Документи на Бисера Пиличева, дф за участие в конкурс за АД „професор“ по научна специалност „Технология на лекарствените форми и биофармация”; област на висше образование 7. „Здравеопазване и спорт“ по професионално направление 7.3. „Фармация“, обявен в ДВ, бр. 56 / 11.07.2025 г.

Резюмета на отпечатаните в пълен текст научни трудове на Бисера Пиличева, дф

1. Резюмета на трудове, представени като хабилитационен труд

1.1. Pilicheva B., Uzunova Y., Bodurov I., Viraneva A., Exner G., Sotirov S., Yovcheva T., Marudova M. Layer-by-layer self-assembly films for buccal drug delivery: The effect of polymer cross-linking (2020) Journal of Drug Delivery Science and Technology, 59, art. no. 101897, DOI: 10.1016/j.jddst.2020.101897

ABSTRACT: The goal of this study was the preparation of multilayer polyectrolyte films intended for buccal delivery of benzydamine (BZ). Another important issue was to evaluate the effect of polymer cross-linking as a means to enhance drug loading and ensure sustained drug release. LbL deposition technique was applied for the multilayer build-up. The deposition was performed by alternative dip-coating of corona pretreated polylactic acid substrates into chitosan and caseine solutions. Chitosan and casein deposition was followed by cross-linking with glutaraldehyde/sodium tripolyphosphate, and calcium chloride, respectively. For drug loading, the multilayers were soaked into benzydamine solution after chitosan cross-linking. The procedure was carried out until eight layers were laid. The formation of polyelectrolyte complex and the cross-linking occurrence were proven by ATR-FTIR. Further investigations on the morphology and topography of the samples were carried out by AFM. Moreover, swelling behavior, content uniformity, drug release and mucoadhesion were investigated. The experimental data displayed differences in the structure and surface properties of the films as per the cross-linking agent used. All the investigated parameters were influenced by the cross-linking mode. Double cross-linking of chitosan with glutaraldehyde and sodium tripolyphosphate proved to be the most appropriate method for the achievement of the desired goals.

1.2. Vlaeva I., Pilicheva B., Marinova A., Bodurov I., Yovcheva T., Viraneva A., Exner G., Uzunova Y., Sotirov S., Marudova M. Investigation of flexible polyelectrolyte multilayered structure by using different techniques (2019) AIP Conference Proceedings, 2075, art. no. 160007, DOI: 10.1063/1.5091334

ABSTRACT: The presented paper deals with the formulation of medical pads with potential use as drug delivery systems via buccal mucosa adhesion. The pads consists of a substrate and deposited on it multilayered structure of alternating casein and chitosan. The method of preparation of this multilayer structure is layer-by-layer deposition. The electrolyte nature of the used polymers (chitosan - polyanion and casein - polycation) was employed in the formulation process. The substrate initial excess charge was provided by corona discharge pretreatment. The formulation process was monitored by index of refraction measurements by using a laser refractometer. The ability of the formulated pads as drug delivery system was proven by drug release tests, where the kinetics of benzydamine hydrochloride (BH) was used. The method of mucin reaction was employed to establish the potential of pads' adhesion.

1.3. Yovcheva T., Pilicheva B., Marinova A., Viraneva A., Bodurov I., Exner G., Sotirov S., Vlaeva I., Uzunova Y., Marudova M. Crosslinked Chitosan/Casein Polyelectrolyte Multilayers for Drug Delivery (2019) Journal of Physics: Conference Series, 1186 (1), art. no. 012030, DOI: 10.1088/1742-6596/1186/1/012030

ABSTRACT: Polyelectrolyte multilayers (PEMs) are widely used as drug delivery systems, but still remain challenging for their small drug immobilizing capacity. One way to increase the immobilized drug amount may be crosslinking of the PEMs, which stabilize them and increase their porosity. The aim of the present study is fabrication and characterization of chitosan/casein PEMs, which are crosslinked with different crosslinking agents - glutaraldehyde, sodium tripolyphosphate, CaCl2 and combinations of two of them. XPS method was used to prove the PEMs crosslinking. SEM was used to observe film morphology and its variation due to cross'linking. Water capacity of PEMs in 100 % relative humidity was investigated. Release of model drug Benzydamine Hydrochloride was monitored spectrophotometrically at 306 nm. The crosslinking improves the PEMs stability and causes formation of porous surface. After crosslinking the amount of the immobilized drug increased several times.

1.4. Exner G., Marudova M., Sotirov S., Marinova A., Viraneva A., Pilicheva B., Bodurov I., Vlaeva I., Uzunova Y., Yovcheva T. Multilayered polyelectrolyte structures with potential for intracavity drug delivery systems (2019) Applied Surface Science, 493, pp. 620 - 627, DOI: 10.1016/j.apsusc.2019.07.039

ABSTRACT: A design of multilayered polyelectrolyte structures consisting of a substrate of poly-DL-lactide film covered with multilayers of chitosan and casein is reported. An innovative method of corona pretreatment of the substrate was used to ensure the required substrate surface excess charge for the electrostatic attachment of the polyelectrolytes on it. The number of layers was varied from 4 to 16 and the ability of the system to load drugs and to adhere to the buccal mucosa was investigated by means of UV-VIS-NIR spectroscopy, atomic force microscopy, and the small sessile drop technique. At the particular pH and ionic strength used, the optimal number of layers is 8, which results from the loose morphology and complex nature of multilayered structural formation. The drug release is accompanied by polyelectrolyte dissolution.

1.5. Zahariev N., Marudova M., Milenkova S., Uzunova Y., Pilicheva B. Casein micelles as nanocarriers for benzydamine delivery (2021) Polymers, 13 (24), art. no. 4357, DOI: 10.3390/polym13244357

ABSTRACT: The aim of the present work was to optimize the process parameters of the nano spray drying technique for the formulation of benzydamine-loaded casein nanoparticles and to investigate the effect of some process variables on the structural and morphological characteristics and release behavior. The obtained particles were characterized in terms of particle size and size distribution, surface morphology, production yield and encapsulation efficiency, drug-polymer compatibility, etc., using dynamic light scattering, scanning electron microscopy, differential scanning calorimetry, and Fourier transformed infrared spectroscopy. Production yields of the blank nanoparticles were significantly influenced by the concentration of both casein and the crosslinking agent. The formulated drug-loaded nanoparticles had an average particle size of 135.9 nm to 994.2 nm. Drug loading varied from 16.02% to 57.41% and the encapsulation efficiency was in the range 34.61% to 78.82%. Our study has demonstrated that all the investigated parameters depended greatly on the polymer/drug ratio and the drug release study confirmed the feasibility of the developed nanocarriers for prolonged delivery of benzydamine.

1.6. Milenkova S., Pilicheva B., Uzunova Y., Yovcheva T., Marudova M. Casein Microgels as Benzydamine Hydrochloride Carriers for Prolonged Release (2022) Materials, 15 (4), art. no. 1333, DOI: 10.3390/ma15041333

ABSTRACT: This research aims to investigate the properties of nano-and micro-sized casein hydrogels crosslinked by sodium tripolyphosphate as drug delivery systems. Benzydamine hydrochloride was chosen as a model hydrophilic drug. The gels were synthesized by varying different parameters: casein concentration, casein/crosslinking ratio, and addition of ethanol as a co-solvent. The electrostatic attractive interactions between the casein and the sodium tripolyphosphate were confirmed by FTIR spectroscopy. The particle sizes was determined by dynamic light scattering and varied in the range between several hundred nanometers and several microns. The yield of the gelation process was high for all investigated samples and varied between 55.3% and 78.3%. The encapsulation efficiency of the particles was strongly influenced by the casein concentration and casein/crosslinker ratio and its values were between 4.6% and 22.4%. The release study confirmed that casein particles are useful as benzydamine carriers and ensured prolonged release over 72 h.

1.7. Pilicheva B., Uzunova Y., Marudova M. Polyelectrolyte Multilayer Films as a Potential Buccal Platform for Drug Delivery (2022) Polymers, 14 (4), art. no. 734, DOI: 10.3390/polym14040734

ABSTRACT: The goal of this research was to study the potential of polyelectrolyte multilayers as buccal dosage forms for drug delivery and to investigate how the properties of the drugs impact the overall performance of the delivery system. Multilayer films based on the polyelectrolyte interaction between casein and chitosan were developed using benzydamine, tolfenamic acid and betahistine as model drugs. The samples were characterized for surface pH, moisture content and moisture absorption, swelling behavior and mucoadhesion. Additionally, surface morphology was investigated, as well as the drugs' physical state after incorporation in the multilayer films. The samples proved to be non-irritant (pH was within the physiological range), physically stable (moisture content and moisture absorption below 5%) and mucoadhesive, adsorbing from 60 to 70% mucin. The release behavior corelated to the swelling index profiles of the samples and was strongly dependent on the drug solubility. The developed multilayer films appeared to be an optimum delivery system for sparingly soluble drugs due to the high drug loading achieved.

1.8. Marudova M., Milenkova S., Zahariev N., Yovcheva T., Pilicheva B. Formulation and characterization of Benzydamine loaded casein/chitosan nanocomplexes (2023) Journal of Physics: Conference Series, 2436 (1), art. no. 012028, DOI: 10.1088/1742-6596/2436/1/012028

ABSTRACT: The objectives of the present study were to synthesize casein/chitosan nanocomplexes, which are able to immobilize and release Benzydamine in a controlled manner, and to investigate the influence of casein/chitosan ratio on their morphological, physico-chemical and drug carrier characteristics. The complexes were obtained by electrostatic interaction at pH 6, at which casein in negatively charged and chitosan is protonated. The yield of the complexation was the lowest for chitosan excess particles (18.4%) and increased to 83.5% for casein excess particles. The particle size varied in the range from 400 nm to several microns depending on the casein/chitosan ratio. Atomic force microscopy (AFM) was used to assess the morphological properties of the nanocomplexes. It was found that the Benzydamine loading was minimal (15%) in the stoichiometric complex and maximal (30%) in complexes with casein/chitosan ratio 5:1. The mechanism of Benzydamine release was defined as Fickian diffusion.

1.9. Milenkova S., Ambrus R., Mukhtar M., Pilicheva B., Marudova M. Spray-Dried Chitosan Hydrogel Particles as a Potential Delivery System for Benzydamine Hydrochloride (2024) Gels, 10 (3), art. no. 189.

ABSTRACT: Chitosan, being a biocompatible and mucoadhesive polysaccharide, is one of the most preferred hydrogel-forming materials for drug delivery. The objectives of the present study are to obtain spray-dried microparticles based on low-molecular-weight chitosan and study their potential application as cargo systems for the orally active drug benzydamine hydrochloride. Three types of particles are obtained: raw chitosan particles (at three different concentrations), cross-linked with sodium tripolyphosphate (NaTPP) particles (at three different chitosan:NaTPP ratios), and particles coated with mannitol (at three different chitosan:mannitol ratios), all of them in the size range between 1 and 10 μm. Based on the loading efficiency and the yields of the formulated hydrogel particles, one model of each type is chosen for further investigation of the effect of the cross-linker or the excipient on the properties of the gel structures. The morphology of both empty and benzydamine hydrochloride-loaded chitosan particles was examined by scanning electron microscopy, and it was quite regular and spherical. Interactions and composition in the samples are investigated by Fourier-transformed infrared spectroscopy. The thermal stability and phase state of the drug and drug-containing

polymer matrixes were tested by differential scanning calorimetry and X-ray powdered diffraction, revealing that the drug underwent a phase transition. A drug release kinetics study of the chosen gel-based structures in simulated saliva buffer (pH = 6.8) and mathematical modeling of the process were performed, indicating the Weibull model as the most appropriate one.

1.10. Viraneva A., Marudova M., Pilicheva B., Grigorov A., Zahariev N., Milenkova S., Yovcheva T. Benzydamine-Loaded Casein Nanospheres Embedded in Polyelectrolyte Multilayers (2025) IEEE Transactions on Dielectrics and Electrical Insulation, 32 (3), pp. 1599 - 1605, DOI: 10.1109/TDEI.2025.3542349

ABSTRACT: Polyelectrolyte multilayers (PEMs) films containing benzydamine hydrochloride (Benz)-loaded casein nanoparticles are developed aiming to increase the structure drug loading efficiency and to prolong the drug release. The multilayers are built on biodegradable polyester substrates, which are previously charged in a corona discharge. Medium viscosity sodium alginate (SA) is used as a partner in the multilayer formulation. The SA and the casein nanoparticles are deposited on the substrate using the layer-by-layer (LbL) deposition technique. The drug loading efficiency and the release kinetics in artificial saliva are determined spectrophotometrically.

2. Резюмета на статии от група показатели Г

2.1. Georgieva Y.Zh., Pilicheva B.A., Kokova V.Yu., Apostolova E.G., Kassarova M.I. Taste Masking of Enalapril Maleate by the Precipitation Method (2019) Folia Medica, 61 (3), pp. 426 - 434, DOI: 10.3897/folmed.61.e39208

ABSTRACT: Background: Taste masking of bitter or unpleasant drugs is an important prerequisite to improve patient compliance, especially for children and elderly patients. We aimed at obtaining taste-masked microparticles intended for incorporation into orodispersible tablets (ODTs). We selected the precipitation method using enalapril maleate (ENA) as a model bitter-tasting drug and Eudragit EPO® as a pH sensitive polymer. Aim: The aim of this study was to obtain microparticles with enalapril maleate by the precipitation method in order to mask the bitter taste of the drug. Materials and methods: Nine models of enalapril maleate – Eudragit EPO® microparticles were prepared by the precipitation method at varied drug-polymer ratios. The models were characterized in terms of size, shape, production yield, drug content, encapsulation efficiency and moisture content. Fourier-transformed infrared spectroscopy, powder X-ray diffraction and differential scanning calorimetry were used to analyze possible interactions in the complex. In vitro drug release in simulated salivary fluid and in vivo taste evaluation in rats were realized to prove taste masking. Results: The particle size distribution varied from 266.9 μm to 410.9 μm. The shape of the resulting particles was irregular. The production yield varied from 23.6% to 78.2%. The drug content ranged between 2.3% to 4.8%, encapsulation efficiency increased from 1.6% to 9.0%. In vitro drug release data indicated significant taste masking. Conclusion: Some of the obtained microparticles by the precipitation method showed satisfactory taste masking efficiency, which proved the taste masking feasibility of this method.

2.2. Georgieva Y., Kassarova M., Kokova V., Apostolova E., Pilicheva B. Taste masking of enalapril maleate by microencapsulation in Eudragit EPO® microparticles (2020) Pharmazie, 75 (2-3), pp. 61 - 69, DOI: 10.1691/ph.2020.9123

ABSTRACT: Microencapsulation is one of the most commonly used taste masking techniques. It can be accomplished by various methods, including coacervation, solvent evaporation, extrusion and spray-drying. Enalapril maleate, a bitter-tasting ACE-inhibitor, is available worldwide in conventional tablet formulations and as oral solution in the USA. The purpose of this study was to develop enalapril-loaded microparticles using spray-drying and to test their taste masking potential. Eudragit EPO® was used as a taste masking polymer for the preparation of a drugpolymer suspension. The suspension was

then spray-dried under the following conditions: inlet temperature 65 °C, outlet temperature 30 °C, aspiration 100% and pump rate 10%. The drug-to-polymer ratio was varied and seven different microparticle models were developed. The yield of spray-dried particles ranged from of 51.3 to 85.4%, drug loading varied from 7.75 to 24.69% and encapsulation efficiency ranged from 58.5 to 95.7%. The particle size varied between 5.00 μm and 17.47 μm and the moisture content varied between 7.1% and 10.3%. In vitro taste assessment revealed minimal or no ENA release in artificial saliva. In vivo studies (with experimental animals and healthy volunteers) were used to evaluate the taste masking potential of spray-dried microparticles of enalapril maleate and Eudragit EPO®.

2.3. Milenkova S., Pilicheva B., Tsoneva S., Marudova M. Chitosan/alginate nano-spheres for curcumin loading and delivery (2020) Bulgarian Chemical Communications, 52, pp. 134 - 140, DOI: 10.34049/bcc.52.A.237

ABSTRACT: In this study curcumin-loaded chitosan-NaTPP nano-spheres were prepared by ionotropic gelation followed by chitosan-alginate polyelectrolyte complex formation for some of the samples. DLS and AFM analysis showed that the particles were nearly spherical in shape with nano-sized diameters. The size of the spheres could be varied by changing the polymer and crosslinker concentrations. FTIR analysis revealed potential interactions among the constituents in the composite nano-spheres. It proved that the curcumin did not interact with the spheres and retained its chemical structure. The loading efficiency (%) of curcumin in the nanospheres was above 60%. The in vitro drug release profile along with kinetics and mechanism of release from the nanospheres were studied under simulated physiological conditions for different incubation periods. The release rate could be changed by varying the chitosan and NaTPP concentrations, as well as by coating the nano-spheres with chitosan-alginate complexes.

2.4. Pilicheva B., Uzunova Y., Katsarov P. Comparative study on microencapsulation of lavender (Lavandula angustifolia mill.) and peppermint (mentha piperita L.) essential oils via spray-drying technique (2021) Molecules, 26 (24), art. no. 7467, DOI: 10.3390/molecules26247467

ABSTRACT: Essential oils have been studied for various applications, including for therapeutic purposes. There is extensive literature regarding their properties; however, their low stability limits their application. Generally, the microencapsulation of essential oils allows enhanced stability and enables the potential incorporation in solid dosage forms. Lavender and peppermint oils were encapsulated in microparticles using a spray-drying technique under optimized conditions: 170◦C temperature, 35 m3/h aspiration

volume flow, and 7.5 mL/min feed flow. Arabic gum and maltodextrin were used as coating polymers individually in varying concentrations from 5 to 20% (w/v) and in combination. The microparticles were studied for morphology, particle size, oil content, and flowability. The formulated powder particles showed a high yield of 71 to 84%, mean diameter 2.41 to 5.99 μm, and total oil content of up to 10.80%. The results showed that both the wall material type and concentration, as well as the type of essential oil, significantly affected the encapsulation process and the final particle characteristics. Our study has demonstrated that the encapsulation of lavender and peppermint oils in Arabic gum/maltodextrin microparticles by spray-drying represents a feasible approach for the conversion of liquids into solids regarding their further use in powder technology.

2.5. Pilicheva B., Boyuklieva R. Can the nasal cavity help tackle COVID-19? (2021) Pharmaceutics, 13 (10), art. no. 1612, DOI: 10.3390/pharmaceutics13101612

ABSTRACT: Despite the progress made in the fight against the COVID-19 pandemic, it still poses dramatic challenges for scientists around the world. Various approaches are applied, including repurposed medications and alternative routes for administration. Several vaccines have been approved, and many more are under clinical and preclinical investigation. This review aims to systemize the available information and to outline the key therapeutic strategies for COVID-19, based on the nasal route of administration.

2.6. Katsarov P., Shindova M., Lukova P., Belcheva A., Delattre C., Pilicheva B. Polysaccharide-based micro-and nanosized drug delivery systems for potential application in the pediatric dentistry (2021) Polymers, 13 (19), art. no. 3342, DOI: 10.3390/polym13193342

ABSTRACT: The intensive development of micro-and nanotechnologies in recent years has offered a wide horizon of new possibilities for drug delivery in dentistry. The use of polymeric drug carriers turned out to be a successful technique for formulating micro- and nanoparticles with controlled or targeted drug release in the oral cavity. Such strategies have the potential to provide an improved therapeutic approach to prevention and treatment of various oral diseases not only for adults, but also in the pediatric dental practice. Due to their biocompatibility, biotolerance and biodegradability, naturally occurring polysaccharides like chitosan, alginate, pectin, dextran, starch, etc., are among the most preferred materials for preparation of micro-and nano-devices for drug delivery, offering simple particle-forming characteristics and easily tunable properties of the formulated structures. Their low immunogenicity and low toxicity provide an

advantage over most synthetic polymers for the development of pediatric formulations. This review is focused on micro-and nanoscale polysaccharide biomaterials as dental drug carriers, with an emphasis on their potential application in pediatric dentistry.

2.7. Milenkova S., Manolov I., Pilicheva B., Nikolova M., Marudova M. Curcumin loaded casein submicron-sized gels as drug delivery systems (2021) Journal of Physics: Conference Series, 1762 (1), art. no. 012009, DOI: 10.1088/1742-6596/1762/1/012009

ABSTRACT: Hydrogels from natural polyelectrolytes possess many important features such as low toxicity, biocompatibility, biodegradability and hydrophilicity. These properties make them suitable for applications such as immobilization and controlled release of drugs and other types of biologically active molecules. In the present study submicron-sized hydrogels made from casein by ionotropic gelation are investigated. For the purpose, two types of crosslinkers are used at different pH. To characterize these submicron gels, their sizes, chemical structures and thermal stability are examined by DLS, FT-IR and DSC respectively. To prove their immobilization ability, active compound, namely curcumin, is immobilized in the hydrogel's structures. DPPH assay is conducted to establish the antioxidant properties of the curcumin before and after immobilization. The loading efficiency of the nanostructures together with the curcumin release kinetics are evaluated and modelled mathematically.

2.8. Marudova M., Zahariev N., Milenkova S., Pilicheva B., Viraneva A., Yovcheva T. Development and In-Vitro Characterization of Benzydamine Loaded Chitosan Nanoparticles (2021) Macromolecular Symposia, 395 (1), art. no. 2000279, DOI: 10.1002/masy.202000279

ABSTRACT: The aim of the present research is the preparation of chitosan nanoparticles intended for Benzydamine (BZ) delivery. The method of ionotropic gelation is applied for the nanoparticle's formation. The crosslinking is performed by NaTPP. Dynamic light scattering analysis shows that particle's sizes are in nano-scale and can be varied by changing the polymer and crosslinker concentrations. The yield of the gelation varies between 23% and 69%. The BZ loading efficiency differs in the range from 10% to 24.3%. Differential scanning calorimetry is applied for investigating the BZ state. It proved that the BZ changes its physical state from crystal to amorphous. The in vitro drug release profile along with kinetics and mechanism of release from the nano-spheres are studied

under simulated physiological conditions for different incubation periods. The release rate can be changed by varying the chitosan and NaTPP concentrations.

2.9. Milenkova S., Pilicheva B., Zahariev N., Shivachev B., Rusew R.I., Yovcheva T., Marudova M. Milk protein-based formulations as controlled delivery systems for tolfenamic acid (2022) Bulgarian Chemical Communications, 54, pp. 64 - 70, DOI: 10.34049/bcc.54.B1.0409

ABSTRACT: Casein-based gels were examined as potential drug carrier for a model drug, namely tolfenamic acid (TA). TA is widely applied as anti-cancer agent along with its ability to induce degradation of specific tumor proteins and decrease metastasis in liver in the case of pancreatic cancer. Casein-based spheres were formulated at high pH by ionotropic gelation in the presence of crosslinker CaCl2. To optimize their chemical content and structure, casein concentration, TA concentration and casein/crosslinker ratio were varied. Sizes and morphology of casein gels loaded with TA were examined. The structure's phase state was tested by differential scanning calorimetry. ATR-FTIR was used to establish the crosslinking process between casein and CaCl2. The efficiency of the loading process of drug was calculated. Studies on the drug release kinetics were conducted under simulated physiological conditions.

2.10. Gvozdeva Y., Kassarova M., Pilicheva B. Formulation of Enalapril-Loaded Microspheres Using Emulsion Solvent Evaporation Technique for Bitter Taste Masking (2022) Indian Journal of Pharmaceutical Sciences, 2022, Vol 84, Issue 6, p1407, DOI: 10.36468/pharmaceutical-sciences.1039, ISSN: 0250-474X

ABSTRACT: The aim of the present study was to formulate polymer microspheres using the emulsion solvent evaporation technique as an approach to mask the bitter taste of enalapril maleate. Five models were prepared with two water-insoluble polymers. Talc was used to stabilize the particle structure. Production yields ranged from 38 % to 53 %; drug loading varied between 17 % and 40 %; the mean particle size ranged from 140 μm to 339 μm. No interaction between enalapril and the polymers was estimated. A drug release study in artificial saliva indicated a negligible amount of enalapril dissolved in the test medium which demonstrated successful taste masking.

2.11. Milenkova S., Zahariev N., Ambrus R., Pilicheva B., Marudova M. A Study on the Stoichiometry of Casein/Chitosan Gel Complexes as a Delivery System for Quercetin (2023) Applied Sciences (Switzerland), 13 (19), art. no. 10868, DOI: 10.3390/app131910868

ABSTRACT: As a well-known plant flavanol, quercetin possesses a diverse range of biological properties. These include its ability to act as an antioxidant, reduce inflammation, and exhibit anticancer effects. Consequently, it finds extensive application in numerous models related to wound healing. However, the poor physicochemical characteristics of the molecule (which include low solubility, stability, and permeability) eventually reduce its bioavailability at the targeted sites. A variety of nano formulations with great therapeutic potential have been created in order to get around these obstacles on the way to successful therapy. The current investigation aims to examine the properties of nano- and micro-sized casein/chitosan gel polyelectrolyte complexes (PECs) with respect to their potential for quercetin loading and release. Four different types of hydrogel particles at pH 6 and different casein/chitosan charge ratios were synthesized; namely, 1:1, 2:1, 4:1, and 6:1 in excesses of casein. The attractive electrostatic interactions between the oppositely charged polyelectrolytes were proved by FT-IR spectroscopy. The process yield increased from 37.5% to 72.5% in excesses of casein. The gel particle’s size varied between 377 nm and 5.72 μm depending on the casein/chitosan stoichiometry. The morphology of the obtained gel polyelectrolyte complexes was found to be spherical, based on scanning electron microscopy and atomic force microscopy analysis. The quercetin loading efficiency was above 95% for all investigated hydrogel complexes. Investigation of the physical state of the loaded polyphenol by the differential scanning calorimetry and X-ray powdered diffraction technique suggested the occurrence of partial recrystallization phenomena. The quercetin release test was performed in phosphate buffer (pH 5.5) at 32 °C and permanent stirring at 50 rpm. A zero-order model was used to describe in the best way the release kinetics. The reported casein/chitosan complexes loaded with quercetin may find application in wound healing as a concomitant treatment.

2.12. Lukova P., Katsarov P., Pilicheva B. Application of Starch, Cellulose, and Their Derivatives in the Development of Microparticle Drug-Delivery Systems (2023) Polymers, 15 (17), art. no. 3615, DOI: 10.3390/polym15173615

ABSTRACT: Micro- and nanotechnologies have been intensively studied in recent years as novel platforms for targeting and controlling the delivery of various pharmaceutical substances. Microparticulate drug delivery systems for oral, parenteral, or topical

administration are multiple unit formulations, considered as powerful therapeutic tools for the treatment of various diseases, providing sustained drug release, enhanced drug stability, and precise dosing and directing the active substance to specific sites in the organism. The properties of these pharmaceutical formulations are highly dependent on the characteristics of the polymers used as drug carriers for their preparation. Starch and cellulose are among the most preferred biomaterials for biomedical applications due to their biocompatibility, biodegradability, and lack of toxicity. These polysaccharides and their derivatives, like dextrins (maltodextrin, cyclodextrins), ethylcellulose, methylcellulose, hydroxypropyl methylcellulose, carboxy methylcellulose, etc., have been widely used in pharmaceutical technology as excipients for the preparation of solid, semi-solid, and liquid dosage forms. Due to their accessibility and relatively easy particle-forming properties, starch and cellulose are promising materials for designing drug-loaded microparticles for various therapeutic applications. This study aims to summarize some of the basic characteristics of starch and cellulose derivatives related to their potential utilization as microparticulate drug carriers in the pharmaceutical field.

2.13. Boyuklieva R., Zagorchev P., Pilicheva B. Computational, In Vitro, and In Vivo Models for Nose-to-Brain Drug Delivery Studies (2023) Biomedicines, 11 (8), art. no. 2198, DOI: 10.3390/biomedicines11082198

ABSTRACT: Direct nose-to-brain drug delivery offers the opportunity to treat central nervous system disorders more effectively due to the possibility of drug molecules reaching the brain without passing through the blood–brain barrier. Such a delivery route allows the desired anatomic site to be reached while ensuring drug effectiveness, minimizing side effects, and limiting drug losses and degradation. However, the absorption of intranasally administered entities is a complex process that considerably depends on the interplay between the characteristics of the drug delivery systems and the nasal mucosa. Various preclinical models (in silico, in vitro, ex vivo, and in vivo) are used to study the transport of drugs after intranasal administration. The present review article attempts to summarize the different computational and experimental models used so far to investigate the direct delivery of therapeutic agents or colloidal carriers from the nasal cavity to the brain tissue. Moreover, it provides a critical evaluation of the data available from different studies and identifies the advantages and disadvantages of each model.

2.14. Zahariev N., Katsarov P., Lukova P., Pilicheva B. Novel Fucoidan Pharmaceutical Formulations and Their Potential Application in Oncology—A Review (2023) Polymers, 15 (15), art. no. 3242, DOI: 10.3390/polym15153242

ABSTRACT: Fucoidan belongs to the family of marine sulfated, L-fucose-rich polysaccharides found in the cell wall matrix of various brown algae species. In the last few years, sulfated polysaccharides have attracted the attention of researchers due to their broad biological activities such as anticoagulant, antithrombotic, antidiabetic, immunomodulatory, anticancer and antiproliferative effects. Recently the application of fucoidan in the field of pharmaceutical technology has been widely investigated. Due to its low toxicity, biocompatibility and biodegradability, fucoidan plays an important role as a drug carrier for the formulation of various drug delivery systems, especially as a biopolymer with anticancer activity, used for targeted delivery of chemotherapeutics in oncology. Furthermore, the presence of sulfate residues with negative charge in its structure enables fucoidan to form ionic complexes with oppositely charged molecules, providing relatively easy structure-forming properties in combination with other polymers. The aim of the present study was to overview essential fucoidan characteristics, related to its application in the development of pharmaceutical formulations as a single drug carrier or in combinations with other polymers. Special focus was placed on micro- and nanosized drug delivery systems with polysaccharides and their application in the field of oncology.

2.15. Todorova M., Milusheva M., Kaynarova L., Georgieva D., Delchev V., Simeonova S., Pilicheva B., Nikolova S. Drug-Loaded Silver Nanoparticles—A Tool for Delivery of a Mebeverine Precursor in Inflammatory Bowel Diseases Treatment (2023) Biomedicines, 11 (6), art. no. 1593, DOI: 10.3390/biomedicines11061593

ABSTRACT: Chronic, multifactorial illnesses of the gastrointestinal tract include inflammatory bowel diseases. One of the greatest methods for regulated medicine administration in a particular region of inflammation is the nanoparticle system. Silver nanoparticles (Ag NPs) have been utilized as drug delivery systems in the pharmaceutical industry. The goal of the current study is to synthesize drug-loaded Ag NPs using a previously described 3-methyl-1-phenylbutan-2-amine, as a mebeverine precursor (MP). Methods: A green, galactose-assisted method for the rapid synthesis and stabilization of Ag NPs as a drug-delivery system is presented. Galactose was used as a reducing and capping agent forming a thin layer encasing the nanoparticles. Results: The structure, size distribution, zeta potential, surface charge, and the role of the capping agent of drug-

loaded Ag NPs were discussed. The drug release of the MP-loaded Ag NPs was also investigated. The Ag NPs indicated a very good drug release between 80 and 85%. Based on the preliminary results, Ag NPs might be a promising medication delivery system for MP and a useful treatment option for inflammatory bowel disease. Therefore, future research into the potential medical applications of the produced Ag NPs is necessary.

2.16. Boyuklieva R., Hristozova A., Pilicheva B. Synthesis and Characterization of PCL-Idebenone Nanoparticles for Potential Nose-to-Brain Delivery (2023) Biomedicines, 11 (5), art. no. 1491, DOI: 10.3390/biomedicines11051491

ABSTRACT: The present work is focused on the preparation of an optimal model of poly-ε-caprolactone nanoparticles as potential carriers for nasal administration of idebenone. A solvent/evaporation technique was used for nanoparticle preparation. Poly-ε-caprolactone with different molecular weights (14,000 and 80,000 g/mol) was used. Polysorbate 20 and Poloxamer 407, alone and in combination, were used as emulsifiers at different concentrations to obtain a stable formulation. The nanoparticles were characterized using dynamic light scattering, SEM, TEM, and FTIR. The resulting structures were spherical in shape and their size distribution depended on the type of emulsifier. The average particle size ranged from 188 to 628 nm. The effect of molecular weight and type of emulsifier was established. Optimal models of appropriate size for nasal administration were selected for inclusion of idebenone. Three models of idebenone-loaded nanoparticles were developed and the effect of molecular weight on the encapsulation efficiency was investigated. Increased encapsulation efficiency was found when poly-ε-caprolactone with lower molecular weight was used. The molecular weight also affected the drug release from the nanostructures. Dissolution study data were fitted into various kinetic models and the Korsmeyer–Peppas model was found to be indicative of the release mechanism of idebenone.

2.17. Milenkova S., Marudova M., Zahariev N., Yovcheva T., Pilicheva B. Crosslinked chitosan-based particles obtained by water-in-oil emulsion technique (2023) Journal of Physics: Conference Series, 2436 (1), art. no. 012027, DOI: 10.1088/1742-6596/2436/1/012027

ABSTRACT: Particles based on emulsified chitosan in oil phase have been reported in the present study. Sodium Tripolyphosphate (NaTPP) was introduced to them as a crosslinker and its effect on their properties was examined. Laser diffraction technique showed that the obtained structures are micronsized particles with single modal distribution. Optical

microscopy has confirmed their size range, together with the fact that their shape is regular and spherical. Swelling studies in simulated saliva conditions (pH = 6.8) has shown a relation between the crosslinking degree of the particles and cycles of swelling and dissolution at different rates. Mucoadhesion test confirmed their potential as delivery systems through a mucosal route and showed dependence of the mucoadhesion properties upon the free amino groups left onto the chitosan chains.

2.18. Grancharova Ts., Simeonova S., Pilicheva B., Zagorchev P. Gold Nanoparticles in Parkinson's Disease Therapy: A Focus on Plant-Based Green Synthesis (2024) Cureus, 22;16(2):e54671. DOI: 10.7759/cureus.54671. PMID: 38524031; PMCID: PMC10960252, ISSN: 2168-8184.

ABSTRACT: Parkinson's disease (PD) is a progressive neurodegenerative disease that affects approximately 1% of people over the age of 60 and 5% of those over the age of 85. Current drugs for Parkinson's disease mainly affect the symptoms and cannot stop its progression. Nanotechnology provides a solution to address some challenges in therapy, such as overcoming the blood-brain barrier (BBB), adverse pharmacokinetics, and the limited bioavailability of therapeutics. The reformulation of drugs into nanoparticles (NPs) can improve their biodistribution, protect them from degradation, reduce the required dose, and ensure target accumulation. Furthermore, appropriately designed nanoparticles enable the combination of diagnosis and therapy with a single nanoagent. In recent years, gold nanoparticles (AuNPs) have been studied with increasing interest due to their intrinsic nanozyme activity. They can mimic the action of superoxide dismutase, catalase, and peroxidase. The use of 13-nm gold nanoparticles (CNM-Au8®) in bicarbonate solution is being studied as a potential treatment for Parkinson's disease and other neurological illnesses. CNM-Au8® improves remyelination and motor functions in experimental animals. Among the many techniques for nanoparticle synthesis, green synthesis is increasingly used due to its simplicity and therapeutic potential. Green synthesis relies on natural and environmentally friendly materials, such as plant extracts, to reduce metal ions and form nanoparticles. Moreover, the presence of bioactive plant compounds on their surface increases the therapeutic potential of these nanoparticles. The present article reviews the possibilities of nanoparticles obtained by green synthesis to combine the therapeutic effects of plant components with gold.

2.19. Keremidarska-Markova M., Sazdova I., Mladenov M., Pilicheva B., Zagorchev P., Gagov H. Sirtuin 1 and Hormonal Regulations in Aging (2024) Applied Sciences, 14 (24), art. no. 12051, DOI: 10.33/app142412051

ABSTRACT: Aging affects the structure and functions of all organs and systems in the organism. In the elderly, significant changes in hormonal levels are observed. These translate to a predisposition for chronic diseases, including cardiovascular, neurodegenerative, and metabolic disorders. Therefore, tremendous scientific effort is focused on investigating molecular mechanisms and drugs with the potential to reduce hormonal changes in old age and their impact. Sirtuin 1 (SIRT1), a member of the sirtuin family of deacetylases, has been extensively studied as a regulator of multiple pathways related to antioxidant properties, optimal immune response, and metabolism. SIRT1 plays a key role in regulating various hormonal pathways and maintaining homeostasis. In the present study, we review the interplay between SIRT1 and hormonal regulations, including the endocrine role of the hypothalamic–pituitary–thyroid, –adrenal, –gonadal, and –liver axes, of other endocrine glands, and of non-endocrine tissues in the aging organism. The application of natural SIRT1 activators, such as resveratrol, curcumin, paeonol, and Buyang Huanwu Decoction, for the treatment of aging and senescence is discussed. SIRT1 activators improve mitochondrial function, reduce oxidative stress, and promote longevity, but their clinical application is limited by low bioavailability and poor permeability across biological barriers. For this reason, advanced delivery strategies are being considered, including nose-to-brain drug delivery and nanotechnology-based formulations.

2.20. Abarova S., Grancharova T., Zagorchev P., Tenchov B., Pilicheva B. Novel Spectroscopic Studies of the Interaction of Three Different Types of Iron Oxide Nanoparticles with Albumin (2024) Nanomaterials, 14 (23), art. no. 1861, DOI: 10.3390/nano14231861

ABSTRACT: In the present work, we studied the interactions of three types of iron oxide nanoparticles (IONPs) with human serum albumin by fluorescence and UV-Vis spectroscopy. The determined binding parameters of the reactions and the thermodynamic parameters, including ΔHo, ΔSo, and ΔGo indicated that electrostatic forces play a major role in the interaction of IONPs with HSA. These measurements indicate a fluorescent quenching mechanism based on IONPs-HSA static complex formation. Our study shows that the interaction between HSA and IONPs depends on the nanoparticle structure. The interaction was found to be spontaneous, exothermic, and entropy-driven. HSA was shown to interact moderately with IONPs obtained with plant

extracts of Uncaria tomentosa L. and Clinopodium vulgare L., and firmly with IONPs prepared with Ganoderma lingzhi (Reishi) extract, via ground-state association. Analysis by modified Stern-Volmer approximation indicates that the quenching mechanism is static. Our study significantly improves our understanding of the mechanisms of interaction, distribution, and transport involved in the interaction between proteins and IONPs. It provides crucial insights into the functional perturbations of albumin binding capacity and the effects of IONPs on the stability and structural modifications of plasma carrier proteins.

2.21. Marudova M., Milenkova S., Zahariev N., Pilicheva B., Yovcheva T. Alginate-Based Emulsion Micro-Spheres as 5-Fluorouracil Carriers (2024) Macromolecular Symposia, 413 (4), art. no. 2300262, DOI: 10.1002/masy.202300262

ABSTRACT: The present study aims to investigate the potential of alginate microspheres, formulated by emulsion technique, as a drug delivery system for 5-Fluorouracil. Three different concentrations of alginate, namely 1%, 2%, and 3%, are used in the synthesis. The spheres are characterized in terms of their size, morphology, process yield, and loading efficiency. Based on these parameters, the model formulated from 1% alginate is chosen and loaded with different concentrations of 5-Fluorouracil achieving polymer to drug mass ratios 2:1, 4:1, and 6:1. The release process of the drug is completed in 3 h, showing burst effect for all models. The faster release from Model 2:1 is associated with the biggest drug amount and its accumulation on the periphery of the spheres.

2.22. Milenkova S., Tashkov S., Zahariev N., Pilicheva B., Marudova M. 5-FLUOROURACIL ENCAPSULATED CHITOSAN MICROSPHERES (2024) Journal of Chemical Technology and Metallurgy, 59 (4), pp. 887 - 896, DOI: 10.59957/jctm.v59.i4.2024.19

ABSTRACT: 5-Fluorouracil (5-FU) is a chemotherapeutic agent used in therapies for both systematically and topical treatment of different types of cancers. Depending on the period of application, its administration may lead to different side effects such as nausea, headache, pain or even photosensitivity. To avoid them, the drug may be encapsulated into polymeric particles. In the present study, bio polymeric spheres based on chitosan, a linear polysaccharide, are presented. The spheres are formulated by an emulsion technique with solvent evaporation. Three types of particles are synthesized: without crosslinker, with sodium tripolyphosphate and glutaraldehyde crosslinker. The

resultant structures are evaluated regarding their size, morphology, and encapsulation efficiency. The crosslinking process and the drug presence in the particles is confirmed by FT-IR. A drug release study is conducted to examine the release kinetics and understand the release behaviour depending on the presence and the type of the crosslinker.

2.23. Grancharova Ts., Zagorchev Pl., Pilicheva B. Green synthesis and photothermal application of superparamagnetic iron oxide nanoparticles for cancer hyperthermia (2025) Bulgarian Chemical Communications, 57, pp. 22 - 26, DOI: 10.34049/bcc.57.A.MU06

ABSTRACT: Cancer remains a major global health challenge, with traditional treatments like chemotherapy and radiation often yielding limited long-term success. Hyperthermia, one of the oldest cancer therapies, is promising due to the disorganized blood supply in tumors, making them more sensitive to heat compared to healthy tissues. Nanomedicine, particularly nanoparticle-mediated hyperthermia, is emerging as an innovative treatment strategy. Iron oxide nanoparticles (IONPs) are of interest due to their ability to convert near-infrared (NIR) laser radiation into localized heat while functioning as contrast agents for imaging. In this study, we investigated the synthesis of IONPs using Ganoderma lingzhi (Reishi) extract (IONP@GL) by green synthesis method. The synthesized IONPs were characterized by their size, morphology, magnetic properties, and photothermal efficiency. IONP@GL exhibited good heat generation under 808 nm laser irradiation. These findings highlight the potential of green-synthesized IONPs for application in photothermal cancer therapy.

3. Резюмета на публикувани глави от книги

3.1. Marudova M., Milenkova S., Pilicheva B., Zahariev N. The nonfood applications of casein (2024) Casein: Structural Properties, Uses, Health Benefits and Nutraceutical Applications, pp.383-397, DOI:10.1016/B978-0-443-15836-0.00009-3

ABSTRACT: Casein, being a major milk protein, has high importance in food and nutritional applications, but not only that. As the demand and seeking for new biodegradable solutions in the nonfood industry continuously grow, casein turns out to be a proper solution. Due to its foaming, film-forming, adhesive, and conductive properties, it is a suitable base material for the preparation of a variety of products. Casein-based coatings, latexes, and bioplastics may substitute the conventional nonenvironmentally friendly options. Along with this, novel soft biosensors with high adhesion and lack of skin irritation containing casein may open the door for a whole new generation of bionic and health-monitoring devices. In addition to these applications, casein is a irretrievable material of interest in dentistry and tooth restoration.

3.2. Pilicheva B., Zahariev N., Milenkova S., Marudova M. Use of casein for pharmaceutical applications (2024) Casein: Structural Properties, Uses, Health Benefits and Nutraceutical Applications, pp. 299 - 314, DOI: 10.1016/B978-0-443-15836-0.00017-2

ABSTRACT: Advances in pharmaceutical research have led to the investigation of a wide variety of natural and synthetic materials and their applicability as drug delivery carriers. In recent decades, the use of naturally derived materials for biomedical applications has increased dramatically. Researchers around the world are investigating new materials that could supplant the use of synthetic and semisynthetic agents. Biobased polymers, which are composed of renewable resources and are found abundantly in nature, represent/have manifested a huge breakthrough in polymer science. Casein has emerged as an optimal carrier for the development of conventional and novel drug delivery systems due to its specific structural and functional characteristics. In this chapter, the main pharmaceutical applications of casein will be discussed. The foremost knowledge and achievements in the field of casein-based drug-delivery systems are summarized, the authors’ personal experience is shared, and the challenges and new perspectives in mastering this research area are outlined.

3.3. Bravo-Arrepol G., Dimitrov Katsarov P., Asenova-Pilicheva B., Kancheva-Lukova P., Escobar-Avello D., Peniche H., García L., Peniche-Covas C., Michaud P., Delattre C., Becheran-Maron L., Castaño J., Lopez M.D., Valdes O., Nesic A., Cabrera-Barjas G. Working principles and use of chitosan for food component encapsulation (2023) Materials Science and Engineering in Food Product Development, pp. 161 - 209, DOI: 10.1002/9781119860594.ch9

ABSTRACT: In Chapter 8, our colleagues have used gelatin as an example to illustrate how polymers can be used to encapsulate food components. Here we will use another polymer, namely chitosan, to further demonstrate the potential use of polymers in food science. Chitosan is a nontoxic, biocompatible, and cationic biopolymer that can be isolated from crustacean shells, fungal biomass, and insects. Nowadays, there are comprehensive data on its use in pharmacy and biomedicine. Also, several applications in the food industry as flocculating agents, antimicrobial, thickeners, and emulsifiers have been suggested and implemented. However, its commercial use for food material encapsulation is still in its infancy but deserves close attention to find new application opportunities. In this chapter, we will review the use of chitosan as a polymeric matrix to encapsulate essential food components that need to be protected before or during food processing due to their oxidation-sensitive nature. Furthermore, the potential applications of chitosan for nano and microencapsulated material to be used in food packaging will be discussed in the context of sustainable production and circular economy. Emerging technologies in food materials, like three-dimensional (3D) printing and wet spinning, will be discussed, and a market overview of chitosan's uses in food will be presented. It is anticipated that this chapter will contribute to widespread chitosan use as a food industry material to improve product shelf life and food-packaging functionality.