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Обзорная статья / Review article

Modern Research in the Field of Microencapsulation (Review)

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Abstract

Introduction. Microencapsulation is one of the promising areas for obtaining new dosage forms. The peculiarity of microencapsulated forms is that the substance is protected from the effects of various environmental factors that can cause their destruction (acidity of gastric juice, the effect of food, joint intake of other drugs, diseases of the gastrointestinal tract, etc.). This method is used for various groups of drugs, such as antibiotics, nootropics, vitamins, probiotics, anticonvulsants, enzymes. Particular attention should be paid to antibacterial drugs, since the possibility of microencapsulation solves one of the most important problems in antibiotic therapy – the resistance of microorganisms.

Text. The purpose of the review is to analyze modern research in the field of microencapsulation, to study trends and directions for the creation of microcapsules with high activity and bioavailability and with minimal side effects. The article provides brief information and main conclusions on the development of techniques and selection of conditions for microencapsulation of individual medicinal substances, on the study of polymers of various natures for use as carriers, on the methods of forming double shells of microcapsules, and also investigated the efficiency of microencapsulation of biologically active substances, such as antibacterial preparations, substances of plant and animal origin and preparations from various pharmacological groups. Variants of microencapsulation techniques for specific compounds that are suitable for substances similar in composition and action, as well as methods for creating microcapsules with double shells for compounds insoluble in water, are presented.

Conclusion. The article shows the achievements and prospects of using microencapsulation of medicinal substances and their advantages over standard dosage forms. The active introduction of the developed methods into production will allow the creation of new dosage forms with known medicinal substances that have a prolonged effect, which will reduce the frequency of use of the drug, as well as retain their activity under the influence of negative factors of the internal environment of the body. Also, in the form of microcapsules, the substances are more active in comparison with non-encapsulated substances.

Keywords: microencapsulation, polymer, prolonged dosage form, antibacterial drugs, enzymes, double shell, double emulsions, bioavailability, release

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

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Современные исследования в области микрокапсулирования (обзор)

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Резюме

Введение. Микрокапсулирование – одно из перспективных направлений получения новых лекарственных форм. Особенность микрокапсулированных форм заключается в том, что вещество защищено от воздействия различных факторов окружающей среды, которые могут вызвать их разрушение (кислотность желудочного сока, влияние пищи, совместный прием других препаратов, заболевания желудочно-кишечного тракта и т. п.). Данный метод применяется для различных групп препаратов, таких как антибиотики, ноотропы, витамины, пробиотики, противосудорожные препараты, ферменты. Особое внимание следует уделить антибактериальным препаратам, так как возможность микрокапсулирования решает одну из важнейших проблем терапии антибиотиками – резистентность микроорганизмов.

Текст. Цель обзора – анализ современных исследований в области микрокапсулирования, изучение тенденций и направлений по созданию микрокапсул с высокой активностью и биодоступностью и с минимальными побочными эффектами. В статье приводятся краткие сведения и основные выводы по разработке методик и подбору условий для микрокапсулирования индивидуальных лекарственных веществ, по изучению полимеров различной природы для использования в качестве носителей, по способам формирования двойных оболочек микрокапсул, а также исследована эффективность микрокапсулирования биологически активных веществ, таких как антибактериальные препараты, вещества растительного и животного происхождения, и препаратов из различных фармакологических групп. Приведены варианты методик микрокапсулирования для конкретных соединений, которые подходят для сходных по составу и действию веществ, а также способы создания микрокапсул с двойными оболочками для нерастворимых в воде соединений.

Заключение. В статье показаны достижения и перспективы использования микрокапсулирования лекарственных веществ и их преимущества перед стандартными лекарственными формами. Активное внедрение разработанных методик в производство позволит создать новые лекарственные формы с известными лекарственными веществами, обладающими пролонгированным действием, что позволит сократить кратность применения препарата, а также сохраняющими свою активность под влиянием негативных факторов внутренней среды организма. Также в форме микрокапсул вещества проявляют большую активность в сравнении с незакапсулированными веществами.

Ключевые слова: микрокапсулирование, полимер, пролонгированная лекарственная форма, антибактериальные препараты, ферменты, двойная оболочка, двойные эмульсии, биодоступность, высвобождение

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. Ю. А. Полковникова осуществила обзор публикаций, написание текста рукописи, проверку конечной версии рукописи и перевод. Н. А. Ковалёва сделала обзор публикаций, оформление рукописи.

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INTRODUCTION

Microencapsulation of active pharmaceutical substances (APS) has been actively investigated. A great number of studies devoted to the selection of microencapsulation conditions for certain APS, particularities of pharmacological action of drug products, their release and bioavailability, selection of polymers for microencapsulation and their influence on a substance, development of methods for APS encapsulation, quantitative and quantitative analysis of microcapsules have been carried out.

The particularity of microencapsulated forms is that a substance is protected from exposure to various environmental factors which may cause their destruction (acidity of the gastric juice, food effect, concomitant administration of drugs, gastrointestinal diseases, etc.). In such a form, a drug product achieves a target organ with maximal bioavailability.

The method is used for various drug products such as antibiotics, nootropics, vitamins, probiotics, anticonvulsants, enzymes, etc.

CONDITIONS FOR MICROCAPSULE PRODUCTION

Adequate conditions for microcapsule production for each object, depending on its physical-chemical properties should be selected [1].

One of the first technologies of microcapsule production is coacervation or phase separation. Coacervation is a process of formation of high molecular compounds enriched with a dissolved substance in a solution. As an example, production of tocopherol acetate microcapsules can be given. Water soluble compounds – mixture of gelatin and gum acacia serve as polymers. In acetic medium, gelatin gets a positive charge and interacts with gum acacia, and forms polyelectrolyte coacervation complex. While cooling slowly, white charcoal is introduced and dehydrated with isopropyl alcohol, dried. Microcapsules with particle diameter 100–200 μm are produced.

Based on microcapsules, such dosage forms as tablets, suspensions, subcutaneous implants can be produced [2].

TYPES OF POLYMERS-CARRIERS

The selection of a polymer for micro-encapsulation plays a great role. As a coating, alginate sodium is often used – water soluble and biodegraded polymer. It is often used as antacid agent, as well as anticoagulant in food industry. APS microencapsulation in alginate sodium was investigated. Formation of coating around developing microcapsules when alginate sodium is introduced as calcium salt, and ion-linked microgels of alginate calcium occur (figure 1).

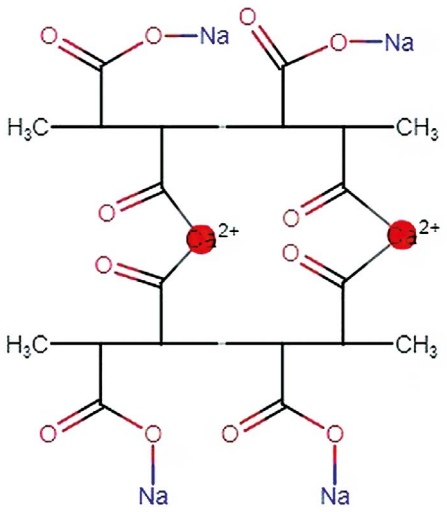

Figure 1. **Ion-crosslinked calcium alginate microgels**

Such drug substances as acridone, furacylline, tetracycline, dibazole and metronidazole were used. The best results of acridone microencapsulation were obtained with ultrasonic dispersion of the mixture which allowed to reduce time for microcapsule production, to increase the number of obtained microcapsules, as well as to improve physical-chemical characteristics of a substance to be microencapsulated. The analysis of the effect of a diluent effect showed that product yield was greater with ethanol used rather than with acetone. The magnetic vortex method was used for microencapsulation, as when an ultrasonic disperser was used then reaction mixture was overheated, and a thin dispersion was coagulated. The test results have shown that individual process conditions should be selected for each encapsulated substance. pH effect on furacylline sedimentation was investigated. With the increase of medium pH, stability of aqueous dispersions is increased [3].

Along with alginate sodium as a coating polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) are used. These are water soluble polymers used for microencapsulation of water insoluble compounds such as antibacterial agents. The bioavailability is increased in water soluble form. Using the method of polymer sedimentation on a substance surface by diluent change, microcapsules of derivative acridone – 4-carboxy acridone with antibacterial, antiviral and antineoplastic activity were obtained. Using IR-spectroscopy method, it was established that the substance was within the capsule and absent on the surface. The analysis of antibacterial activity of microcapsules of 4-carboxyacridone has proven that the activity of the encapsulation did not different from reference activity. The investigation confirms that PVA and PVP coatings do not reduce activity of an encapsulated APS, and its full introduction within a polymer protects from the exposure of hazardous factors of body internal environment [4].

Water soluble polymers are also widely used for microencapsulation – cellulose esters such as acetyl cellulose, ethyl cellulose and nitrocellulose. The methods for APS encapsulation with their use were developed. It is represented by microencapsulation of acridine acetic acid with antiviral, immunostimulatory and anti-inflammatory action. Microcapsules are produced with the physical-chemical method using polymer re-precipitation. As a precipitation agent, aqueous acetone solution is used, the precipitation within occurs not so actively, and a dense polymer membrane is formed on the surface. The method of IR spectroscopy has established that particles of an encapsulated substance are absent on the membrane surface. UV spectrophotometry is used for the assay. Nitrocellulose microcapsules are characterized with the largest yield [5].

PRODUCTION OF MICROSENCAPSULATED FORMS OF ANIMAL CELLS AND TISSUES

Microencapsulation of animal cells allows not only to reduce immunogenicity but also to prevent migration of encapsulated cells. Alginate/poly-L-lysine microspheres are most often used for microencapsulation of such cells [6].

Cells are placed to a solution of alginate sodium, and microcapsules are produced with calcium chloride solution due to formation of an ion-linked complex. The obtained microcapsules are placed to poly-L-lysine solution forming their outer coating. The interaction between positively charged amino groups of poly –L-lysine and free negatively charged alginate groups underlies the process. The membrane thickness is regulated by concentration of poly-L-lysine. The obtained microcapsules should be retained in alginate sodium solution for neutralization of free positively charged poly-L-lysine amino groups.

The main method for hypothyroidism treatment is oral administration of thyroid hormones. The method has several disadvantages including low bioavailability and adverse effects. The study on microencapsulation of pig thyroid cells as the method for substitution of thyroid hormones was carried out. The polymer in which the tissue is embedded prevents penetration of antibodies which allows to implant organoids with thyroid cells. Thyroid gland cells are separated and encapsulated to

alginate-poly-L-lysine-alginate microcapsules with the microfluoride device. The method principle is described above on the example of alginate-poly-L-lysine-alginate microcapsules. The study results have shown that the release of thyroxin from encapsulated cells was higher than the one from non-encapsulated ones ($P < 0.05$) and remained throughout the experiment period (> 28 days). The results suggested that microencapsulated organoids of pancreatic cells may have potential for the use in therapy and/or drug screening [7].

Overcoming immunogenicity and refusal from immunosuppressive agents in microencapsulation of tissues was proven in the study on immunoisolation of auricular chondrocytes by rat pancreatic islets (CMI-islet). The obtained microcapsules were treated with glucose. The study has shown that cells are able to release the sufficient amount of insulin in accordance with glucose concentration. Compared to naked islets they reacted better. Insulin secretion was confirmed within 100 days which indicates survival and secretory activity.

Using microencapsulation of pancreatic islets with cells of the recipient ear cartilage, long-term insulin secretion may be supported, and reaction to glucose problems may be improved. The new immunodilution technology differs from other methods of immunoisolation as donor tissue is embedded to a recipient's tissue which allows transplanted cells to be recognized as recipient's cells. The microencapsulation method may lead to the development of sustainable methods of xenotransplantation not using immunosuppressants [8].

In one of the studies, albumin microcapsules with lisozyme were obtained. The aim of microencapsulation is to decrease immunogenicity. To produce microcapsules, albumin solution was chemically cross-linked with glutaric dialdehyde, then APS – lysozyme was added. To prevent cross-linking of APS with albumin matrix, the excess of glutaric dialdehyde was neutralized with sodium bisulfite. The process was finished with spray drying. The evaluation of bioactivity confirmed that lysozyme preserved its properties and activity The studies in vivo have shown the absence of increase of antigen-specific serum IgG level which indicates biosafety and biocompatibility of cross-linked albumin matrix [9].

IMMOBILISATION OF ENZYMES WITH MICROENCAPSULATION METHODS

Microencapsulation is used not only for APS but also for various enzymes. The study on immobilization of thiamine kinase from pig liver was carried out. The study has established that enzyme immobilization to a hydrophobic carrier based on butadiene rubber increases its stability and thermal resistance. Hydrophilic coating from cellulose nitrate due to nitro group with a negative charge accumulates near positively charged thiamine molecules and magnesium cations which decreases their contents near a catalytic enzyme center. Therefore a hydrophobic carrier of thiamine kinase may be used as a drug product with a prolonged action [10].

Also, a study was carried out on the encapsulation of pepsin in a 10% solution of maltodextrin. To carry out microencapsulation of enzymes, a special apparatus was developed in which jet dispersion was carried out. This apparatus differed from those previously developed in that due to the diffusion of maltodextrin into the enzyme, its high long-term activity is ensured. The microcapsules obtained with this apparatus were evaluated for proteolytic properties and activity. Analysis of the research results showed that the thicker the maltodextrin layer, the longer its initial activity remains. It was confirmed that maltodextrin provided a high hardness of the capsule walls, which makes it possible to use only this substance as an effective encapsulating material without additional additives [11].

Transglutaminase (TG) is an important enzyme that increases hardness, viscosity and water-binding capacity by catalyzing the cross-linking of proteins. Obtaining a microencapsulated form of TG allows maintaining the enzyme activity for a long time. In the study, the TG enzyme was microencapsulated using freeze-drying. Covering – beckons, gum arabic and casein. Optimum conditions: homogenization speed – 11200 rpm, homogenization time – 1.27 min, a mixture of mannitol, gum arabic and casein in a ratio of 38.2, 40.2 and 21.6 %, respectively. The residual activity of the microencapsulated TG enzyme was determined by photocolorimetry. It was 93 %, and the uncoated enzyme was 64 % under the same conditions. Since this parameter is an important characteristic of the enzyme, it can be concluded that the use of freeze drying contributes to the preservation of TG activity [12].

MICROCAPSULES – NEW DOSAGE FORM KNOWN DRUGS

The creation of microencapsulated forms is used for nootropic APIs. In addition to the selection of process conditions, a comprehensive analysis of the resulting product is carried out. In this area, a computer was carried out. lexical study of alginate-chitosan microcapsules with vinpocetine. Microcapsules were obtained by extrusion

using sodium alginate of various concentrations as a polymer, as well as a medium viscosity chitosan solution. The alginate complex was crosslinked with a calcium chloride solution according to the mechanism described above.

The rock capsules were placed in a chitosan solution, where the molecules formed a polyelectrolyte alginate-chitosan complex due to the interaction between the amino groups of chitosan and free, unbound calcium ions, carboxyl groups of sodium alginate. Complexation occurs by the mechanism of electrostatic interaction between oppositely charged functional groups. The method of atomic force microscopy was used to compare microcapsules with and without a chitosan shell. As a result, it was found that the surface of the particles has characteristic differences with an increase in the concentration of alginate natrya (Figure 2).

It was determined with spectrophometry method that microencapsulation efficiency was maximal in concentration of alginate sodium 2.5 % (86.8 %) (figure 3).

The greatest release of vinpocetine was observed in samples with concentration of alginate sodium 1 % (41.7 %), but the gradual APS was found in concentration 2.5 % which allowed to use the microcapsules for production of drugs with a prolonged action.

As a result of complex studies it is established that microcapsules can be used in production of capsule dosage forms [13].

Microencapsulation methods are used for production of drugs with a prolonged action. The study was carried out on the example of simulated compounds – *o*-crezoacetic and acridione acetic acids – with immunostimulatory and antiviral action. Coating – polymer Eudragit® L100 representing cation co-polymer of metacrylic acid, methyl methacrylate (1:1). APS release kinetics was investigated with the spectrophotometric method. It was established that the longest release time was observed with the use of carboxymethylcellulose (192 h) and guar gum (168 h). The study confirms that it is possible to produce drugs with a prolonged action using the polymers [14].

The production of microcapsules of ursodeoxycholic acid (UDCA) was developed. The study aim was to develop oral UDCA microcapsules for patients with 1 type diabetes mellitus. UDCA for microencapsulation was taken as a suspension in concentration 1 mg/ml. As a matrix, 2 % solution of sodium alginate was used. Ions were cross-linked with 2 % calcium chloride solution. The obtained microcapsules were tested

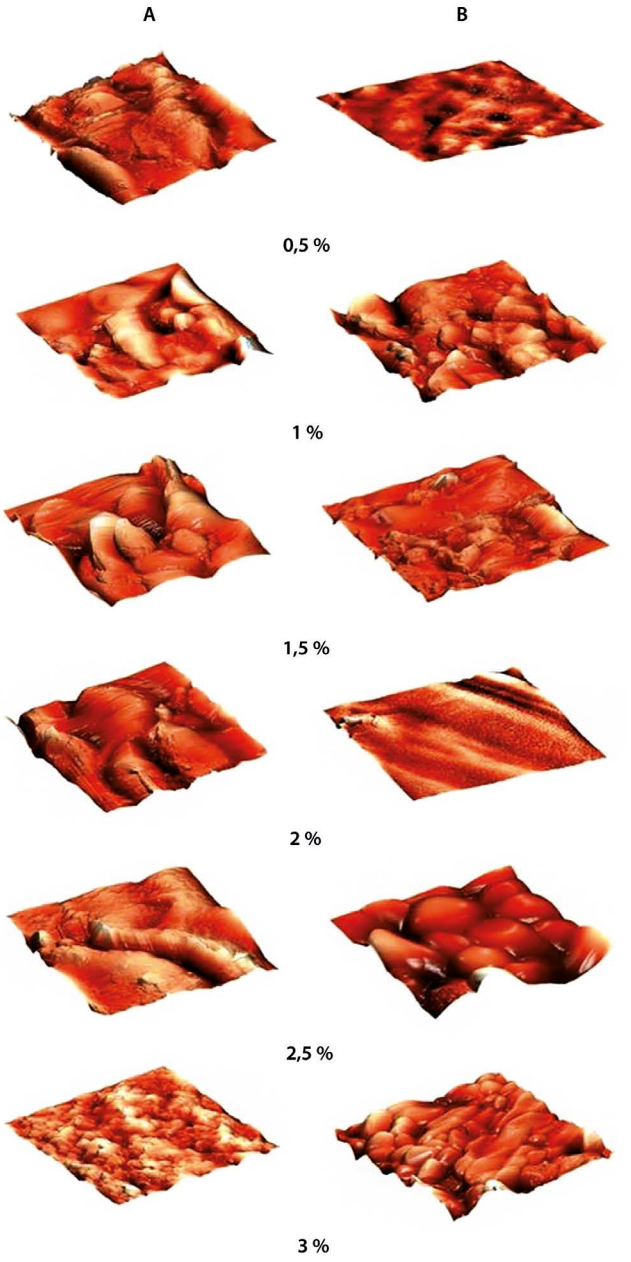

Figure 2. Three-dimensional AFM images of the surface of microcapsules with different concentrations of sodium alginate treated with a solution of chitosan (A) and without chitosan (B) at a scanning area of 5 × 5 microns²

on 3 equal groups of mice: 1-st took UDCA; 2-nd – empty microcapsules; 3-rd – UDCA microcapsules. The experiment showed that UDCA microcapsules led to the decrease of the increased glucose level in blood, decrease of inflammation and change of concentrations of primary and secondary gall acid. UDCA has a direct protective action on pancreatic β-cells [15].

The studies on dutasteride microencapsulation – a drug for treatment of prostate gland diseases were also carried out. Ethyl cellulose in ratio with the drug 1:1, 1:3 and 1:5 was a polymer. APS and ethyl cellulose

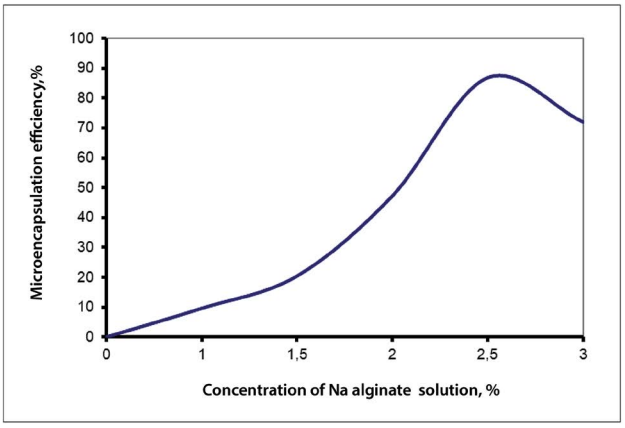

Figure 3. Dependence of the microcapsulation efficiency on the concentration of sodium alginate

were dissolved in dichloromethane, then emulsified in an aqueous solution of polyvinyl alcohol (PVA) to produce homogeneously sized microcapsules with. The size of obtained microparticles was 95–119 μm with good efficacy and release. With the method of Fourier spectroscopy, it was established that hydrogen bond was formed between APS and the polymer. The type of interaction increases solubility of hydrophobic molecules of dutasteride which, in its turn, increases its release and bioavailability. Considering physical-chemical properties and molecular interactions of substances, microcapsules with assigned properties and release intensity may be produced [16].

Embedding of substances to microcapsules allows not only to increase bioavailability and decrease probability of adverse effects but also to mask unpleasant taste of drugs. The prominent example is encapsulation of an allergic agent – cetirizine dihydrochloride – to chitosan nanoparticles. To produce microcapsules, the method of ionotropic gel formation with further spray drying was used. Chitosan in concentration 0.5–2.0 % was produced by dissolution in diluted acetic acid. APS was dissolved in the solution with a magnetic mixer till a homogeneous mixture was obtained. To form ionic gel, tripolyphosphate sodium was added to the mixture. Microcapsules were formed as a result of interaction of negatively charged phosphate groups of tripolyphosphate sodium and positively charged amino groups of cetirizine. The obtained microcapsules had a smooth surface and diameter 0.5–5 μm. It was established with HPLC that efficiency of encapsulated APS was about 70 % regardless of cetirizine concentration and APS/polymer ratio. To determine degree of the release, microcapsules were dried with spray drying, placed to a membrane with size pore 8 kDa with phosphate buffer solution and incubated at 37 ± 0.5 °C for 12 hours. Then a high level of the substance release within first two hours, and then deceleration and gradual elimination of the remaining component was found with HPLC method. The method is applicable for APS with a good solubility in water as aqueous solution of chitosan is used for microencapsulation process, and the substance within should dissolve [17].

MICROENCAPSULATION OF PROBIOTICS

The studies on the possibility of microencapsulation of probiotics were carried out. For that, enteric soluble copolymer Eudragit® L100 and product containing strains of probiotic microorganisms were used: *Bacillus subtilis, Bacillus licheniformis*. Microcapsules were produced with the physical-chemical method of polymer re-precipitation on the surface of an encapsulated substance via diluent substitution. Carrier – Polysorb® and oil base. As a qualitative analysis of microcapsules, the presence of nitrogen containing compounds was established which proved the protein presence. The study confirmed the possible use of copolymer Eudragit® L100 for microencapsulation of probiotics [18].

Probiotic bacteria *Lactobacillus plantarum* were microencapsulated in a biocomposite consisting of alginate sodium, pectin and gelatin (1.06, 0.55 and 0.39 %, respectively). The obtained microparticles showed a greater survival of bacteria by 88.66 % compared to non-encapsulated bacteria. Then microcapsules containing a probiotic and fatty acid – docosahexanoic acid (DHA) were analyzed. The results confirmed that bacteria were fully captured by the matrix, and DHA increased smoothness of particle surface. It was established with the method of Fourier spectroscopy that hydrogen bonds were formed between the nucleus from alginate-pectin-gelatin complex and DHA. It allowed to provide thermal stability of such microcapsules compared to the bacteria not embedded to microcapsules [19].

MICROENCAPSULATION AS THE METHOD TO OVERCOME RESISTANCE TO ANTIBACTERIAL DRUGS

Microencapsulation is actively used for antibacterial drugs. As antibiotics should be administered several times a day (orally and/or parenterally), and as they have as a rule low bioavailability, embedding of the substances to microcapsules allows to solve such problems.

In this field, the study on microencapsulation of such antibacterial drugs such as oxytetracycline and ceftriaxone, to double coatings including two types of polymers: water soluble (alginate sodium, polyvinyl alcohol and polyvinylpyrrolidone) – outer coating and water insoluble (acetyl cellulose) – inner coating was carried out. The use of inner water insoluble coating allows to provide various rate of APS release varying microcapsule sizes. Outer water soluble coating protecting from exposure to negative factors, increases APS bioavailability. Acetone solution of acetyl cellulose was administered to antibiotic suspension and precipitated on APS surface due to a gradual addition of distilled water (diluent substitution). Then the second coating was formed with the addition of aqueous solution of alginate sodium or PVA (PVP) adding a saturated solution of sodium chloride (to achieve full precipitation).

With the method of IR-spectroscopy, the presence of all components in microcapsules is established, as well as layer-by-layer application of polymers to APS is confirmed. The yield of antibiotics from microcapsules with PVA and alginate sodium is higher than the one with PVA (ratio 1:1:1). The use of double coatings provides a prolonged drug action and optimal therapeutic concentration of a drug substance in blood [20].

Nowadays, there is the trend of bacterial resistance to administered antibacterial drugs. Due to that, one of the treatment methods for infectious diseases is phagotherapy. The microencapsulated form of the bacteriophage on the model of Klebsiella bacteriophage was developed. The bacteriophage was pre-concentrated for 100 times with the method of membrane ultrafiltration. As a carrier, sodium alginate was used. Microcapsules were produced with several methods. In the first case, the hydrogel with bacteriophage based on alginate sodium in concentration 0.25 to 3 % dispersed to drops of various size was instilled to vaseline oil (dispersion medium). Cross-linking was performed with chloride calcium, and the coating hardened. The obtained microcapsules were 26 to 300 μm in dimensions. In the second case, microencapsulation was performed similarly but not using alginate sodium. The size of microcapsules was 66 to 1250 μm. It is not appropriate to use the second method as such microcapsules were of irregular form, had unsatisfactory technological characteristics (flowability, dosing uniformity). Microcapsules produced on a polymer carrier with concentration 1.0 to 2.0 % have optimal technological characteristics. Then stabilizers for a bacteriophage were selected. The best stability was observed in the presence of sugars – sorbit, lactose, mannit and methylcellulose. The next stage – selection of drying type and mode. It is experimentally established that a vacuum single drying at moderate vacuum depth is the most optimal. The standardization was performed by the parameters: appearance, moisture, average weight and deviation from average weight, lytic activity. Pharmacological activity of developed microcapsules with Klebsiela bacteriophage was evaluated *in vitro* in a beaker with magnetic vortex for liquid Klebsiela bacteriophage and in the laboratory identifier of degradability process for an microencapsulated product. The studies have shown that microcapsules with a bacteriophage after exposure to acidic medium have a greater bioavailability than a bacteriophage in a liquid dosage form. The production of a microencapsulated bacteriophage form with enteric soluble coating allows to use it for production of complex drugs or as a drug product alone [21].

During the study, microencapsulation technology was used for the increase of phage resistance to physiological conditions, and the obtained microcapsules were tested in the media simulating body conditions. *Bacillus subtilis, Salmonella enterica subsp* were used for this purpose. Phages *Enterica serovar Typhimurium (Salmonella Typhimurium)* were isolated from various sources and then microencapsulated to 1.33 % alginate sodium solution with spray drying to minimize damage of the physiological environment. The stability of microcapsules in the simulated gastric fluid and gall salt environment was investigated. To examine stability in gastric environment, the solution consisting of 0.2 % solution of sodium chloride and pepsin, with pH 2.4 was prepared, microcapsules were added and incubated at temperature 37 °C. Samples were withdrawn at 0, 15, 30, 60 and 90 minutes of the incubation. Phage titers were determined with the method of two-layer agar. The maximal decrease of the titer of encapsulated phages shown after two-hour incubation was 2.29 logarithmic units for phage *B. subtilis*, 1.71 logarithmic unit for phages *S. Enteritidis* and 0.60 logarithmic unit for phages *S. Typhimurium*, meanwhile free phages lost their viability even after 15-minute incubation. To examine stability in gall salt environment, microcapsules were placed to 2 % gall extract and incubated at 37 °C. Samples were withdrawn in the first and third hours of incubation. A titer was determined like in the gastric environment. It was found that microencapsulation increased phage stability in gall salt environment – after three hours of incubation, the difference between titers of microencapsulated phages and free phages may achieve up to three logarithmic units [22].

The study was published which was devoted to production of microparticles containing rifampicin, with polymer poly(3-hydroxybutirate-co-3-hydroxyvalerate) for oral administration. The selection of the polymer is associated with its good biocompatibility and biodegradability. Microparticles of poly(3-hydroxybutirate-co-3-hydroxyvalerate) with rifampicin were produced with the method of emulsification and diluent evaporation where as diluent and emulsifier, chloroform and polyvinyl alcohol are used, respectively. Microparticles were produced in the range of sizes 20–60 μm with the change of primary concentrations of poly(3-hydroxybutirate-co-3-hydroxyvalerate), polyvinyl alcohol and rifampicin. With the method of UV-spectrophotometry, the value of encapsulation efficiency of 14 % was obtained. The evaluation of antimicrobial activity of microcapsules showed the same inhibition of *Staphylococcus aureus* growth like the one of free rifampicin incubated for 20 and 24 h, but toxic effects manifested to a lesser extent. To evaluate release kinetics, microcapsules were incubated at 36.5 ± 0.1 °C in a phosphate buffer medium. The concentration was determined spectrophotometrically at wavelength 473 nm. It was established that almost 90 % of product loaded to microparticles was released in 24 hours [23].

With microencapsulation, a new prolonged dosage form of furacilin was developed. The drug substance was embedded to a double coating consisting of copolymer Eudragit® L100 (inner coating) and PVP, and PVA (outer coating). It is inner coating that provides a prolonged APS release. Microcapsules were produced with the method of re-precipitation of the polymer (1 % acetone solution of Eudragit® L100) on APS surfaced with diluent change (dimethylformamide/distilled water). The obtained dispersion was left for one day at temperature 0–5 °C. Then the second coating was produced with the addition of 2 % aqueous PVA or PVP solution. For complete precipitation, a saturated solution of sodium chloride was added to the obtained mixture. It was dispersed with a magnetic mixer and dried in a drying cabinet. As a result of the study of release kinetics with the method of UV-spectroscopy (maximum at 367 nm), it was established that complete furacilin release occurred in 140 hours. According to the quantitative analysis, APS was fully within a microcapsule. With the method of IR-spectroscopy, layer-by-layer polymer application to furacilin was confirmed. The use of the method allows to influence the rate of APS release from microcapsules changing coating thickness [24].

PRODUCTION OF MICROENCAPSULATED FORMS OF MEDICINAL PLANTS

Prospectiveness of studies on microencapsulation of APS derived from medicinal plants should be stated. The study object is oil extract of carotinoids from the tunic of purpur ascidian. The particularity of encapsulation of oil extracts is the development of methods for production of stable emulsions not flaking for a long time. As a polymer, alginate sodium was used, outer coating – chitosan. Microcapsules were produced with the method of extrusion. The cross-linking was performed with calcium chloride solution. Chitosan forms a water soluble polyelectrolyte complex by interaction between free carboxyl groups of alginate and inherent amino groups. It is appropriate to use alginate sodium/chitosan pair for the increase of solubility of microcapsules by chitosan and gradual APS release by alginate sodium which increases APS bioavailability. To optimize emulsification and improve emulsion structure and stability, it was ultrasonified. Owing to that, the emulsion has an optimal structure and lowest viscosity which allows to produce microcapsules of the smaller size. Therefore, the method of microencapsulation of carotinoid oil extract can be used for production of oil extracts of other plants [25].

Oil from unroasted coffee grains is widely used for cosmetic purposes. Production of its microencapsulated form is of interest for improvement of antioxidant activity exposed to the light, heat and oxygen. Acacia gum was a polymer, production method – spray drying of oil-aqueous emulsion. Obtained microcapsules had a spherical form with smooth surfaces which confirms appropriateness of the use of acacia gum as an encapsulating material. With the active oxygen method (adapted from conductometry), the established antioxidant activity was significantly higher compared to reference antioxidant α-tocoferol, and compared to pure oil, 7 and 3-fold increase of activity was observed. The results indicate the prospective industrial use of the method of coffee oil microencapsulation [26].

Guarana is well-known for its nutritional and pharmaceutical potential, and semi-purified guarana extract has antidepressant and antipanic action. However, low solubility, bioavailability and stability of the semi-purified extract limit its use as a component of pharmaceutical agents. The delivery of the semi-purified extract as microparticles may help in optimization of its stability. In this study, microparticles containing semi-purified guarana extract were produced with the method of spray drying with the combination of gum acacia (GA)

and maltodextrin (MD) in ratio 30:70, 40:60 and 50:50. The solution of the extract and GA/MD dispersion, mixed separately for 20 min. Then the extract solution was added to GA/MD dispersion, mixed for about 5 min and spray dried. The drying technology: input temperature 190 °C, aspiration 80 %, pressure 2 Bar, pump from 6 %, outlet temperature 120–130 °C. The technology of spray drying and the selected processing conditions gave satisfactory efficiency of encapsulation (80–110 %) and product yield (55–60 %). The average diameter of microparticles was about 4.5 μm. DPPH ability to radical blowing showed that microparticles may protect semi-purified guarana extract from exposure to high temperatures as antioxidant ability was maintained. The dissolution studies in vitro show that all preparations fully dissolve within 60 min. Microencapsulation has improved technological characteristics of powders and preserved antioxidant properties. The study has demonstrated the appropriateness of production of the microparticles for one-stage process with spray drying. The composition of each product influenced physical-chemical characteristics. The method of spray drying can be used as an effective and economical approach to production of the semi-purified extract of guarana microparticles [27].

The study on microencapsulation of lavender oil to protect oil components from evaporation was carried out. To produce microcapsules, the mixture of gum acacia and maltodextrin was used as a polymer, and spray drying was the method used. The method of spray drying is similar to the one for guarana extract. The size of obtained particles was 12.42 ± 1.79 μm, microencapsulation efficiency – 77.89 %. The obtained data shows that the mixture of gum acacia and maltodextrin as a polymer protects lavender oil from evaporation of components [28].

One of the problems for the use of herbal products is a bitter taste. To solve the problem, microencapsulation is used. The study of encapsulation of quercetin to carnauba wax, shellac or zein with the method of hot melt extrusion showed that microcapsules dissolved significantly to a lesser extent in saliva at pH 6.8 compared to pure quercetin. The complex, correspondingly, masks well a taste in the order of zein > carnauba wax > shellac. The bitterness analysis *in vitro* with the electronic tongue confirmed a good taste masking efficacy of microencapsulated powders. Therefore, thermal extrusion microencapsulation may become an attractive method for production of biologically active powders with taste masking [29].

MICROENCAPSULATION OF VITAMINS

The study was carried out to examine stability of microencapsulated ascorbic acid in the simulated gastrointestinal tract in vitro and influence of microencapsulated acid on iron bioavailability. As covering materials, polyglycerol monostearate (PGMS) and medium-chain triacyl glycerin (MST) were used, and as main materials, L-ascorbic acid and ammonium iron sulfate. When ascorbic acid is microencapsulated with MST, the release of ascorbic acid was 6.3 % at pH 5 and 1.32 % at pH 2 in simulated gastric fluids for 60 min. When ascorbic acid was encapsulated by PGM, then more ascorbic acid was released in the range of 9.5 to 16.0 %. For comparison, the release of ascorbic acid covered by MST and PGM was significantly increased by 94.7 and 83.8 %, correspondingly, within 60 minutes of incubation in artificial intestinal fluid [30].

α-tocoferol is a well-known fat soluble antioxidant and widely is used in food industry for stabilization of free radicals. Its introduction to food and stability is an additional problem as α-tocoferol directly added tends to be inactivated by food components. The study was aimed to optimize the conditions for encapsulation of α-tocoferol combined with alginate sodium (0.5, 1.0, 1.5 and 2.0 %) as the main wall material and pectin (2.0 %) as a carrier. Solutions of pectin and alginate sodium were mixed till a homogeneous mixture was obtained. As an emulsifier, tween-80 and 1 % tocoferol solution were added. The resulting mixture was further homogenized for 30 minutes on a homogenizer. A stable emulsion was poured with a needle to 5 % calcium chloride solution. Microcapsules were rinsed with water and maintained at room temperature for 24 hours (syringe method). Efficiency of α-tocoferol encapsulation established with HPLC to microcapsules produced in optimal conditions was 52.91 % with the use of alginate sodium 1.5 % and pectin 2.0 %. α-tocoferol was encapsulated with an encapsulating unit in standard conditions and compared with the syringe method. Greater efficiency (55.97 %) of encapsulation was found in microcapsules prepared with the encapsulating unit and 52.11 % in microcapsules prepared with the syringe [31].

DOUBLE EMULSIONS – PRODUCTION AND STABILISATION METHODS

Microencapsulation of biologically active substances with the method of double emulsions is applicable for hydrophilic substances. Emulsions are stabilized by polyelectrolytes are conversely charged surfactants to increase surface activity. Complexes of chitosan

and XathanGum (xanthan gum) were prepared with the mixing of solutions of individual compounds with various concentrations. The compounds interacted due to formation of ion bonds between positively charged chitosan molecules (amino groups) and negatively charged XanthanGum (carboxyl groups). The solutions were used in 24 hours after mixing. Emulsions were prepared with ultrasound. The resulting emulsions had interphase tension at the border of phase separation which was measured with tensiometry. Interphase steady-state surface tension was decreased with the increase of polymer concentrations in a diluent. The use of the polycomplexes allows to produce microcapsules with a high enzymatic resistance. As a result of the studies it is established that the use of complexes Chitosan-XanthanGum is appropriate for production of a stable double emulsion [32].

For microencapsulation of biologically active substances, surfactants of various chemical nature are used, and cation and anion polyelectrolytes are also selected. Due to that, the study on microencapsulation of rosemary oil with the method of double emulsions was carried out. For oil microencapsulation, two types of polyelectrolytes were used: positively charged (cation) chitosan and negatively charged (anion) gums (xanthan, guar, acacia). APS was mixed with the surfactant, and cation (chitosan) and anion (gum) polyelectrolytes were alternatively introduced to the preparation. To establish the influence of polyelectrolyte nature on aggregative stability of the emulsion, zeta potential was measured. As a result, it was determined that emulsions with xanthan and guar gums were stable for 30 days, zeta potential was –25.86 and –19.66 mV, correspondingly. Emulsions with gum acacia were not unstable, and precipitation was observed within 24 hours (zeta potential –0.66 mV). Correspondingly, the higher was zeta potential of anion polyelectrolyte, the more stable would be the emulsion [33]. For microencapsulation of biologically active substances, surfactants of various chemical nature are used, and cation and anion polyelectrolytes are also selected. Due to that, the study on microencapsulation of rosemary oil with the method of double emulsions was carried out. For oil microencapsulation, two types of polyelectrolytes were used: positively charged (cation) chitosan and negatively charged (anion) gums (xanthan, guar, acacia). APS was mixed with the surfactant, and cation (chitosan) and anion (gum) polyelectrolytes were alternatively introduced to the preparation. To establish the influence of polyelectrolyte nature on aggregative stability of the emulsion, zeta potential was measured. As a result, it was determined that emulsions with xanthan and guar gums were stable for 30 days, zeta potential was –25.86 and –19.66 mV, correspondingly. Emulsions with gum acacia were not unstable, and precipitation was observed within 24 hours (zeta potential –0.66 mV). Correspondingly, the higher was zeta potential of anion polyelectrolyte, the more stable would be the emulsion [33].

The method was developed for microencapsulation on anti-tuberculosis drugs with the technology of covering with polyelectrolyte multilayers and investigation of solubility of microcapsules with pH values simulating various regions of the gastrointestinal tract. To produce microcapsules, polymers were used – gellan (1 and 3 %), pectin (1 and 2 %) and alginate sodium (2 and 3 %) were used. To obtain multilayers, cation polyelectrolyte – chitosan and anion polyelectrolytes – dextrane sulfate and Eudragit® S. APS – isoniaside, pirasinamide, moxifloxacine hydrochloride were selected. The method for microcapsule production – ionotropic gel formation. The polymer solution was heated with APS up to 90 °C, and the resulting mixture was introduced by drops to 1 % calcium chloride solution. In 10 minutes, microcapsules were rinsed with distilled water and air dried. The contents of pyrazinamide and moxifloxacine was established with the method of UV-spectrometry, the contents of isoniaside – with the method of bromatometry. At pH = 7.4, APS release from microcapsules, without multilayers applied, was about 30 % of the active ingredient within 4 hours, about 50 % within 8 hours, over 80 % within 12 hours. The prolongation was 12 hours which provided the presence of therapeutic drug doses during a day. Anti-tuberculosis drugs with the controlled intestinal release can be produced with the method [34].

CONCLUSION

Microencapsulation is the perspective method for production of resistant and prolonged dosage forms. Drug substances as microcapsules have a greater bioavailability, stability exposed to gastric acidic environment, as well maximum delivery to the target organ. Production of microcapsules with antibacterial drugs as the problem of microorganism resistance to existing drugs and dosage forms is rather challenging. Meanwhile, microencapsulation process is rather labor consuming and requires prior preparation of all components, as well as selection of adequate conditions.

REFERENCES

1. Dolçà C., Ferrándiz M., Capablanca L., Franco E., Mira E., López F., García D. Microencapsulation of Rosemary Essential Oil by Co-Extrusion/Gelling Using Alginate as a Wall Material. *Journal of Encapsulation and Adsorption Sciences*. 2015;5:121–130.
2. Stepanova E. F., Kim M. E., Murzagulova K. B., Evseyeva S. B. Microcapsules: use prospects inmodern pharmaceuticalpractice. *Sovremennye problemy nauki i obrazovaniya*. 2014;5:767. (In Russ.)
3. Grekhneva E. V., Kudryavtseva T. N. *Osobennosti mikrokapsulirovaniya nekotorykh lekarstvennykh preparatov v al'ginat natriya* [Features of microencapsulation of some drugs in sodium alginate]. *AUDITORIUM*. 2014;3(3):12–16. (In Russ.)
4. Markovich Yu. D., Grekhneva E. V., Kudryavtseva T. N., Efanov S. A., Klimova L. G. *Mikrokapsulirovanie 4-karboksiakridona v vodorastvorimye polimery. Uchenye zapiski. Elektronnyy nauchnyy zhurnal kurskogo gosudarstvennogo universiteta* [Microencapsulation of 4-carboxyacridone into water-soluble polymers]. *Uchjonye zapiski. Jelektronnyj nauchnyj zhurnal Kurskogo gosudarstvennogo universiteta*. 2013;3-2(27):1–5. (In Russ.)
5. Grekhneva E. V., Belokon' V. L., Orlova S. V. *Mikrokapsulirovanie biologicheski aktivnykh veshchestv v vodonerastvorimye polimery* [Microencapsulation of biologically active substances in water-insoluble polymers]. *AUDITORIUM*. 2016;2(10):15–19. (In Russ.)
6. Aijaz A., Perera D., Olabisi R. M. Polymeric Materials for Cell Microencapsulation. *Cell Microencapsulation*. 2017:79–93. DOI: 10.1007/978-1-4939-6364-5_6.
7. Yang Y., Opara E. C., Liu Y., Atala A., Zhao W. Microencapsulation of porcine thyroid cell organoids within a polymer microcapsule construct. *Experimental Biology and Medicine*. 2017;242(3):286–296. DOI: 10.1177/1535370216673746.
8. Lee J. I., Kim H. W., Kim J. Y., Bae S. J., Joo D. J., Huh K. H., Fang Y. H., Jeong J. H., Kim M. S., Kim Y. S. Microencapsulation of pancreatic islets with canine ear cartilage for immunoisolation. *Transplantation Proceedings*. 2012;44(4):1091–1094. DOI: 10.1016/j.transproceed.2012.02.015.
9. Patel N., Addo R. T., Ubale R., Uddin M. N., D'Souza M., Jobe L. The effect of antisense to NF-κB in an albumin microsphere formulation on the progression of left-ventricular remodeling associated with chronic volume overload in rats. *Journal of Drug Targeting*. 2014;22(9):796–804. DOI: 10.3109/1061186X.2014.921927.
10. Chernikevich I. P., Zimatkina T. I., Sorokopyt E. M. Immobilization of thiamine kinase from pig liver by the microencapsulation method. *Zhurnal Grodnenskogo gosudarstvennogo medicinskogo universiteta = Journal of the Grodno State Medical University*. 2015;4(52):33–38. (In Russ.)
11. Kudryashov L. S., Tikhonov S. L., Tikhonova N. V., Dyachkova A. V. Microcapsulation of pepsin and estimation of its proteolytic properties. *ESSUTM Bulletin*. 2019;3(74):35–41. (In Russ.)
12. Isleroglu H., Turker I., Koc B., Tokatli M. Optimization of microencapsulation conditions of transglutaminase by freeze drying. *Journal of Food Science and Technology*. 2019;56(11):4925–4937. DOI: 10.1007/s13197-019-03962-9.
13. Polkovnikova Yu. A., Severinova N. A., Koryanova K. N., Tulskaya U. A, Grechkina M.V. Morphological, technological and biopharmaceutical studies of alginate-chitosan microcapsules with vinpocetine. *Pharmacy & Pharmacology*. 2019;7(5):279–290. (In Russ.) DOI: 10.19163/2307-9266-2019-7-5-279-290.
14. Grekhneva E. V., Kometiani I. B., Gusel'nikova A. O., Petrova Yu. I. *Vozmozhnosti ispol'zovaniya metodov mikrokapsulirovaniya i adsorbtsii dlya sozdaniya lekarstvennykh preparatov prolongirovannogo deystviya. Obrazovatel'nyy, nauchnyy i innovatsionnyy protsessy v nanotekhnologiyakh* [Possibilities of using microencapsulation and adsorption methods to create drugs with prolonged action]. Obrazovatel'nyj, nauchnyj i innovacionnyj processy v nanotehnologijah. Sbornik dokladov uchastnikov VIII Vserossijskoj konferencii. 2017. 136–138 p. (In Russ.)
15. Mooranian A., Zamani N., Ionescu C. M., Takechi R., Luna G., Mikov M., Goločorbin-Kon S., Kovačević B., Al-Salami H. Oral gavage of nano-encapsulated conjugated acrylic acid-bile acid formulation in type 1 diabetes altered pharmacological profile of bile acids, and improved glycaemia and suppressed inflammation. *Pharmacological Reports*. 2020;72(2):368–378. DOI: 10.1007/s43440-019-00030-z.
16. Kim K. H., Cho S.-A., Lim J. Y., Lim D. G., Moon C., Jeong S. H. Preparation of microcapsules with the evaluation of physicochemical properties and molecular interaction. *Archives of Pharmacal Research*. 2014;37(12):1570–1577. DOI: 10.1007/s12272-013-0306-0.
17. Li F-Q., Ji R.-R., Chen X., You B.-M., Pan Y.-H., Su J.-C. Cetirizine dihydrochloride loaded microparticles design using ionotropic cross-linked chitosan nanoparticles by spray-drying method. *Archives of Pharmacal Research*. 2010;33(12):1967–1973. DOI: 10.1007/s12272-010-1212-3.
18. Uymanova T. N., Grekhneva E. V., Kudryavtseva T. N., Gorobets A. Yu. Vozmozhnost' mikrokapsulirovaniya probiotikov v kishechnorastvorimyy polimer Eudragit® L100. *Obrazovatel'nyy, nauchnyy i innovatsionnyy protsessy v nanotekhnologiyakh. Sbornik dokladov uchastnikov VIII Vserossiyskoy konferentsii* [Possibility of microencapsulation of probiotics in the enteric polymer Eudragit®L100. *Educational, scientific and innovative processes in nanotechnology. Collection of reports of the participants of the VIII All-Russian conference*]. 2017. 138–142 p. (In Russ.)
19. Vaziri A. S., Alemzadeh I., Vossoughi M., Khorasani A. C. Co-microencapsulation of Lactobacillus plantarum and DHA fatty acid in alginate-pectin-gelatin biocomposites. *Carbohydrate Polymers*. 2018;199:266–275. DOI: 10.1016/j.carbpol.2018.07.002.
20. Grekhneva E. V., Vlasova V. V. *Osobennosti mikrokapsulirovaniya oksitetratsiklina i tseftriaksona v dvoynye obolochki* [Features of microencapsulation of oxytetracycline and ceftriaxone in double membranes]. *AUDITORIUM*. 2017;2(14):19–23. (In Russ.)
21. Kovyazina N. A., Nikolaeva A. M., Funkner E. V., Yefimovap M. G. Studying of possibility of microencapsulation of the bacteriophage. *Nauchnye vedomosti Belgorodskogo gosudarstvennogo universiteta. Serija: Medicina. Farmacija = Scientific bulletins of Belgorod State University. Series: Medicine. Pharmacia*. 2014;24(195):205–210. (In Russ.)
22. Soykut E. A., Tayyarcan E. K., Evran Ş., Boyacı İ. H., Çakır İ., Khaaladi M., Fattouch S. Microencapsulation of phages to analyze their demeanor in physiological conditions. *Folia Microbiol.* 2019;64(6):751–763. DOI: 10.1007/s12223-019-00688-1.
23. Durán, N., Alvarenga, M. A., Da Silva E. C., Melo P. S., Marcato P. D. Microencapsulation of antibiotic rifampicin in poly(3-hydroxybutyrate-co-3-hydroxyvalerate). *Archives of Pharmacal Research*. 2008;31(11):1509–1516. DOI: 10.1007/s12272-001-2137-7.

24. Grekhneva E. V., Kudryavtseva T. N. *Razrabotka novoy lekarstvennoy formy furatsilina metodom mikrokapsulirovaniya* [Development of a new dosage form of furacillin by microencapsulation]. *AUDITORIUM*. 2018;2(18):17–23. (In Russ.)
25. Pivnenko T. N., Buntush A. E. Experimental foundetion of microcapsulation of oil extract of carothinoids from tunics of purple ascidian. *Nauchnye trudy Dal'rybvtuz*a. 2017;42:82–89. (In Russ.)
26. Nosari A. B. F. L., Lima J. F., Serra O. A., Freitas L. A. P. Improved green coffee oil antioxidant activity for cosmetical purpose by spray drying microencapsulation. *Revista Brasileira de Farmacognosia*. 2015;25(3):307–311. DOI: 10.1016/j.bjp.2015.04.006.
27. Klein T., Longhini R., Bruschi M. L., de Mello J. C. P. Microparticles containing guaraná extract obtained by spray-drying technique: development and characterization. *Revista Brasileira de Farmacognosia*. 2015;25(3):292–300. DOI: 10.1016/j.bjp.2015.03.010.
28. Burhan A. M., Abdel-Hamid S. M., Soliman M. E., Sammour O. A. Optimisation of the microencapsulation of lavender oil by spray drying. *Journal of Microencapsulation*. 2019;36(3):250–266. DOI: 10.1080/02652048.2019.1620355.
29. Khor C. M., Ng W. K., Kanaujia P., Chan K. P., Dong Y. Hot-melt extrusion microencapsulation of quercetin for taste-masking. *Journal of Microencapsulation*. 2017;34(1):29–37. DOI: 10.1080/02652048.2017.1280095.
30. Lee J.-B., Ahn J., Lee J., Kwak H.-S. The microencapsulated ascorbic acid releasein vitro and its effect on iron bioavailability. *Archives of Pharmacal Research.* 2003;26(10):874–879. DOI: 10.1007/BF02980035.
31. Singh J., Kaur K., Kumar P. Optimizing microencapsulation of α-tocopherol with pectin and sodium alginate. *Journal of Food Science and Technology*. 2018;55(9):3625–3631. DOI: 10.1007/s13197-018-3288-6.
32. Kudasova D. E., Mutaliyeva B. Zh., Saparbekova A. A., Elemanova Zh. R. Microencapsulation of biologically active substances by the method of double emulsions. *Sovremennye nauchnye issledovanija i razrabotki*. 2018;9(26):188–191. (In Russ.)
33. Antonova A. S., Noskova Yu. V. *Vliyanie anionnogo polielektrolita na ustoychivost' emul'sii pri mikrokapsulirovanii BAV* [Effect of anionic polyelectrolyte on the stability of the emulsion during microencapsulation of biologically active substances]. *Molodye uchjonye – razvitiju Nacional'noj tehnologicheskoj iniciativy*. 2019;1(1):67–69. (In Russ.)
34. Mussabayeva B. H., Murzagulova K. B., Kim M. E., Izumrudov V. A., Aripzhanova Z. Zh. Encapsulation of antitubercular drugs by biopolymers and polyelectrolyte multilayers. *Farmatsiya i farmakologiya = Pharmacy & Pharmacology*. 2017;5(2):164–176. (In Russ.)