9. Author's reference for the original scientific contributions in the publications presented in the competition by Assoc. prof. Viktoria Milkova Nakova

My scientific expertise focuses on the investigation of polyelectrolyte adsorption on model particles (colloidal particles, liposomes, and emulsion droplets) in an aqueous medium, as well as the analysis of the electrical properties of the produced colloid-polymer suspensions and their stability.

The most significant results are obtained with the application of electrokinetic methods – electric light scattering, dynamic light scattering and microelectrophoresis. The combination of these methods allowed a detailed description of the surface electrical properties and the stability of the studied systems.

I have contributions in three main research topics:

1. **investigation of the correlation between the physicochemical characteristics of polysaccharides (chitosan and alginate) and their ability to stabilise model colloid-polymer suspensions;**
2. **formation and characterisation of multilayer films of polysaccharides and carbon nanomaterials (carbon dots) on non-spherical particles;**
3. **development and characterisation of model polysaccharide-based systems for delivery and controlled release of active components.**

The investigations from the first two topics have a completely fundamental character, while the third topic is devoted to a design and characterisation of model systems with potential application. All three problems are important for colloidal science, as they are related to modification of the particle surface and affect both the stability of dispersed systems and the possibility of their applications. The topic belongs to an extremely actual and dynamic area of modern science in accordance with the possibilities for application in the fields of nanotechnology, medicine, pharmacy and electronics. Stable polymer-containing structures with different shapes, composition, size and functionality can be formed by appropriate selection of components and precise control over the conditions for preparation.

The research included in the scientific papers presented for participation in the competition is in accordance with one of the main scientific priorities of the Institute of Physical Chemistry: *Design, characterization and optimization of complex liquid media and nanostructured materials for applications in medicine, pharmacy, food and oil industries: • Functionalized surfaces, decorated micro- and nanostructured materials; applications in the pharmaceutical, chemical and oil industries; • Properties and structure of complex liquid compositions containing synthetic and biosurfactants, peptides, proteins and polymers, bacteria and viruses; applications for medical and environmental purposes; • Electrokinetic spectroscopy of colloid-polymer systems; • Design of (nano)capsules by layer-by-layer adsorption of polymers on colloidal particles and emulsion droplets.*

I participate in the competition with scientific results obtained mainly in the last 5 years. On the topic of the competition are presented 16 publications, which have been published in international scientific journals, referenced and indexed in world-renowned databases of scientific information: Q1 (8 publications), Q2 (6 publications), Q3 (1 publication) and 1 book chapter. I participate in the competition with 5 independent publications (4 publications in journals from quartile Q1 and 1 book chapter). The results included in the publications have been presented at 25 scientific forums.

To date, my scientific research has been summarised in 41 scientific papers, of which 36 have been published in international refereed scientific journals: Q1 (21 publications), Q2 (13 publications), Q3 (1 publication) and one book chapter. The Hirsch index on Scopus is 12. The results were obtained during the implementation of 12 research projects and have been presented at 81 scientific forums.

1. Investigation of the correlation between the physicochemical characteristics of polysaccharides (chitosan and alginate) and their ability to stabilise model colloid-polymer suspensions (publications № 1, 3, 9, 11, 16)

1.1. Adsorption of chitosan on lecithin-stabilised emulsion droplets.

(publications № 1, 11, 16: 1. **Milkova, V.** Electrosteric stabilization of oil/water emulsions by adsorption of chitosan oligosaccharides - an electrokinetic study. *Carbohydrate polymers* **2021**, *265*, 118072. http://doi.org/10.1016/j.carbpol.2021.118072. (JIF=10.723, 2021, 2/56, Q1); 11. Goycoolea, F.M.; **Milkova, V.** Electrokinetic behavior of chitosan adsorbed onto o/w nanoemulsion droplets. *Colloids and Surfaces: A.* **2017**, *519*, 205-2011. https://doi.org/10.1016/j.colsurfa.2016.05.093. (JIF=2.829, 2017, 70/147, Q2); 16. **Milkova, V.** Chitosan-stabilized oil-in-water nanoemulsions: electrokinetic properties. In K. Ramalingam (Ed.), Handbook of Research on Nanoemulsion Applications in Agriculture, Food, Health, and Biomedical Sciences, **2022** (pp. 44-58). IGI Global Scientific Publishing. https://doi.org/10.4018/978-1-7998-8378-4.ch003)

Chitosan is a copolymer of β-(1-4)-2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose, which is obtained by deacetylation of chitin, a polysaccharide extracted from insects, crustaceans and some fungi. Chitosan is a weak polyelectrolyte (pKa ~6.5). Its unique properties (low toxicity to cells and tissues, biocompatibility, biodegradability, antibacterial, anti-tumour activity, etc.) make it an indispensable component in the composition of platforms for various applications in bionanotechnology.

As a polycation, chitosan forms complexes with negatively charged biomolecules, enzymes, interacts with cell membranes and oppositely charged particles (solid particles, liposomes and many others). As a weak polyelectrolyte, its charge density depends on the pH of the medium and this is an advantage for many therapeutic applications. The high charge density of chitosan (pH < pKa) favours its polyelectrolyte behaviour, while at low charge density (pH above 7) its low cytotoxicity facilitates the intracellular release of bioactive molecules or drugs. However, the low charge density of chitosan leads to poor solubility in water, aggregation in solution and poor stability of chitosan-based structures. Many theoretical and experimental studies in the literature have shown that the factors that affect the stability of chitosan-based systems are the concentration and charge density (i.e. adsorption conditions), as well as the size of the produced structures. The biological activity of chitosan in these structures is determined by the molecular weight, degree of acetylation (DA), pH, ionic strength concentration of the polymer, which organism (e.g., crustaceans or fungi) it is obtained, and other factors related to the procedure for formation of the structures.

Therefore, even small changes in the molecular structure of chitosan, for example molecules with the same molecular weight but different DA (or vice versa), will lead to a different charge distribution or different charge density and correspondingly different electrical properties (resp. stability) or biological activity of the produced chitosan-based structures.

In addition, it is known that in a solution of fully charged polyelectrolytes, some of the counterions undergo "counterion condensation". This phenomenon is expected when the average distance between two charges along the polyion chain is less than the Bjerrum length (0.714 nm in water at room temperature). The "condensed" counterions have lower mobility compared to the counterions from the diffuse part of the double electric layer. Since in the experimental conditions of the studies (pH 4.0–4.7), chitosan is considered as a highly charged polyelectrolyte, the presence of counterions with lower mobility in the vicinity of the polyions can be expected.

Compared to the properties of polysaccharides, chitosan oligosaccharides (COS) have many advantages in their physicochemical characteristics and remarkable bioactive properties: very low molecular weight (below 3 900 g/moL), low degree of polymerization (up to 20 monomers), low degree of acetylation (about 10 %), low viscosity and high solubility in water. COS offer a unique opportunity to perform model studies on the adsorption and distinguish the contribution of different interactions between chitosan and an oppositely charged surface by screening in DA and molecular weight of the molecules.

<u>The studies summarised in this topic aim</u> to analyse the correlation between the physicochemical characteristics (molecular weight and DA) of poly- and oligosaccharides of chitosan and the electrokinetic behaviour of emulsions in the presence of different concentrations of polymer. In the studies are used ultra-pure and well-characterised chitosans. The samples are dissolved in water or HCl solution with molarity calculated according to the characteristics of each of the chitosans. In the studies (in the pH range 4.0–4.7), the molecule can be considered as a fully charged polycation. Since chitosans are dissolved in HCl, the presence of Cl^- counterions is expected in the solution. Oil-in-water nanoemulsions (o/w) stabilized with soy lecithin, obtained by the solvent displacement method (primary emulsion), are used. Each sample is prepared separately by adding a different amount of chitosan to a small volume of the primary emulsion, not by titration (secondary emulsion).

Main contributions:

- For the first time, the statement about the dominant contribution of hydrophobic (at high DA) and electrostatic (at low DA) interactions between chitosan monomers and a charged surface is experimentally confirmed.
- For the first time, it is experimentally proven that an electrokinetic model for analysing the change in the electrical properties of dispersion in the presence of COS is a useful approach for distinguishing the contribution of electrostatic and hydrophobic interactions responsible for the achievement of electrosteric stabilisation of the studied systems.
- The electrokinetic behaviour of secondary nanoemulsions stabilised with lecithin in the presence of different concentrations of chitosan oligosaccharides (COS) with completely uncharged 4 or 5 monomers shown that the ζ-potential of the droplets gradually decreases (in absolute value), but overcompensation of the surface charge is not achieved. It is shown that the adsorption of short uncharged chains is a result of hydrophobic interactions with the hydrophobic domains of the lecithin layer, stabilising the emulsion droplets, and the decrease in the electrokinetic charge is due to a shift of the slip plane of the double electric layer. At the same time, it is registered two regimes of increase in the thickness of the polymer layer with increasing COS concentration. According to the estimation, it is found that at low COS concentrations, a thin monolayer is formed on the lecithin layer, while with increasing chitosan concentrations, several molecular layers can be formed on the surface due to secondary hydrophobic interactions between the molecules themselves.
- The registered electrokinetic response from a primary nanoemulsion in the presence of different concentrations of a mixture of COS containing charged and uncharged monomers is similar to that of emulsion droplets in the presence of polysaccharides. It is suggested that the observed behaviour results from the balance between electrostatic interactions between the charged monomers and the negatively charged domains of the surface, and hydrophobic interactions between the uncharged monomers and the hydrophobic domains of the lecithin layer. It is found that the electrostatic interactions have a dominant effect on the electrical properties of the COS-stabilised emulsion droplets.
- A critical charge density of chitosan polysaccharides is defined, denoted as DA 28 %. Electrokinetic studies shown that with a gradual increase in the concentration of chitosan added to the primary emulsion, the isoelectric point of the droplets and the surface charge do not

depend on the molecular weight of the polymer when DA<28 %. This is due to the measured effective, not the real, charge of the particles with adsorbed polymer. At DA>28 %, the isoelectric point of the droplets and the overcharging of the surface shift to a higher polymer concentration, since U_{ef} depends on the number of adsorbed charges, not on the molecular weight (or the contour length of the polymer).

- It is assumed that the electrical properties of the adsorbed polysaccharides on the model surface depend on the balance of the effect of molecular weight and DA. It is found that the electrical polarizability is a complex parameter that allows distinction of the properties of chitosans with DA<28 %, when the electrokinetic behaviour of particles (emulsion droplets) is almost the same. The fraction of released Cl^- counterions upon the adsorption of chitosans on the surface is estimated. Based on the assumption that the polarisation of the fraction of "condensed" counterions near the polyion chain defines the electrical properties of the studied systems, the electrical polarizability of one polyelectrolyte molecule is calculated.
- The mass ratio between chitosan and lecithin (g chitosan/ 1 g lecithin) is estimated. It is found that the adsorbed amount of chitosan (about 0.22 mg/m^2) in the stabilised emulsion does not depend on the molecular weight and DA of the polysaccharide.

1.2. Adsorption of chitosan on model non-spherical colloidal particles.

(publication № 9. **Milkova, V.**; Radeva, Ts. Influence of charge density and calcium salt on stiffness of polysaccharides multilayer film. Colloids and Surfaces: A **2015**, *481*, 13-19. https://doi.org/10.1016/j.colsurfa.2015.03.061. (JIF=2.760, 2015, 56/144, Q2).

The studies summarised in this topic aim to investigate the influence of the charge density of polysaccharides and the presence of a divalent electrolyte on the electrical properties and "stiffness" of a multilayer formed from chitosan and pectin. These studies aim to supplement the findings of the electro-optical behavior of multilayer films of these polysaccharides on model particles, which were the subject of study in previous studies (not participating in the competition). Ellipsoidal nanocrystals of β-FeOOH are chosen as a model surface, because the preparation, electrical properties and stability of which have been studied in detail in our group.

Main contributions:

- A higher excess of small counterions in the film is found when the last adsorbed layer is formed of chitosan compared to those of pectin: the electro-optical effect of suspensions of particles with an adsorbed polymer film with an outermost layer of chitosan, at low orientation energies, is higher than that of pectin, but at high intensity of the applied field (i.e. at high degrees of orientation close to the full orientation in the system) it becomes smaller than that of pectin layer. It is suggested that this is due to the appearance of an elasto-hydrodynamic effect, which dominates at high field intensity and favours the perpendicular orientation of the "soft" particles to the direction of the applied electric field. This effect becomes even more pronounced with increasing film thickness. Therefore, the electro-optic method can provide information, albeit indirectly, about the mechanical properties of multilayer films based on the dependence of the electro-optical effect on the intensity of the applied electric field.

- It is reported that the addition of divalent counterions to a suspension of particles with an adsorbed film with an outermost pectin layer leads to an increase in the electro-optical effect. It is suggested that this is an indicator of denser packing and a corresponding increase in the stiffness of the film compared to a film without added electrolyte.

1.3. Adsorption of alginate on model non-spherical colloidal particles.

(publication № 3. **Milkova, V.** Comparative electrokinetic study of alginate-coated colloidal particles. *Gels* **2023**, *9*, 493. https://doi.org/10.3390/gels9060493. (JIF=5, 2023, 14/95, Q1)

Alginates are a family of polysaccharides isolated from seaweed. They are water-soluble linear block copolymers composed of (1-4)-linked poly–L– guluronate (G) and poly–D–mannuronate (M) residues. The structure of alginate can be described as randomly or blockwise distributed homopolymeric sections of G-residues (G-block) and M-residues (M-block) separated by sections in which the two types of residues alternate (MG-block). Therefore, the molar ratio of the blocks (M/G ratio) and the molecular weight are the two main characteristics that determine the physicochemical and bioactive properties of alginates. The presence of carboxyl groups in the structure of the alginate molecule determines its polyelectrolyte behaviour and therefore the molecule can be considered as a highly charged polyion.

Alginates have remarkable potential in pharmacy and tissue regeneration, as they are biocompatible, biodegradable, have very low toxicity, are a relatively inexpensive product, and have the ability to form gels due to a strong affinity to divalent cations.

The studies summarised in this topic aim to analyse the correlation between the physicochemical characteristics of selected alginate samples on the electro-optical behaviour and stability of an aqueous suspension of model non-spherical colloidal particles coated with a monolayer of polymer. In the studies, alginates with different molecular weights and M/G ratios are used. Ellipsoidal β-FeOOH nanocrystals are chosen as a model surface.

Main contributions:

- Electrokinetic studies shown that with a gradual increase in the concentration of alginate added to a suspension of particles, a gradual decrease (in absolute value) in the electrophoretic mobility, U_{ef}, of the particles is registered. This is a consequence of the partial screening of the surface charge as a result of the adsorption of the polymer. It is found that the isoelectric point of the particles in the presence of polymer does not depend on its characteristics (it is achieved at an alginate concentration of about 10^{-4} mg/mL). With a further increase in the alginate concentration, overcharging of the surface charge and re-stabilisation of the suspension are achieved (at about 2×10^{-2} mg/mL).
- Alginate is known to have an affinity for divalent cations. Therefore, an influence of the polymer characteristics on these interactions is expected. The gradual increase in the concentration of Ca^{2+} to a stabilised suspension of particles coated with a layer of alginate showed a gradual decrease in U_{ef}. It is found that the decrease in U_{ef} is higher for alginates with a lower the M/G ratio or a higher molecular weight. Furthermore, it is found that the electro-optical effect (at 1 kHz) registered from stabilised suspensions of particles coated with alginate increases until reaching a critical concentration of Ca^{2+} and decreases at higher concentrations. This critical concentration of Ca^{2+} (3×10^{-5} M) does not depend on the characteristics of the alginate.
- The analysis of the electro-optical behaviour of stabilised suspensions of particles with adsorbed polymer shown that the experimentally obtained value of the critical frequency, ν_{cr}, of relaxation of the effect (at 1 kHz) is significantly lower than that for particles without polymer. At the same time, the experimental value of ν_{cr} is higher than the calculated theoretical value (according to the Schwartz formula for polarisation of condensed counterions with lower mobility along the polyionic chain of a highly charged polyelectrolyte in a solution of an electrolyte). Therefore, it is proposed that the polarisation of condensed Na^+ counterions at short distances (e.g. G-, M- M/G block) along the length of the polyion is responsible for the creation of the electro-optical effect. The fraction of condensed counterions released upon the alginate adsorption (without the addition of Ca2+ ions) is estimated. This analysis is

performed based on theoretical models, according to which highly charged polyelectrolytes retain some of their condensed counterions upon adsorption onto a weakly charged surface.

- A correlation is established between the increase in the electro-optical effect (at high orientation energies, without the addition of Ca^{2+}) and the increase in the fraction of G-residues (or a decrease in the M/G ratio), as well as with an increase in the molecular weight of the polymers. At low energies of orientation, the behaviour of the suspension in the absence of Ca^{2+} does not depend on the characteristics of the alginates. In the presence of a very low concentration of Ca^{2+}, a correlation is established between the increase in the electro-optical effect (at 1 kHz) and the increase in the fraction of G-residues, which is explained by a stronger interaction between the divalent ions and the alginate molecules.

2 Preparation and characterisation of multilayer films of polysaccharides and carbon nanomaterials (carbon dots) on non-spherical particles

(publication № 2. **Milkova, V.** Polysaccharide/carbon quantum dots composite film on model colloidal particles - an electro-optical study. *Polymers* **2023**, *15(18)*, 3766. https://doi.org/10.3390/polym15183766. (JIF=4.7, 2023, 19/95, Q1)

The design of multicomponent structures is a promising approach for formation of functional materials with potential applications in biotechnology, pharmacy and medicine. Among the different methodologies for the development of new biocompatible platforms (capsules, drug carriers, artificial cells, etc.), procedures based on the layer-by-layer method are extremely attractive because they provide incredible flexibility in terms of the selection of components in order to achieve the desired functionality of the produced structures.

Essentially, the layer-by-layer technique is a process of sequential electrostatic adsorption of oppositely charged components on a charged surface, with the final multicomponent complex stabilised by strong electrostatic interactions. A significant interest has films formed on particles, because the procedure allows the incorporation of different small, charged components in the film, and this leads to the formation of a multicomponent shell with desired properties (functional core-shell structures).

Carbon dots (Cdots) are spherical structures with a size of less than 10 nm, which have significant advantages over classical semiconductor quantum dots containing atoms of heavy metals – high solubility and stability in water, bright photoluminescence in the visible spectrum, low toxicity, biocompatibility, high chemical stability, photostability, easy surface functionalization and low-cost synthesis. Their remarkable properties enable various applications (biosensors, cell imaging, drug tracking, metal ion detection and many others).

The studies summarised in this topic aim to obtain and characterise a stable multilayer film composed of alginate, chitosan, and Cdots. The study is performed based on the working hypothesis that stable composite structures can be formed despite the low density of the negative charge of carbon nanomaterials. In addition, despite their low concentration in the film, the presence of Cdots will affect the electro-optical behaviour and electrical properties of the suspension of composite particles.

Main contributions:

- The electric field light scattering method is applied for the first time for the investigation of the electrical properties and stability of carbon-polymer colloidal structures.
- Electrokinetic measurements shown an oscillation in the sign of the electrophoretic mobility value, U_{ef}, of the particles after the adsorption of alginate and chitosan, which is proof for the overcharging of the surface charge, successful adsorption of each of the components and re-stabilisation of the system after each adsorption step. At the same time, the absolute value of U_{ef} of the particles with the last adsorbed alginate layer is higher compared to that for particles with an outer chitosan layer. The results confirmed previous studies that the value of U_{ef} depends only on the type of component adsorbed in the last adsorption step, but almost does not depend on the number of layers formed by a given component. These results are in good agreement with previous studies in the group focused on the investigation of the electrical properties and stability of multilayer films formed only from polyelectrolytes on model colloidal particles.
- The incorporation of Cdots into the film is a result of the electrostatic interaction between the negatively charged Cdots and the positively charged chitosan monomers adsorbed on the film surface in the previous adsorption step. The addition of a low concentration of Cdots does not lead to an overcompensation of the surface charge of the particle surface, but only to a partial screening of the chitosan charge, which is registered by a slight decrease (in absolute value) of the U_{ef} of the particles.
- The analysis of the registered electro-optical behavior of the suspension of particles with an adsorbed polymer film shown that the electric polarizability, γ, (similar to U_{ef}) determined for

a suspension of particles with an outermost layer of alginate is significantly higher than that of particles with an outermost layer of chitosan, which is due to the higher charge density of alginate. This result confirmed previous suggestions that the electrical properties of the component adsorbed in the last adsorption step define the electro-optical behaviour of a suspension of particles covered by a multicomponent film.

- An oscillation in the dependence of the electric polarizability and the film thickness on the number of deposited layers is registered. The results indicate that the higher value of γ for a suspension of particles with a polymer film with an outer layer of alginate correlated with a larger layer thickness. At the same time, for particles with an adsorbed layer of chitosan, as well as after the addition of Cdots, γ, and the film thickness decreased. It is proposed a mechanism for film formation and the anomalous behaviour in the oscillation of the film thickness and polarizability is explained by the manifestation of additional compensatory effects due to the formation of non-stoichiometric complexes in the suspension, which are adsorbed on the film surface during the next adsorption step.
- The analysis of the dynamics of the counterion charge of the particles coated with a multilayer film confirmed the assumption that upon the adsorption of a highly charged polymer, where a "condensation" of a part of the counterions is expected, the polarizability of the layer of "condensed counterions" with lower mobility near to the polyions surface, is responsible for the registered electro-optical behavior of the suspension. The participation of diffuse H^+ counterions from Cdots in the mixed layer of chitosan/Cdots is negligible.
- • The concentration of Cdots in each layer of the film is determined by UV-vis spectroscopy.

3 Development and characterisation of model polysaccharide-based carriers for delivery and controlled release of active components (publications 4, 5, 6, 7, 8, 10, 12, 13, 14, 15)

(publications № 4. **Milkova, V.**, Martinov, P., Vilhelmova-Ilieva, N., Iliev, I. Role of chitosan characteristics on the properties of curcumin-loaded carriers and their potential application in ophthalmologic infection therapy. *Polysaccharides* **2025**, *6*, 22. https://doi.org/10.3390/ polysaccharides6010022 (JIF=4.7, 2023, Q1, 19/95, 2023); 5. **Milkova, V.** Experimental study of the interaction of silica nanoparticles with a phospholipid membrane. *Sci* **2025**, *7*, 6. https://doi.org/10.3390/sci7010006. (Q1); 6. Nikolova, I.; Paunova-Krasteva, T.; Petrova, Z.; Grozdanov, P., Nikolova, N.; Tsonev, G.; Triantafyllidis, A.; Andreev, S.; Trepechova, M.; **Milkova, V.**; Vilhelmova-Ilieva, N. Bulgarian medicinal extracts as natural inhibitors with antiviral and antibacterial activity. *Plants* **2022**, *11*, 1666.

https://doi.org/10.3390/ plants11131666 (JIF=4.5, 2022, 43/239, Q1); 7. Kamburova, K.; Dimitrov, I.L.; Hodzhaoglu, F.; **Milkova, V.** Investigation of the aggregation of Aβ peptide (1-40) in the presence of κ-carrageenan-stabilised liposomes loaded with homotaurine. *Molecules* **2024**, *29*, 3460. https://doi.org/10.3390/molecules29153460. (JIF=4.2, 2023, Q1); 8. Gyurova, A.; **Milkova, V.**; Iliev, I.; Lazarova-Zdravkova, N.; Rashev, V.; Simeonova, L.; Vilhelmova-Ilieva, N. Anti-coronavirus activity of chitosan-stabilized liposomal nanocarriers loaded with natural extracts from Bulgarian flora. *Life* **2024**, *14*, 1180. https://doi.org/10.3390/life14091180. (JIF=3.2, 2023, 26/109, Q1); 10. **Milkova, V.**; Goycoolea, F. M. Encapsulation of caffeine in polysaccharide oil-core nanocapsules. *Colloid and Polymer Science* **2020**, *298*, 1035-1041. https://doi.org/10.1007/s00396-020-04653-0. (JIF=1.931, 2020, Q2); 12. **Milkova, V.**; Kamburova, K.; Martinov, P.; Vilhelmova-Ilieva, N.; Rashev, V. Chitosan-based nanocarriers for delivery of remdesivir. *Sci. Pharm.* **2023**, *91*, 37. https://doi.org/10.3390/scipharm91030037. (JIF=2.3, 2023, Q2); 13. **Milkova, V.**; Boshkova, N.; Grancharov, G.; Stoilova, O.; Boshkov, N. Corrosion behavior of hybrid zinc coatings based on chitosan and corrosion inhibitor BTA: effect of the molecular weight and ζ-potential. *Coatings* **2024**, *14*, 495. https://doi.org/10.3390/coatings14040495. (JIF=2.9, 2023, 68/179, Q2); 14. **Milkova, V.**; Vilhelmova-Ilieva, N.; Gyurova, A.; Kamburova, K.; Dimitrov, I.; Tsvetanova, E.; Georgieva, A.; Mileva, M. Remdesivir-loaded nanoliposomes stabilized by chitosan/hyaluronic acid film with a potential application in the treatment of coronavirus infection, *Neurol. Int.* **2023**, *15*, 1320-1338. https://doi.org/10.3390/neurolint15040083. (JIF=3.2, 2023, 82/280, Q2); 15. **Milkova, V.**; Goycoolea, F.M. Polysaccharide-stabilized capsules for delivery of indomethacin. *ChemistrySelect* **2023**, *8(26)*, e202204420. https://doi.org/10.1002/slct.202204420. (JIF=1.9, 2023, 142/231, Q3).

The studies summarised in this topic aim to produce and characterise model polysaccharide-based carriers for delivery and controlled release of active components. In these studies, oil-core nanocapsules, liposomes and hydrogels loaded with a drug or other active molecules, stabilised with one or more layers of oppositely charged polysaccharides are produced. To achieve targeted action of the carriers, appropriately selected aptamers (RNA) with pronounced affinity towards specific proteins are additionally adsorbed on the surface of the polymer film. In addition, in order to investigate the influence of nanostructures on living cells, a model study of the interaction between model nanoparticles and a lipid membrane (as a model of a biological membrane) is performed.

Main contributions:

- Stable liposomes suitable for encapsulation of bioactive components (Veklury®, homotaurine or extracts of Bulgarian medical plants) stabilised with a monolayer of poly- and oligosaccharides of chitosan, as well as with multilayer films formed from chitosan and hyaluronic acid or κ-carrageenan, are obtained. By using software with open access, were selected aptamers (RNA) with a pronounced affinity towards the spike protein of human coronavirus HCoV-OC43 and amyloid β (Aβ) peptide. An analysis of the effect of the physicochemical characteristics of the polysaccharides, the experimental conditions (concentrations, pH, ionic strength, temperature, the combination of polysaccharides) on the size, stability, and the amount of encapsulated agent is performed. Microbiological assays and estimation of the oxidative stress in the presence of the produced structures are also performed. It is established that with appropriate selection of polysaccharides and experimental conditions, stable, fully biocompatible liposomes can be produced that successfully preserve the bioactive properties of the encapsulated substances and ensure their targeted transport and controlled release.

- For the first time, the aggregation of amyloid peptides is studied in the presence of composite structures loaded with an anti-amyloid agent. The kinetics of the process is analysed by monitoring the change in the polydispersity of a mixed suspension of Aβ peptide and liposomes containing homotaurine. The originality of the study is that for the first time the appearance of the first aggregates and the evolution in the aggregation process of Aβ has been indirectly monitored by changing the standard percentiles (D10, D50 and D90) obtained by analysing the measured particle size distribution in the dispersion.
- For the first time, stable liposomes loaded with a multicomponent aqueous extract of medical Bulgarian plants (*Glycyrriza glabra L.; Sambucus nigra; Aesculas hippocastanum; Potentilla reptans; Allium sativum*) are successfully produced and characterised, and their effect on human coronavirus HCoV-OC43 is analysed, as well.
- For the first time, the medicine Veklury® (Gilead Science Inc., Foster City, CA, USA) is encapsulated in polymer capsules. The main ingredient of the drug is remdesivir, a substance that was among the candidates for a drug against COVID-19.
- Stable oil-core nanocapsules suitable for encapsulation of active molecules and drugs (indomethacin, Veklury®, curcumin and caffeine) are produced. For this purpose, o/w nanoemulsions (primary emulsions) stabilised with lecithin are prepared and additionally stabilised by adsorption of a thick chitosan layer or a multilayer chitosan/hyaluronic acid film (secondary emulsions). A new procedure is developed for the formation of films on the emulsion droplets by using a low concentration of droplets and polysaccharides at a low ionic strength of the medium. In these studies, two approaches are applied: encapsulation of poorly water-soluble substances in the oil phase and encapsulation of water-soluble substances in the

polymer shell. By systematically studying the influence of the physicochemical characteristics of the polysaccharides, stable nanocapsules suitable for encapsulating a high concentration of the active component are obtained. The stability of the resulting structures and the kinetics of release of the active component in simulated biological environments (saliva, ocular fluids) are studied.

- It is studied the effect of the charge density of model nanoparticles and lipid layer on the interactions between them. For this purpose, composite films are formed on a macroscopic, flat (hydrophilic or hydrophobic) silica surface by sequential adsorption of zwitterionic liposomes and nanoparticles. The kinetic experiment confirmed that the lipid adsorption is a diffusion-limited process that depends on the pH and properties of the substrate. The adsorption of negatively charged particles on the lipid mono- or bilayer is a result of the electrostatic interaction with the positively charged domains on the membrane surface. It is found that the number of adsorbed lipid molecules and particles depends on the charge of the particles and the net charge of the membrane: with an increase in the charge of the particles and the membrane, as well as the hydrophobicity of the substrate, the adsorbed amount decreases.
- Chitosan particles (nanogels), respectively unloaded and loaded with a corrosion inhibitor (benzotriazole), are prepared and characterized by ion-induced gelation. In order to increase the corrosion resistance of zinc coating, the produced particles are incorporated in a suitable zinc coating.

17.07.2025 Viktoria Milkova