



Changes of metabolism in animals due to conditions of a purulent wound process while using photomineralisation as dressing tools

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Abstract

Annotation: the wound process is difficult, multicomponent and has its own a phase of character. Recently, there has been an active search for new products for the treatment and prevention of purulent wounds. The effect of sorbents on the process of wound healing is being studied. The impact of the damaging factor causes the development of inflammation, which leads to immediate biochemical changes. During primary aeration, cells are destroyed and their contents are easily transported to the external environment. Previously, a decrease in the concentration of total protein in the blood has already been noted. At the first stage of the wound process, one part of the protein is transferred to the exudate, and the other goes to decay to amino acids. In terms of energy, disbalance amino acids enter into deamination and transamination reactions, as indicated by the high activity of alanine aminotransferase and aspartate aminotransferase as well as increased concentration of urea. The products of amino acid breakdown enter the Krebs cycle for subsequent energy production, which is necessary for further regeneration processes. Violations of the integrity of muscle tissue leads to the fact that the concentration of creatinine in the blood increases. Creatine phosphate due to the destruction of muscle fiber does not mobilize the generation of ATP, but reacts to non-enzymatic dephosphorylation with the formation of creatine anhydride - creatinine.

Keywords: wound process, metabolism, phytonitratosorbents, montmorillonite, biochemical changes, metabolism, inflammation process

Krut UA, Oleynikova II, Radchenko AI, Kuzubova EV (2019) Changes of metabolism in animals due to conditions of a purulent wound process while using photomineralisation as dressing tools. Eurasia J Biosci 13: 1-5.

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INTRODUCTION

Treatment of patients with purulent wounds is still one of the most important problems of clinical surgery. Currently, most practitioners and researchers of purulent processes follow to phase treatment of purulent wounds (Abaev 2006, Gaidul and Mukhin 2005, Gorokhov 2016, Kondratenko and Sobolev 2007, Pervushkin et al. 1998, Vinik et al. 2013). During inflammation, metabolism changes not only in the area of damage, but also in other organs and tissues. Previously, a study of changes in the wound focus (Shaposhnikov et al. 2016), was carried out, there was an increase in the concentration of total protein in wound exudate and glucose, the pH of the exudate was changed to the acidic side. In that way, the part of the protein which has entered the wound surface is exposed to microorganisms. As a result, of processes of decay products of amino acids are formed, in particular such toxic substances such as phenol and indole, which easily penetrate into the internal environment (Ohunov et al. 2018). Considering the infection of the wound, the vast majority of practitioners

use antibiotics. However, considering the high rate of mutation of hospital strains of microorganisms, antibiotics can lead to resistant resistance and reinfection of the wound. Practitioners are increasingly in alternative methods of treatment of purulent wounds (Mayorov et al. 2018). By now, developed and implemented a variety of different methods and methods of treatment of wound processes, but none of them fully satisfy the surgeons (Gatalullin et al. 2018, Solov'ov et al. 2003). Therefore, the flow of new proposals does not decrease, and the issues of treating wounds and wound infections continue to attract the attention of authors and practitioners (Pods 1982). The success of treatment of patients with purulent wounds depends largely on local treatment. In this connection, it remains important to search for new methods and means of such treatment with multidirectional action, providing antimicrobial, anti-

Received: February 2019

Accepted: May 2019

Printed: June 2019

Inflammatory and reparative effect (Magomedov 2011). In particular, there is an increasing interest in sorption wound coverings, which allow reducing exudations. Also in a number of publications (Shaposhnikov et al. 2016) it is noted that many sorption wound coverings inhibit the growth of pathogenic microflora, which allows us to consider sorbents as antimicrobial agents.

The aim of our research was to study the dynamics of the General biochemical parameters of blood of laboratory rats in accordance with the phases of the wound process using phytonitratosorbents.

MATERIALS AND METHODS

The research of wound healing properties of phytonitratosorbents in the form of powder and gel, as well as spray Dexpanthenol (Pharmstandard, Russia) was carried out on white linear rats Wistar (80 PCs.), which were selected as a biological model (Tiganov et al. 2018).

Phytonitratosorbents is a complex sorption composite based on inorganic minerals of the montmorillonite group and of the extract of the medicinal plants *Thymus serpyllum*. Due to the high sorption capacity, this agent has a depressing effect on the pathogenic microflora (Vezenshev and Trubitsin 2012, Vezenshev et al. 2010) and is a good neutralizer of the acidic environment (Bukhanov et al. 2014). Biologically active substances included in *Thymus serpyllum* extract have antiseptic properties and stimulate regeneration processes (Garrick et al. 2009, Shitaba et al. 2008). For ease of use in the application of wounds phytonitratosorbents used in two forms: powder and gel.

Rats were simulated purulent wounds. For 4 hours before the operation was canceled feed and water. Anesthesia was carried out by chloral hydrate (300 mg/kg) and zolteil (150 mg/kg) intraperitoneally. Wounds were inflicted in the interscapular area with an average diameter of 2.83 cm², which was 1% of the entire surface of the skin of rats. A special port was sewn into the injured area using a purse-string suture (for the collection of exudate and local study of the wound), and a certain means was added to it that corresponded to the experimental groups. Then the strain of *Escherichia coli* was applied to the wound area in a fixed dose of 2·10⁶ microbial cells.

All studies were carried out in compliance with the requirements of the "Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" adopted by the Council of Europe (Strasbourg, France, 1986) and Council Directive 86/609/EEC of 24.11.19.

The size of the wound surface was measured of animals during the whole observation period. The study of blood of laboratory animals was carried out on each phase of the wound process on the third, ninth and

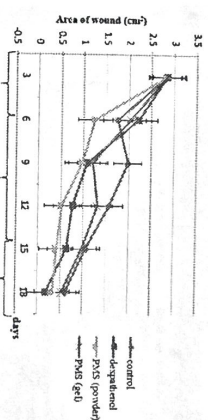


Fig. 1. Change in the area of the wound defect I - inflammatory, II - regeneration, III - reorganization of scar phase of wound healing

fifteenth days. Blood sampling for General and biochemical analysis was carried out from the tail vein into tubes with EDTA anticoagulant, under the action of anesthesia stage I, using analgesics.

Blood tests were performed on a hematological analyzer "Sysmex XE-300" (Germany), and biochemical "Sobas 4000" (Germany) with standard reagent kits.

Statistical analysis of the results was performed using the method of variance analysis, we calculated the average and the variance of the factorial (D_{max}) and deviation from the middle (m). The reliability of the results of the study was determined by the Fisher method by the criterion of t-student. The significance level $p < 0.05$ was taken as reliable (Samson et al. 2018).

Correlation analysis was performed with the determination of the correlation coefficient by the criterion of Pearson's agreement. Multiple regression model was constructed from the most statistically significant indicators, data on the change in the wound defect were selected as the effective trail. All calculations were performed using MS Excel XP software (Lapach et al. 2001).

RESULTS AND DISCUSSION

On the 18th day in the group, where wounds were treated with the gel form of phytonitratosorbents, the area of the wound surface was significantly lower than the initial value by 81% (Fig. 1). The area of the wound surface in animals where Dexpanthenol was used was the lowest (on average 65% less than other groups). However, the use of phytonitratosorbents in the form of powder stimulated a earlier decrease in the area of the wound defect, than in other animals. Biochemical changes in the body of animals and humans underlie the development of various types of external and internal inflammatory processes.

The biochemical status of laboratory animals on the 3rd day of the experiment is presented in Table 1. The total protein content was higher than normal only in the control group. By 16%, however, physiological fluctuations of total protein in the blood serum mostly depend on changes in the volume of the liquid part of the

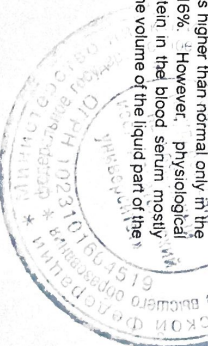


Table 1. Biochemical analysis of rats blood on the 3rd day after the application of a model wound (Mx.m), n=5

Indicator	Experimental group		Control			
	Basal index	I	II	III	IV	V
Total protein (g/l)	74.78 ± 7.232	68.75 ± 1.410	70.87 ± 4.247	65.00 ± 3.372*	68.13 ± 1.922	68.13 ± 1.922
Albumin (g/l)	37.75 ± 1.842	35.53 ± 0.849	35.50 ± 1.777	35.00 ± 1.686*	38.13 ± 0.592	38.13 ± 0.592
Albumin aminotransferase (u/l)	3840 ± 6.380	42.03 ± 0.843	42.03 ± 0.843	42.03 ± 0.843	42.03 ± 0.843	42.03 ± 0.843
Aspartate aminotransferase (u/l)	97.80 ± 8.652	285 ± 1.613	67.20 ± 3.372	67.20 ± 3.372	67.20 ± 3.372	67.20 ± 3.372
Urea (mmol/l)	15.93 ± 5.054	33.03 ± 1.651	15.93 ± 5.054	15.93 ± 5.054	15.93 ± 5.054	15.93 ± 5.054
Creatinine (mmol/l)	150.33 ± 34.108	322.00 ± 7.847	27.63 ± 3.372	15.07 ± 1.686*	295.20 ± 4.292*	295.20 ± 4.292*
Bilirubin total (mmol/l)	2.63 ± 1.310	5.40 ± 0.521	350.67 ± 42.427	158.00 ± 16.858*	154.00 ± 6.743*	154.00 ± 6.743*
Glucose (mmol/l)	5.40 ± 0.521	3.51 ± 0.583	2.90 ± 0.153*	3.13 ± 0.098*	3.30 ± 0.160	3.30 ± 0.160
Note: here and everywhere below - significant differences from the corresponding indicators in animals with baseline, p < 0.05; * - significant differences in performance compared with group I of rats, p < 0.05						

Table 2. Biochemical analysis of blood of rats on the 9th day after application of a model wound (Mx.m), n=5

Indicator	Experimental group		Control			
	Basal index	I	II	III	IV	V
Total protein (g/l)	74.78 ± 7.232	56.70 ± 6.743	63.43 ± 5.150	59.71 ± 1.686	63.43 ± 5.150	63.43 ± 5.150
Albumin (g/l)	37.75 ± 1.842	27.33 ± 0.506*	31.63 ± 1.686	24.93 ± 1.369	31.63 ± 1.686	31.63 ± 1.686
Albumin aminotransferase (u/l)	38.40 ± 6.380	50.65 ± 6.380	51.41 ± 5.054	49.83 ± 6.743	51.41 ± 5.054	51.41 ± 5.054
Aspartate aminotransferase (u/l)	97.80 ± 8.652	135.93 ± 7.586	121.74 ± 8.555	120.43 ± 9.386	121.74 ± 8.555	121.74 ± 8.555
Urea (mmol/l)	15.93 ± 5.054	10.54 ± 5.058	13.41 ± 5.921	16.40 ± 5.064	15.93 ± 5.054	15.93 ± 5.054
Creatinine (mmol/l)	150.33 ± 34.108	168.00 ± 31.717	211.03 ± 28.808	201.50 ± 31.717	198.20 ± 2.752	198.20 ± 2.752
Bilirubin total (mmol/l)	2.63 ± 1.310	5.03 ± 1.686	5.14 ± 0.681	4.86 ± 0.506	4.86 ± 0.506	4.86 ± 0.506
Glucose (mmol/l)	5.40 ± 0.521	3.62 ± 0.592*	3.46 ± 0.341*	3.65 ± 0.506	3.43 ± 0.315	3.43 ± 0.315

blood and are associated with the synthesis or loss of protein. Also hemolysis which is observed in all experimental groups, gives a false positive increase in total protein by 3% for every 1 g of free hemoglobin per liter of blood serum (Gaimov et al. 2008). Thus, it can be assumed that the total protein in the experimental groups is significantly reduced. Albumins have a great influence on osmotic and oncotic pressure of the blood and, consequently, on the exchange of water between it and tissues. The amount of albumin was significantly higher than the background index in group III by 18%, which indicates dehydration of the organism.

In the blood serum of rats at this stage of the experiment increased alanine aminotransferase activity in groups I, III and IV by an average of 37%. The activity of aspartate aminotransferase was significantly higher than the background indicator in all groups by 27 times. An increased of activity of aminotransferases was noted in a number of pathological processes in which the liver is involved.

Urea concentration was higher than the initial value only in groups I and IV by 2.1 times and 1.7 times, respectively. The creatinine index was significantly higher than the norm in the control group in 2.1 times, and in group III in 2.3 times. Simultaneous increase in serum creatinine and urea levels is a sign of ammonia utilization in inflammatory process.

Bilirubin is a tetrapyrrole pigment formed in the process of catabolism of the heme portion of hemoglobin (protoporphyrin IX) of red blood cells that have completed their life cycle. The amount of total bilirubin corresponded to normal physiological parameters in all groups except control. In group I was observed an unreliable increase in bilirubin, possibly due to strong hemolysis of erythrocytes.

The level of glucose in the blood depends on the physiological state of the animal. Stress and changes in body temperature can simultaneously shift the glucose index, both in the direction of increasing concentration and in the direction of lowering. Glucose concentration in the I, II and III groups was below the norm by an average of 44%.

Analysis biochemical parameters of blood of rats on the 9th day after application of a model wound are presented in Table 2, showed that there was a tendency to decrease the concentration of total protein, while hemolysis of erythrocytes was absent and hematocrit was normal. The concentration of albumin was also significantly lower than the background value in all groups by an average of 28%. Thus, catabolic processes were dominated at the elimination of tissue damage prevailed in animals.

The catabolic processes in the organism of animals are also evidenced by the increased activity of aminotransferases. Alanine aminotransferase activity was above normal only in the II experimental group, by 34%. The activity of aspartate aminotransferase was significantly higher than the background value by an average of 34% in all groups of animals.

The concentration of urea and creatinine corresponded to normal physiological parameters in all experimental groups of animals. This may indicate a reduction in nitrogen metabolism.

It is worth noting that there was a tendency to a general increase in bilirubin, perhaps this may be due to intoxication of the body through the metabolic products of pathogenic bacteria.

On the 9th day of the experiment, as well as on the 6th day, the hypoglycemic state of animals was observed. Glucose concentration in all experimental rats

was significantly lower than the background value by an average of 35%.

The biochemical status of rats on the 15th day of the experiment is presented in Table 3. The index of total protein begins to recover to the norm, however, in group IV total protein was below the norm by an average of 33%. It can be assumed that in some groups anabolic processes begin to dominate over catabolic processes. Albumin content was below normal in groups I, III and IV by an average of 32%. Alanine aminotransferase activity was significantly higher than the background in group II by 1.8 times. The activity of aspartate aminotransferase was significantly higher than normal in all groups of experimental animals by an average of 2.2 times.

The decrease in muscle mass of laboratory animals and regular stresses during wound dressings may have led to a significant decrease in urea in groups I and II by an average of 3.4 times. The value of creatinine, also, was below the norm in animals of experimental groups on average 2.9 times.

The content of total bilirubin was higher than the background only in the group where Panthenol was used 2 times.

On the 15th day of the experiment glucose concentration increased 1.7 times in the II and III groups of rats. This this phenomenon regenerative processes.

CONCLUSION

Studies of biochemical analysis of the blood of rats with model skin-muscle wounds showed that the concentration of total protein on the 3rd and 6th day was reduced, by the 9th day there is an increase in the total protein content in the blood plasma, which indicates anabolic processes, about the processes of regeneration. Catabolic process is caused by primary and secondary tissue necrosis, phagocytosis, active proteolysis.

The albumin content at the beginning of the experiment was sharply high, which indicates dehydration of the body, as well as drying up. The fluid from the blood plasma rushed to the epicenter of the injury, thus causing swelling of the wound. By the end of the experiment, its concentration decreased, indicating that the infectious disease had been transferred.

The activity of aminotransferases throughout the experiment was increased, with a decrease by the end of the experiment. It should be noted that according to the research activity of alanine aminotransferase and aspartate aminotransferase increased by 2.5 times when using Panthenol, while the use phytonitralsorbits activity of these enzymes was only 2 times in the acute stage of the wound healing process.

Violations of nitrogen balance were observed on the 3rd day of the experiment. By the 9th day, urea and creatinine values were within the physiological norm.

Due to the strong erythrocyte hemolysis, the bilirubin index was increased in the first stage of the wound healing process. The hypoglycemic state of animals was observed for 3-9 days, which indicates depletion of the body and the fight against infection. By the end of the experiment, glucose levels recovered.

The results of this research convincingly indicate the prospects for the development of pharmacological approaches to the correction of purulent wounds photomineralization on the basis of montmorillonite enriched with extract of Thyme. In addition, in the role of phyto component can be extracts of other medicinal plants with antiseptic properties.

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Table 3. Biochemical analysis of blood of rats at 15 days after application of the model wound (Mx.m), n=5

Indicator	Experimental group		Control			
	Basal index	I	II	III	IV	V
Total protein (g/l)	74.78 ± 7.232	68.69 ± 0.951	69.53 ± 7.260	64.04 ± 1.490	68.13 ± 1.922	68.13 ± 1.922
Albumin (g/l)	37.75 ± 1.842	33.24 ± 0.692	35.05 ± 0.903	29.91 ± 1.490*	38.13 ± 0.592	38.13 ± 0.592
Albumin aminotransferase (u/l)	38.40 ± 6.380	67.80 ± 0.622	51.01 ± 0.700*	55.04 ± 0.892	46.71 ± 0.557	46.71 ± 0.557
Aspartate aminotransferase (u/l)	97.80 ± 8.652	137.60 ± 20.84	180.07 ± 1.901*	130.04 ± 0.193	138.02 ± 2.016*	138.02 ± 2.016*
Urea (mmol/l)	15.93 ± 5.054	4.63 ± 1.612	4.01 ± 2.250*	9.30 ± 5.753	7.70 ± 4.001*	7.70 ± 4.001*
Creatinine (mmol/l)	150.33 ± 34.108	67.05 ± 16.123	62.18 ± 30.407	59.04 ± 30.230	42.07 ± 3.892*	42.07 ± 3.892*
Bilirubin total (mmol/l)	2.63 ± 1.310	3.30 ± 1.357	5.23 ± 0.502	2.63 ± 1.301	2.99 ± 1.238	2.99 ± 1.238
Glucose (mmol/l)	5.40 ± 0.521	7.76 ± 0.527*	6.32 ± 0.584	10.44 ± 0.219*	6.03 ± 0.305	6.03 ± 0.305

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The biochemical status of rats on the 15th day of the experiment is presented in Table 3. The index of total protein begins to recover to the norm, however, in group IV total protein was below the norm by an average of 33%. It can be assumed that in some groups anabolic processes begin to dominate over catabolic processes. Albumin content was below normal in groups I, III and IV by an average of 32%. Alanine aminotransferase activity was significantly higher than the background in group II by 1.8 times. The activity of aspartate aminotransferase was significantly higher than normal in all groups of experimental animals by an average of 2.2 times.

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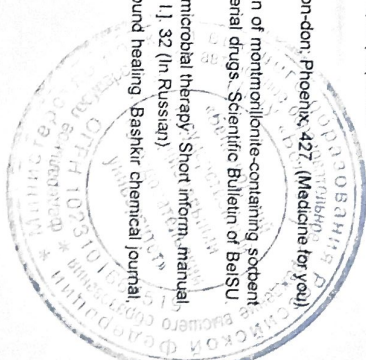
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2019

АНАЛИЗ ФАРМАЦЕВТИЧЕСКОГО РЫНКА РФ ПРОТИВОДИАРЕЙНЫХ

ПРЕПАРАТОВ

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Проанализирован ассортимент противодиарейных препаратов на российском рынке. Изучены лекарственные формы, действующие вещества, страны происхождения средств для лечения диарей.

Ключевые слова: лоперамид, диарея, лекарственная форма

Самым распространенным заболеванием в настоящее время, которое имеет пищевое и водное происхождение, является диарея. По данным ВОЗ, данное заболевание — одна из главных причин смертности в мире. Диарея занимает 8 место по частоте причин смерти. В 2018 году от этого заболевания погибло 1,39 млн человек, большую часть которых составляют дети. Ежегодно регистрируется около 1,7 млн случаев заболеваемости диареей. Этот факт делает данное заболевание глобальной проблемой [1].

И препаратов для лечения данного заболевания огромное количество. Так, например, для купирования диарей применяют лоперамид, гидрохлорид, неасваивающийся антибиотик рифаксимин, диоксазаринический смектит, пробиотики и др. [2].

Был проведен анализ противодиарейных препаратов промышленного производства.

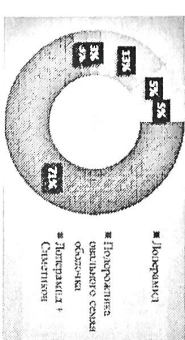


Рисунок 1. Анализ противодиарейных препаратов по действующему веществу

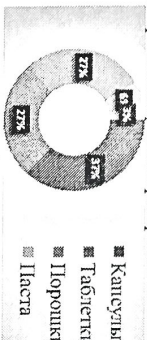


Рисунок 2. Анализ противодиарейных препаратов по лекарственной форме

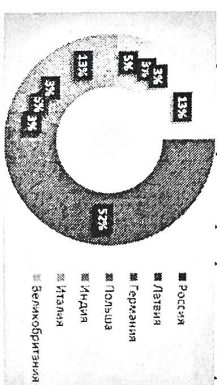


Рисунок 3. Анализ противодиарейных препаратов по стране происхождения

На сегодняшний момент основой терапии диарей являются энтеросорбенты [3].

На Рис. 3 представлена структура рынка противодиарейных препаратов по странам происхождения. Лидером среди стран производителей является Россия.

Такой скачок связан с выходом в апреле этого года на рынок новой формы препарата — суспензии для приема внутрь с действующим веществом — лоперамид.

Как видно из Рис. 2 Самыми популярными лекарственными формами являются капсулы 37% и таблетки 27%. Это связано с простотой и удобством применения данных препаратов.

По данным проведенного исследования можно сделать вывод, что действующее вещество — лоперамид занимает лидирующее место (рис. 1) на рынке противодиарейных средств. Данный факт можно объяснить механизмом действия данного препарата и его доказанной эффективностью.

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ANALYSIS OF THE RUSSIAN PHARMACEUTICAL MARKET OF ANTI-DIARRHEAL DRUGS

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The range of anti-diarrheal drugs on the Russian market is analyzed. Studied dosage forms, active substances, manufacturers of drugs for the treatment of diarrhea.

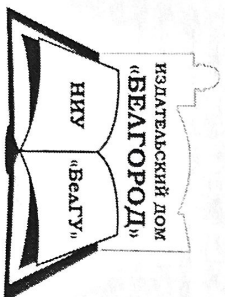
Keywords: loperamide, diarrhea, dosage form



Миниобнаучки России
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предположить, что с целью наиболее полного усвоения биологически активных веществ, целесообразно при создании функциональных продуктов питания использовать наноструктурированный экстракт пустырника.

С учетом положительного влияния на организм, предлагается вводить наноструктурированный экстракт пустырника в состав пробиотического лактомега – мармелада. При этом органолептические и физико-химические показатели мармелада остаются без изменений, что очень важно для потребителя, а биологическая ценность повышается, что актуально в условиях, когда организм человека не получает БАВ в достаточных количествах.

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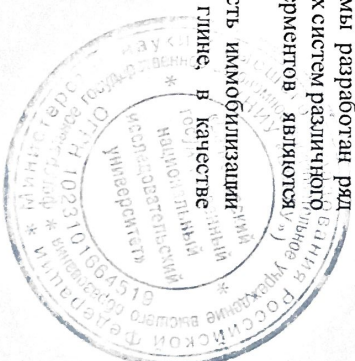
МОНТОРИНГОВЫЕ МИНЕРАЛЫ КАК ПЕРСПЕКТИВНЫЕ НОСИТЕЛИ ДЛЯ ИММОБИЛИЗАЦИИ СЕРИНОВЫХ ПРОТЕАЗ

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Сериновые протеазы широко используются для лечения заболеваний желудочно-кишечного тракта, однако эффективность их применения снижается при контакте с кислой средой желудка за счет ферментативной и кислотной дегградации. Для решения данной проблемы разработан ряд подходов, основанных на создании ферментсодержащих систем различного типа [1]. Одним из перспективных носителей ферментов являются бентонитовые глины, в частности, монтмориллонит.

Цель нашего исследования: изучить возможность иммобилизации сериновых протеаз на монтмориллонитсодержащей глине, в качестве действующего вещества выбран трипсин [2].



Для удаления примесей карбонатов и отгледения монтмориллонитовой фракции проводили обработку бентонитовой глины 10% соляной кислотой. Отмученную глину декантировали и высушивали.

Сорбционную активность монтмориллонитового минерала определяли по стандартной методике, в качестве маркера использовали раствор метиленового синего [3].

Сорбционная ёмкость исходного монтмориллонита до иммобилизации фермента составила 150мг/г; после иммобилизации - 20 мг/г. Что соответствует эффективной концентрации трипсина 3мг/г.

Протеолитическую активность трипсина проверяли по методике Ансона [4]. Ферментативная активность контрольного образца составляет 1.61 ПЕ/мл; протеолитическая активность иммобилизованного фермента составила 1.48 ПЕ/мл (рис.1.).

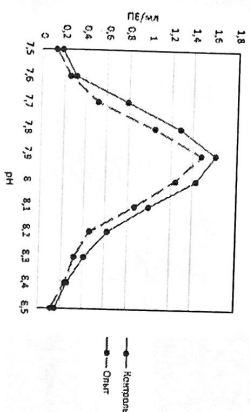
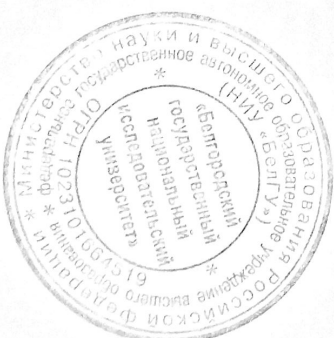


Рис.1. Протеолитическая активность трипсина.

Таким образом, использование монтмориллонитового минерала, матрицы является целесообразным для иммобилизации трипсина.

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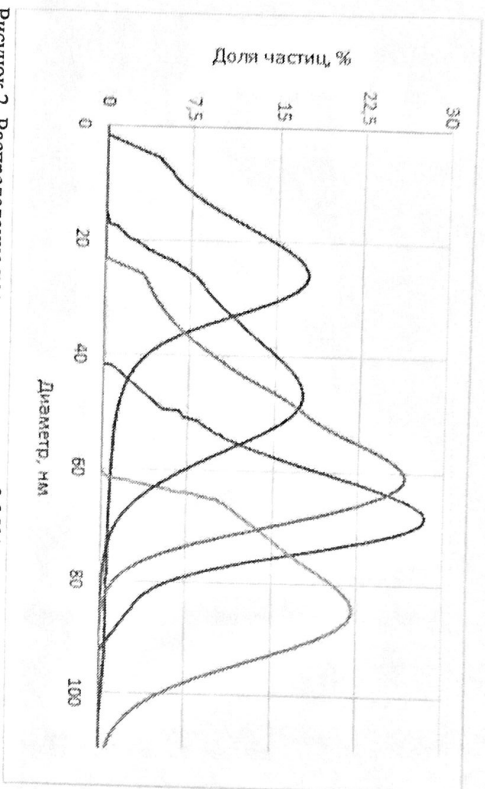


Рисунок 2. Распределение частиц по размерам в 0,35% растворе фосфолипидов 91-е сутки эксперимента.

Таким образом, можно сказать, что ультразвуковой метод является перспективным направлением в создании наноразмерных контейнеров для лекарственных препаратов. По сравнению с тепловым методом он показывает высокий диспергирующий результат, образуя устойчивую суспензию липосом со средним гидродинамическим размером частиц около 70 нм.

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