“VICTOR BABEȘ” UNIVERSITY OF MEDICINE AND PHARMACY FROM TIMISOARA

FACULTY OF PHARMACY

Department I

BORCAN FLORIN

PHD THESIS

POLYURETHANE STRUCTURES APPLICABLE IN THE TRANSMEMBRANE TRANSFER OF BIOLOGICALLY ACTIVE COMPOUNDS

A B S T R A C T

Scientific Coordinator:

PROF. UNIV. DR. ȘOICA CODRUȚA MARINELA

T i m i ș o a r a

2 0 2 3

TABLE OF CONTENTS

A B S T R A C T

The development of nanotechnologies capable of transporting biologically active compounds in the body and releasing them in a specific way at the site of action makes it possible to increase the therapeutic activity and reduce the toxicity of many drugs. These "nanovectors" are capable of protecting the active molecule from degradation by the body's enzymes, selectively targeting it to the target tissue or cell, and controlling its release. More specific than traditional pharmaceutical formulations, the "nanodrugs" make possible the design of new therapeutic strategies in the fight against severe diseases, such as cancers, intracellular infections, metabolic or neurodegenerative diseases, etc.

The prefix "nano" evokes strange scenarios, as if taken from science-fiction, but nanoscience simply refers to a series of techniques for manipulating particles on a nanometric or molecular scale. This technology should inspire hope rather than fear, according to Prof. Dr. C. Palivan, a researcher of Romanian origin from the Department of Physical Chemistry of the University of Basel, member of the Swiss Institute of Nanotechnologies.

Polyurethanes (PU) were initially developed as flexible foams obtained at the laboratory scale by the group of Prof. Otto Bayer in the 1930s at the German company I.G. Farben; the foam obtained then was a more flexible material, more resistant to moisture and with a relatively low production cost compared to all other materials known at that time. Very quickly, new improvements were introduced to further reduce the cost of production and to increase the flexibility of the material. Studies aimed at greater resistance to deformation and reduced flammability followed, and later the modification of some physical-mechanical characteristics such as rigidity and hardness led to the opening of multiple possibilities of use in the automotive industry. The city of Timișoara produced polyurethanes on an industrial scale within the S.C. Spumotim S.A. presenting an extensive catalog of products, the most famous of which belong to the foaming section of Dacia car seats, the section of shoe soles, camping products, films and various sound and thermal insulation solutions.

Due to its versatility, polyurethane is widely used in the medical and pharmaceutical industries. There are biodegradable polyurethanes without health risks that offer excellent performance in terms of durability, flexibility and strength. These materials are also characterized by impeccable biocompatibility, bending resistance and versatility, which allow them to adapt to different types of uses.

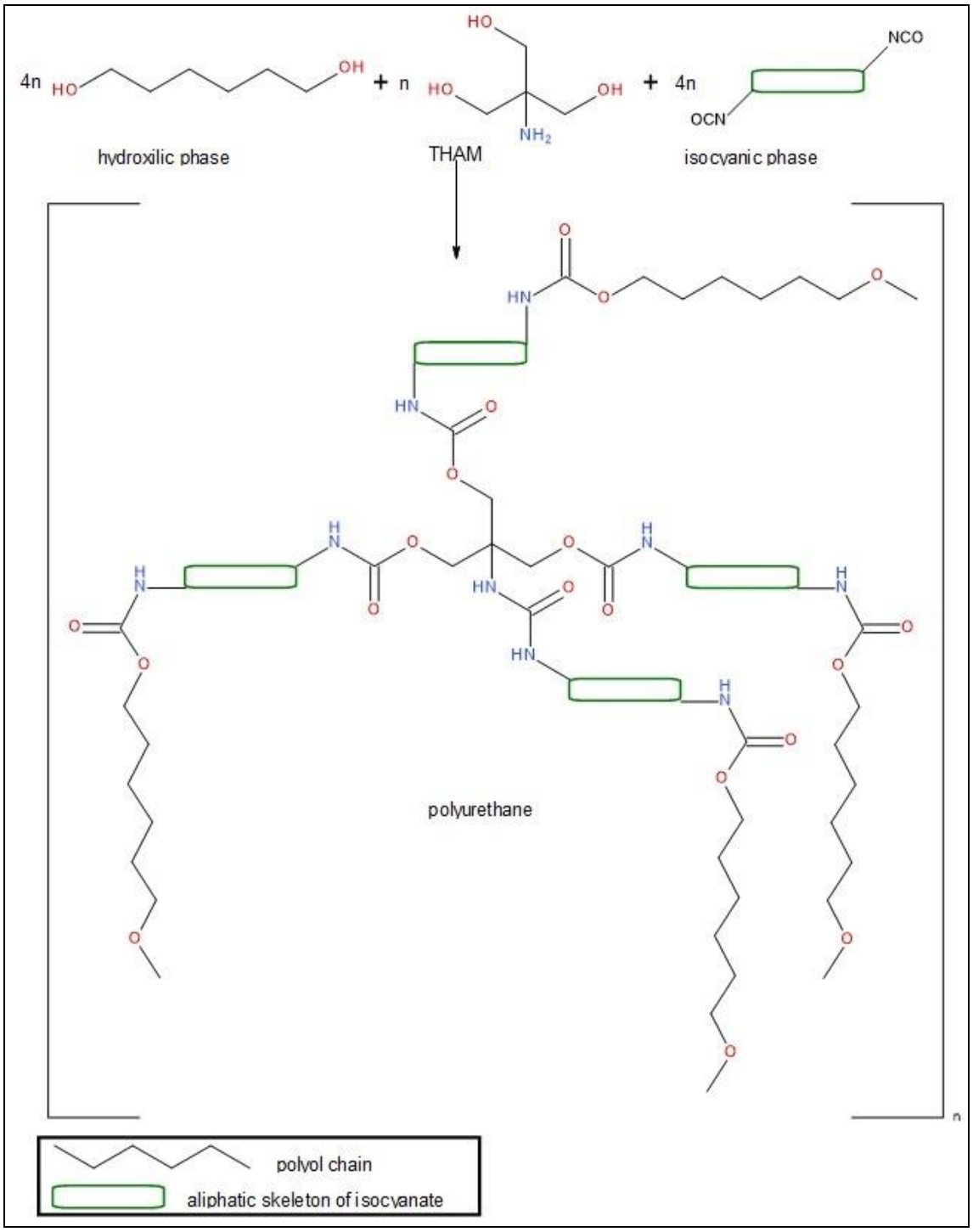

Fig. 1. Schematic representation of the chemical equation of the synthesis of a branched polyurethane

Drug delivery systems based on polyurethane particles began to appear in the last decade of the last century, but the respective studies were based on the use of industrial precursors such as diphenylmethane-4,4'-diisocyanate (MDI), the mixture of isomers toluylene-2,4-diisocyanate and toluylene-2,6-diisocyanate (TDI), respectively catalysts such as stannous octoate and 1,4-diazabicyclo[2.2.2]octane (Dabco), and all of these substances are highly toxic compounds. In addition, it was not then possible to reduce the sizes of the obtained

macromolecular structures below the threshold of 1000 nm, so as to ensure the desired transmembrane permeability. The collaborations between K. Bouchemal from Chimie ParisTech, PSL University and Prof. H. Fessi from the University of Lyon from 2003-2006, led to a series of scientific papers describing the synthesis of polyurethane capsules based on aliphatic isocyanates, obtained by a polyaddition process combined with a strong emulsification in the presence of two surfactants (one lipophilic and the other hydrophilic), with sizes between 200 and 600 nm, usable as carriers for alpha-tocopherol.

Concerns for obtaining and characterizing drug carriers constituted a research direction within the Faculty of Pharmacy of the "Victor Babeș" University of Medicine and Pharmacy Timișoara before 2010; first, a series of scientific articles on the encapsulation of betulinic acid in cyclodextrin complexes were published, and later, our team reported the obtaining and optimization of some polyurethane structures, but also based on metal nanoparticles. In our university, almost 80 scientific papers about different transporters usable as a delivery system for biologically active compounds have been published, more than half of them by researchers from the Faculty of Pharmacy according to Clarivate® Web of Science.

Based on all the previously stated aspects, this doctoral thesis is part of the concerns and research directions of the various collectives and research centers in our university, the central theme being the synthesis and preliminary characterization of some biomaterials based on polyurethane structures that can be used as carriers of biologically active substances. **The aim of this PhD thesis** was to make an important contribution to the development of new polyurethane particles that have improved physico-chemical and biological properties compared to what was already reported in the specialized literature. At the level of 2019, when the studies included in this thesis started, the following aspects were known: (1) the precursors for the synthesis of polyurethane materials are relatively cheap organic compounds, (2) there is the possibility of obtaining very varied products depending on the raw materials and of the ratio between them, but (3) the finished materials have a low solubility and (4) they have not been studied much in the chosen field of applicability. Regarding the novelty and originality of the studies included in the doctoral period, the results obtained and reported in prestigious journals indicated the obtaining of polymeric particles that had not been studied until that moment, whose size can be easily adjusted according to the targeted load. Through the multidisciplinary methods approached, from simple determinations of solubility, pH and refractive index, spectral characterizations, thermal analyzes and up to *in vitro* and *in vivo* tests, this doctoral thesis is constituted as a complex research aimed at completing the general

picture on some polymer products already established in various industrial fields. The synthesis method, friendly to the environment, based on low electricity consumption and the absence of aggressive chemical agents (strong acids and bases), has been modified several times in order to reduce the intake of auxiliary agents (surfactants, catalysts of reaction, polymerization initiators, chain extenders, etc.), to remove all potentially carcinogenic compounds (aromatic isocyanates), but without affecting the intended properties of the reaction products.

General part of the thesis describes in turn, in ***Chapter 1***, several polymeric systems usable as carriers of biologically active substances, both natural and synthetic. Thus, collagen appears in self-administered treatments in several ophthalmic diseases, as hydrogels and drug delivery systems used to target chondrocytes in cartilage repair. Along with collagen in hydrogels, gelatin is also used, with the help of which the encapsulation efficiency, the electric charge density and the hydrophobicity of the carrier could be adjusted. Albumin, chitosan and cyclodextrins are other substances frequently encountered in specialist studies. In contrast to these compounds, synthetic biodegradable polymers such as polyethylene glycol, poly(lactic-co-glycolic acid), some polyamides, poly(ortho-esters) and polyanhydrides are among the preferred macromolecular compounds in this field. The presentation concludes with a brief history of polyurethanes, their chemical structure and current commercial data regarding their areas of applicability.

Chapter 2 describes epithelial membranes by presenting the cells that make up the multi-stratified epithelium and the functions that this tissue performs. Emphasis is placed on describing the junction and interdependence of cognitive tissue, the complex molecular organization, the differentiations that occur, and the thickness of the layers. Articular cartilage, a non-vascularized tissue that covers the bony components of joints, is responsible for pain-free movement of the body by absorbing various shocks. It and the synovial membranes are important to study when the target of treatment is joint pain.

Special part of the thesis, in which the personal contributions of the doctoral student can be found, was built on the basis of four studies presented one after the other in distinct chapters. Thus, ***Chapter 3*** presents an investigation on the changes that pH can produce on some solutions containing polyurethane structures. Previous studies have shown us that in most cases the aqueous solutions containing the polyurethane particles synthesized by our research group have pH values located in a narrow range (almost neutral acid-base, 6.6-7.2) and that they are perfect from this point of view for use regardless of the intended mode of

administration. However, oral administration, the most convenient, frequently results in the inactivation of many biologically active substances due to the very low pH values of the gastric juice and due to the presence of fermentative activities and microbial populations that reduce the stability and absorption of some drugs such as sulfonamides, tetracyclines, chloramphenicol, etc. The study published by the PhD student in the *Journal of Medicine and Life* in 2020 is based on a sample of polyurethane microstructures for which the initial determinations revealed the achievement of a mono-population colloidal suspension with a Gaussian distribution of dimensions between 139 and 151 nm, pH = 6.78 and Zeta potential of +24.6 mV. Reference and test aliquots were placed in buffer solutions of pH 3, 7 and 10, respectively, and maintained in the medium for a period of 15 days. 1.5 mL of each medium was replaced every other day with the respective buffer solution and changes in particle size and surface charge were measured using a Zetasizer type instrument from Cordouan Technol. (France). It has been observed that there is an accelerated decrease in the size of the polyurethane structures in an acidic environment (pH = 3) accompanied by an increase in the values of the Zeta potential in the same environment, which leads to greater stability against the agglomeration tendency, a phenomenon frequently encountered in colloidal solutions. Already knowing that polyether-urethane structures degrade slowly in environments that simulate body fluids, this study has made a major contribution to understanding the potential that such structures have as carriers of biologically active substances.

Chapter 4 indicates in the initial part the role that antiviral medication has in the treatment of some infections, with a special emphasis on acyclovir as the basic substance. Acyclovir was discovered in the mid-1970s and led to a 1988 Nobel Prize in Medicine awarded to American biochemist and virologist Gertrude Belle Elion based on her research into new drug development. Unfortunately, over time, along with the advantages of using acyclovir in infections with the Herpes simplex virus, Herpes zoster, chicken pox, etc., an important series of disadvantages were also discovered, among which we only mention the low absorption upon oral administration, local hypersensitivity reactions and renal toxicity with transient nephropathy. In addition to a number of advantages such as masking the lipophilic/hydrophilic nature of the encapsulated agent and protecting it from the external environment, carriers of biologically active substances present the possibility of gradual and targeted release of their cargo. Thus, the scientific literature presents many transport systems developed to reduce the adverse effects of acyclovir. Our study combined the accumulated knowledge with a series of carefully selected primary raw materials (mixture of two aliphatic isocyanates: hexamethylene

diisocyanate and isophorone diisocyanate, polyethylene glycol and 1,4-butanediol) to obtain a controlled release delivery vehicle.

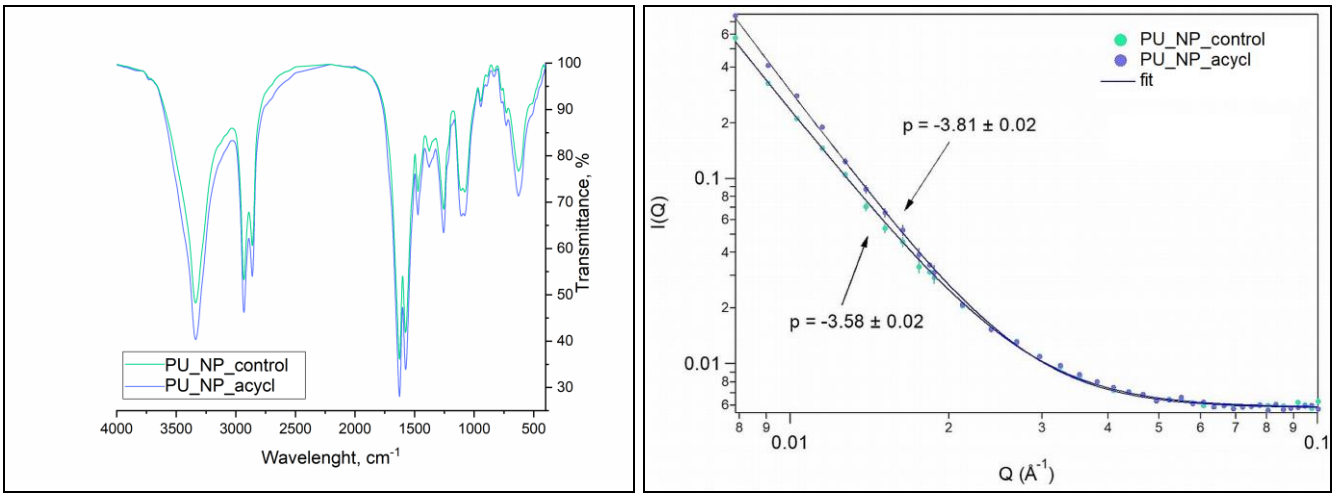

Fig. 2. Comparative characterization of samples by FT-IR spectroscopy and SANS

The results of this research highlighted the obtaining of polyurethane nanoparticles with sizes between 78 and 91 nm, with low polydispersity indices, characteristics to almost homogeneous samples, with Zeta potential values between +26 and +29 mV, very stable in the investigated temperature range. The research was disseminated in the journal *Nanomaterials* belonging to MDPI in the year 2021 (journal located in the first quartile / 25th percentile according to *Journal Citation Reports 2022*, **I.F. = 5.719**), and the spectral analysis indicated an encapsulation efficiency of 78% of the mass of acyclovir introduced into the reaction, while its release, which was tested in a simulated environment, coincided with the profiles characteristic of prolonged and sustained release pharmaceutical forms. A major drawback of the synthesized product was the low aqueous and organic solubility value of the polyurethane carrier used as the vehicle for acyclovir.

Based on the results obtained and especially because of the drawback found and stated previously, the next study was directed towards the structural modification of the polymer chains so that the finished products present improved solubility values. Thus, ***Chapter 5*** describes a research that formed the basis of an invention, registered at the State Office for Inventions and Trademarks on Oct. 23, 2020 and for which the national authority issued Invention Patent No. 134816 / on Jun. 30, 2023. The invention describes a process for improving the solubility of a polyurethane-type carrier, usable for acyclovir. Compared to what was already known at that time, namely: (1) the bioadhesive patches described by patents *EP 2136781 A1* and *US 20130303556 A1* which required repeated change at short time intervals,

(2) the polymer implants of the invention *US 20100303883 A1* for which it is need the intervention of qualified personnel and increase the risk of local infection, (3) the lipid matrices described in *US 20160354470 A1* which had as disadvantages the very rapid degradation and release of some antibiotics, antifungals or anti-cancers, the invention of our research team was based on a polyether-urethane carrier in whose macromolecular chains polar groups have been inserted through the intercalation of 2-acetoxy-1-propan-1-ol ($C_5H_{10}O_3$), respectively 2,3-diacetoxy-propan-1-ol ($C_7H_{12}O_5$). The conclusions of the study were that the solubility increased 1.5-2.0 times compared to a reference sample, while the average particle size and their Zeta potential remained almost unchanged from the benchmark.

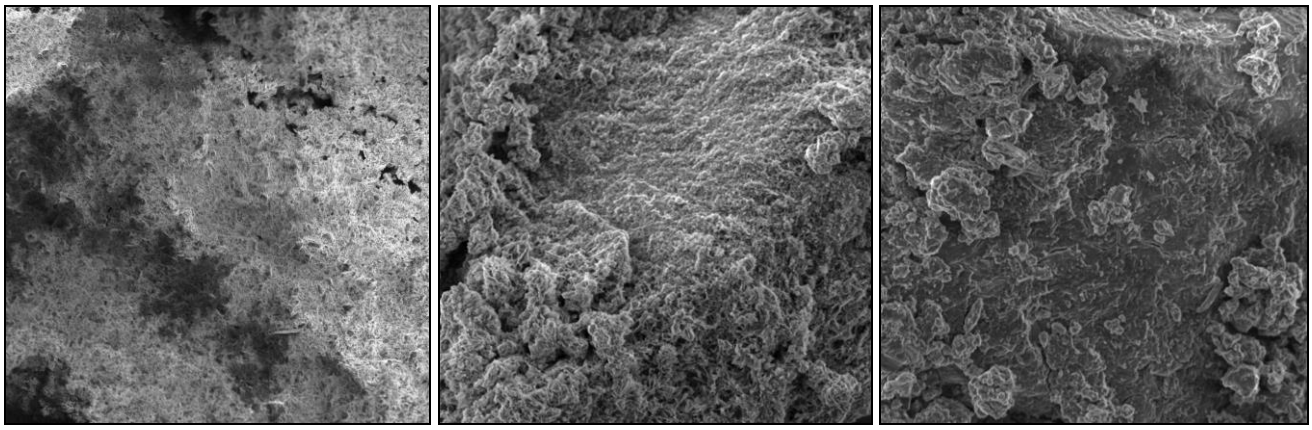

Fig. 3. Appearance of the three polyurethane carrier samples

Chapter 6 presents the latest study of our research team and constitutes a starting point in the transport of genetic material using polyurethane structures as vectors. The paper published in MDPI's *Journal of Functional Biomaterials* in Fall 2023 (a journal ranked in the second quartile / 50th percentile by *Journal Citation Reports 2022*, **I.F. = 4.800**) describes the synthesis and characterization of a carrier usable for 2'-deoxycytidine-5'-monophosphate (dCMP), a nucleotide based on a pyrimidine backbone and a deoxy-beta-D-ribofuranose. Nucleotides link together forming polynucleotide chains which are the segments of biological macromolecules called nucleic acids. It is also very important to mention that nucleotides and their derivatives are involved in many biochemical processes having an important role in cellular metabolism. Although these compounds are synthesized in the body, under certain conditions an exogenous supply is required to cover the body's needs. Our study describes the synthesis and preliminary characterization of a dCMP carrier, the research can be later extended to other compounds that lend themselves to a genetic approach in the treatment of some diseases.

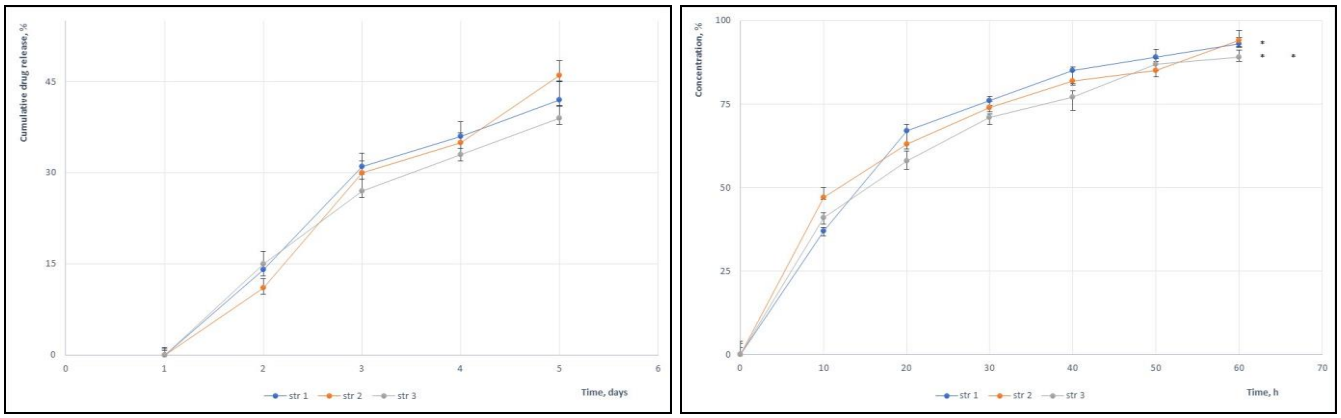

Fig. 4. The rate of release of the active agent and penetrability through an artificial membrane

The obtained results indicate the obtaining of structures with dimensions between 132 and 190 nm, with an average stability against the agglomeration tendency, demonstrated both by Zeta potential measurements and by SEM imaging. The samples proved very stable over time, stable over a wide range of temperatures, showed a nucleotide encapsulation efficiency of approx. 70% and the same profile of delayed release of the active agent that was also encountered in our previous studies. In non-invasive *in vivo* evaluations in which professional probes from Courage-Khazaka (Germany) were used to determine the level of erythema and hydration of the stratum corneum, no side effects were observed on animal skin (laboratory mouse) and in models of *in vitro* testing on human dermal fibroblast culture found that the polyurethane-based polymeric carrier leads to an increase in cell viability, an aspect previously observed and presented by other research groups.

The last chapter of the doctoral thesis highlights the main conclusions of the previously described studies. It is particularly important to remember that nanomaterials and biomaterials are current products, generated by a multi-disciplinary collaboration between the research teams of prestigious universities in the world. The sequence of studies presented in the thesis follows a logical sequence, an algorithm determined by the need to develop carriers of biologically active substances that meet all current requirements.