red blood cell, less often two. Small micronucleus were found in the amount of two, rarely three in one erythrocyte. In 5% of the total number of cells with micronucleus, there was a large micronucleus and one or two small sizes next to it. Only 10% of the total number of cells with micronucleus revealed the formation of small and medium sizes. Thus, a micronucleus test was carried out on the instability of the genetic apparatus in the erythrocytes of the blood of two autochthonous sheep breeds from the Russian Federation and the Republic of Kazakhstan. In Romanov sheep, the relationship between the level of micronucleus in erythrocytes and the age of animals was determined. A comparative characteristic is given for the occurrence of micronuclei in known sheep breeds of two countries: the Russian Federation and the Republic of Kazakhstan.

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Table 1. The frequency of occurrence of erythrocytes with micronucleus in the studied sheep of the Romanov breed (in ‰)

No.	Group of discovered animals	n	The average number of micronucleus per 1000 cells
1	Rams	8	5.6 ± 0.3
2	Ewes	20	4.8 ± 0.3
3	Lambs 2 months age	7	8.7 ± 0.9
4	Group average	35	5.8 ± 1.8

Table 2. The frequency of occurrence of erythrocytes with micronucleus in studied sheep breeds (‰)

Sheep breeds and interbreeding types	n	Number of micronucleuses per 1000 cells
Edilbay interbreeds types:		
Birlik	16	4.6 ± 0.3
Suyunduk	18	3.5 ± 0.2
Romanov	35	5.8 ± 1.8

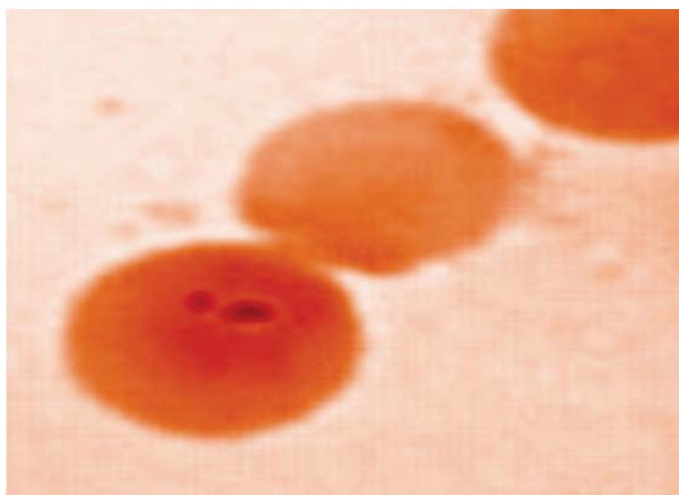


Figure 1. The sheep in the erythrocyte are represented large and medium micronucleuses

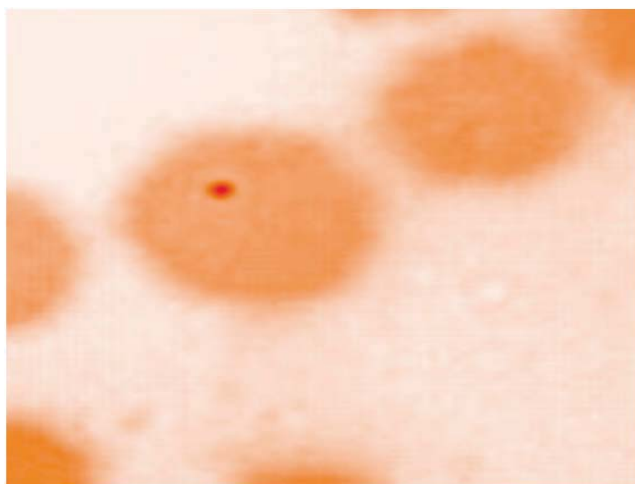


Figure 2. Middle micronucleus in a sheep erythrocyte

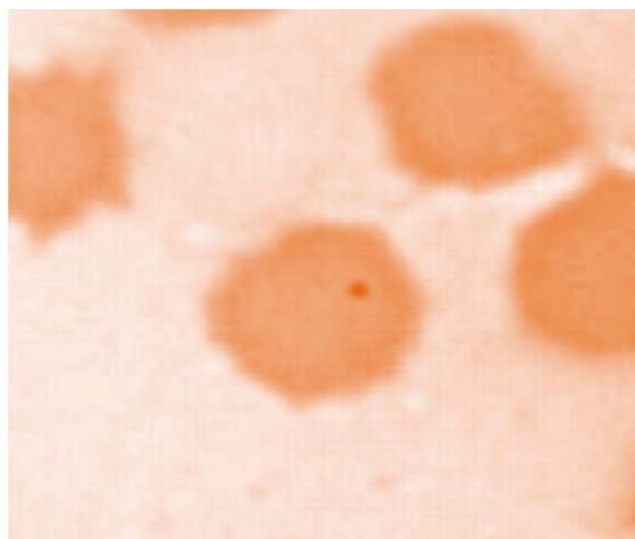


Figure 3. Erythrocyte in a sheep with a small micronucleus

ESTUDO DA PLASMASORÇÃO *IN VITRO* DE LIPOPROTEÍNA E PROPRIEDADES FÍSICO-QUÍMICAS DE SORBENTES POLIMÉRICOS COM GRUPOS AMINO SUBSTITUÍDOS**STUDY OF THE *IN VITRO* LIPOPROTEIN PLASMASORPTION AND PHYSICO-CHEMICAL PROPERTIES OF POLYMERIC SORBENTS WITH SUBSTITUTED AMINO GROUPS****ИЗУЧЕНИЕ СОРБЦИИ ЛИПОПРОТЕИНОВ ИЗ ПЛАЗМЫ *IN VITRO* И ФИЗИКО-ХИМИЧЕСКИХ СВОЙСТВ ПОЛИМЕРНЫХ СОРБЕНТОВ С ЗАМЕЩЕННЫМИ АМИНОГРУППАМИ**DORSKAIA, Elena Vladimirovna^{1*}; PESTOV, Sergei Mihaylovich²

¹ Mendeleev University of Chemical Technology of Russia, Faculty of Technology of Organic Substances and Chemical Pharmaceutical Compounds, Department of Expert Examination in Drug Testing and Narcoanalysis. Russia.

² Russian Technological University, Institute of Fine Chemical Technology, Department of Physical Chemistry. Russia.

* Correspondence author
e-mail: edrhtu@mail.ru

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RESUMO

Atualmente, o método de plasmassorção é amplamente utilizado na prática clínica para remover quantidades excessivas de metabólitos que causam várias patologias. No entanto, os plasmassorbentes aplicados muitas vezes podem fornecer vários efeitos colaterais para o paciente, como a coagulação do sangue, a liberação de partículas de poeira e a absorção de componentes úteis do plasma. Além disso, eles podem ser bastante caros. O objetivo do trabalho foi criar plasmassorbentes disponíveis com boa hemocompatibilidade para a adsorção de lipoproteínas do plasma. Foram sintetizados vários trocadores de ânions com grupos de trimetilamina, monoetanolamina e trietanolamina. Para os sorventes obtidos, foram investigadas as propriedades de adsorção e físico-química. Densidade aparente, volume específico no intumescimento, coeficiente de intumescimento em vários solventes (água, etanol, isopropanol, propilenoglicol, acetona) foram determinados e os cálculos teóricos da capacidade total de troca e massa de reticulação e frações molares foram realizados. Verificou-se que o melhor inchaço é alcançado no propilenoglicol. Para os sorventes LP27 e LP29, foram determinadas as capacidades nas frações de triglicérides e lipoproteínas e o coeficiente de distribuição dos componentes do perfil lipídico entre as fases sólida e líquida. É mostrado que ambos os sorventes têm boas propriedades de sorção, sendo a amostra LP29 bastante promissora para a extração dos componentes do perfil lipídico. Presume-se que os sorventes desenvolvidos sejam mais baratos que os atualmente utilizados, pois são sintetizados com base na matriz disponível comercialmente. Espera-se que os resultados obtidos sejam úteis para investigações adicionais visando à melhoria da qualidade da moderna tecnologia de plasmassorção.

Palavras-chave: *Extração de LDL e VLDL, sorção de colesterol, aterosclerose, hipercolesterolemia, doenças cardiovasculares.*

ABSTRACT

Currently, the plasmadsorption method is widely used in clinical practice to remove excessive amounts of metabolites that cause various pathologies. However, the plasmadsorbents applied often can give a number of side effects for the patient, such as the blood clotting, the release of dust particles, and the sorption of useful components from the plasma. Besides that, they can be quite expensive. The aim of the work was to create available plasmadsorbents with a good hemocompatibility for the lipoprotein adsorption from the plasma. A number of anion exchangers with groups of trimethylamine, monoethanolamine, and triethanolamine were synthesized. For the sorbents obtained, adsorption and physico-chemical properties were investigated. Bulk

density, specific volume at swelling, the swelling coefficient in various solvents (water, ethanol, isopropanol, propylene glycol, acetone) were determined and the theoretical calculations of total exchange capacity and crosslinking mass and molar fractions were made. It was found that the best swelling is achieved in propylene glycol. For the sorbents LP27 and LP29, the capacities on triglyceride and lipoprotein fractions and the coefficient of distribution of lipid profile components between the solid and liquid phases were determined. It is shown that both sorbents have good sorption properties, the sample LP29 being quite promising for the lipid profile components extraction. The developed sorbents are assumed to be cheaper than currently used ones as they are synthesized on the basis of commercially available matrix. The results obtained are expected to be useful for further investigations aimed at the modern plasmasorption technology quality improvement.

Keywords: *LDL and VLDL extraction, cholesterol sorption, atherosclerosis, hypercholesterolemia, cardiovascular diseases.*

АННОТАЦИЯ

В настоящее время метод плазмсорбции широко используется в клинической практике для удаления избыточного количества метаболитов, вызывающих различные патологии. Однако используемые плазмсорбенты зачастую могут вызывать ряд побочных эффектов, таких как повышение свертываемости крови, выделение частиц пыли и извлечение полезных компонентов из плазмы. Кроме того, они могут быть достаточно дороги. Целью данной работы было создание доступных плазмсорбентов, обладающих хорошей гемосовместимостью, для извлечения липопротеинов. Был синтезирован ряд анионитов с группами триметиламина, моноэтаноламина и триэтаноламина и исследованы их адсорбционные и физико-химические свойства. Определены насыпная плотность, удельный объем при набухании, коэффициенты набухания в различных растворителях (вода, этанол, изопропанол, пропиленгликоль, ацетон), а также рассчитаны теоретические значения полной обменной емкости и массовой и мольной долей сшивающего агента. Показано, что наилучшее набухание достигается при использовании в качестве растворителя пропиленгликоля. Для образцов ЛБ27 и ЛБ29 определены адсорбционные емкости по триглицеридам и фракциям липопротеинов и рассчитаны коэффициенты распределения компонентов между твердой и жидкой фазами. Показано, что оба сорбента имеют хорошие сорбционные свойства. Наиболее перспективным для извлечения компонентов липидного спектра является образец ЛБ29, имеющий достаточно высокие емкости по всем компонентам. Предполагается, что разрабатываемые сорбенты будут дешевле, чем используемые в настоящее время, благодаря использованию для их синтеза доступного промышленного анионита. Полученные результаты могут быть полезны для дальнейших исследований, направленных на улучшение качества и доступности современных плазмсорбционных технологий.

Ключевые слова: *извлечение ЛПНП и ЛПОНП, сорбция холестерина, атеросклероз, гиперхолестеринемия, сердечно-сосудистые заболевания.*

1. INTRODUCTION

The plasmasorption method is widely used in clinical practice for the treatment of pathologies caused by the increased content of certain substances in the blood. In particular, it is prescribed for the treatment of atherosclerosis caused by an increased content of low- and very low-density lipoprotein cholesterol (LDL and VLDL) in blood plasma.

The surface of lipoprotein particles is negatively charged; accordingly, copolymers with substituted amino groups having a positively charged quaternary nitrogen atom were chosen as plasmasorbents. Various nitrogen atom substituents affecting the electron density shift were used. In that way, it was possible to adjust the magnitude of the positive charge of the sorption center and its hydrophilic-

hydrophobic properties.

There are a number of studies into this subject that have been carried out in recent times; (Bereli *et al.*, 2011; Cao *et al.*, 2011; Dorskaia *et al.*, 2019a; Dorskaia *et al.*, 2019b; Gan *et al.*, 2016; Hou *et al.*, 2013; 2015; Huang *et al.*, 2010; Huang *et al.*, 2011; Huang *et al.*, 2009; Khattari, 2016; Lan *et al.*, 2012; Li *et al.*, 2014; Li *et al.*, 2011; Li *et al.*, 2013; Liu *et al.*, 2016; Liu *et al.*, 2012; Liu *et al.*, 2015; Lu *et al.*, 2013; Lu *et al.*, 2011a; Lu *et al.*, 2011b; Ma *et al.*, 2011; Ma *et al.*, 2008; Teruel *et al.*, 1995; Wang *et al.*, 2016; Wang *et al.*, 2014; Wang & Lan, 2014; Yu, 2013; Yu *et al.*, 2014; Zheng *et al.*, 2011) can be noted. In (Lu *et al.*, 2011a; Lu *et al.*, 2011b) the extraction of lipid profile components by sorbents based on carbon nanotubes modified with various agents was studied. Papers (Hou *et al.*, 2013; 2015; Huang