Tomas Bata University in Zlín
Faculty of Technology

Habilitation Thesis Summary

Polymers as Carriers of Active Agents

Polymery jako nosiče aktivních látek

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I would like to thank all the colleagues for their valuable cooperation on our research and Tomas Bata University for providing me with the scientific background during my work. Special thanks go to my family for their continuous love, support and encouragement.

SUMMARY

The habilitation thesis presents the topics of active polymer systems that could prevent the risk of microbial infections and contaminations. The introductory part reviews the potential forms of carriers, active molecules, as well as the characterization techniques of prepared systems. The second part is devoted to author's contribution to the subject covering the problems of antimicrobial polymer carriers based on both synthetic and natural polymers in the form of solutions, films, thin layers and nanofibrous membranes. The main goal of the commented research papers was to overview the approaches leading to the design of functional, cost effective and environmentally friendly polymer systems applicable in the food, cosmetic, medical or water treatment industry.

RESUMÉ

Habilitační práce se zabývá problematikou aktivních polymerních systémů, které mají sloužit jako prevence vzniku a šíření mikrobiálních infekcí. V úvodní části jsou představeny potenciální formy nosičů, typy aktivních látek a metody charakterizace připravených systémů. Druhá část je věnována diskuzi výsledků studií antimikrobiálních systémů na bázi syntetických i přírodních polymerů ve formě roztoků, filmů, tenkých vrstev nebo nanovlákenných membrán. Hlavní podstatou diskutovaných publikačních výstupů autora bylo zhodnotit přístupy vedoucí k vývoji funkčního, ekonomicky i environmentálně příznivého polymerního systému využitelného v potravinářství, kosmetice, zdravotnictví nebo procesech úpravy vody.

CONTENT

INTRODUCTION

Microbial infections represent a serious problem, due to the persistence of pathogenic microorganisms present in various environments and applications, including especially pharmaceutical, food and cosmetic industry. Moreover, a number of bacterial strains become highly resistant to various kinds of drugs and are capable of facile spreading into different environments, such as air, soil and water. This is the reason why many of scientific groups constantly search for new possibilities and strategies in order to eliminate above-mentioned risks and thus prevent associated undesirable economic impacts. Some of up-to-know approaches suffer from various negative aspects, such as insufficient and short-term efficiency, low stability, residual toxicity or high volatility. Application of active polymer materials is the perspective strategy how to fight with multi-drug resistant microorganisms and eliminate the above-mentioned drawbacks. These systems can be classified according to their mechanism of action to either passive of active type, which can be affected by many factors, such as molecular weight or polymer chain length. An emphasis is put on the character of base materials applied for the preparation of such systems, especially on their biodegradability, biocompatibility, non-toxicity and safety.

Presented habilitation thesis includes the results of author’s scientific papers regarding the long-term goal consisting in the preparation and study of an effective and economically favourable antimicrobial material with potential applications in pharmaceutical, cosmetic, or food industry as active packaging, dressings, or for water treatment processes. The purpose of this work was to utilize the simple preparation methods for the design of the functional systems that are safe and do not promote bacterial resistance yet. The thesis also gives an overview of the polymer materials utilized as the carrier matrices, the potential active substances, as well as methods of preparation and characterization of the final systems, along with some critical issues associated with this topic. The carriers based on synthetic and natural polymers, namely poly (vinyl chloride), poly (vinyl alcohol), polyethylene oxide, poly lactide, and chitosan were of particular interest. The mutual interactions between the polymer matrix, active molecule and eventual “third” component (surfactant) affecting the stability were investigated.

1. LIST OF PUBLICATIONS RELEVANT TO THESIS

P1 GALYA, T., SEDLARIK, V., KURITKA, I., NOVOTNY, R., SEDLARIKOVA, J., SAHA, P. Antibacterial poly(vinyl alcohol) film containing silver nanoparticles: preparation and characterization. *Journal of Applied Polymer Science* 110, 2008, 3178.

P2 GALYA, T., SEDLARIK, V., KURITKA, I., SEDLARIKOVA, J., SAHA, P. Characterization of antibacterial polymeric films based on poly(vinyl alcohol) and zinc nitrate for biomedical applications. *International journal of Polymer Analysis and Characterization* 13, 2008, 24.

P3 SEDLARIK, V., GALYA, T., SEDLARIKOVA, J., VALASEK, P., SAHA, P. The effect of hydrolysis degree on the properties of antibacterial polymeric films based on poly(vinyl alcohol) and zinc sulphate for biomedical applications. *Journal of Biomaterials Science* 21, 2010, 1421.

P4 MERCHAN, M., SEDLARIKOVA, J., SEDLARIK, V., MACHOVSKY, M., SVOBODOVA, J., SAHA, P. Antibacterial polyvinyl chloride/antibiotic films: The effect of solvent on morphology, antibacterial activity and release kinetics. *Journal of Applied Polymer Science* 118, 2010, 2369.

P5 MERCHAN, M., SEDLARIKOVA, J., VESEL, A., SEDLARIK, V., PASTOREK, M., SAHA, P. Characterization of antibacterial, mechanical, and structural properties of polyvinyl chloride/silver nitrate composites prepared by thermoplastic compounding. *International Journal of Polymer Analysis and Characterization* 15, 2010, 360.

P6 MERCHAN, M., SEDLARIKOVA, J., FRIEDRICH, M., SEDLARIK, V., SAHA, P. Thermoplastic modification of medical grade polyvinyl chloride with various antibiotics: effect of antibiotic chemical structure on mechanical, antibacterial properties, and release activity. *Polymer Bulletin* 67, 2011, 997.

P7 MERCHAN, M., SEDLARIKOVA, J., VESEL, A., MACHOVSKY, M., SEDLARIK, V., SAHA, P. Antimicrobial silver nitrate-doped polyvinyl chloride cast films: Influence of solvent on morphology and mechanical properties. *International Journal of Polymeric Materials* 62, 2013, 101.

P8 SEDLARIKOVA, J., DOLEZALOVA, M., EGNER, P., PAVLACKOVA, J., KREJCI, J., RUDOLF, O., PEER, P. Effect of oregano and marjoram essential oils on the physical and antimicrobial properties of chitosan based systems. *International Journal of Polymer Science* 2017.

P9 KOLAROVA RASKOVA, Z., STAHEL, P., SEDLARIKOVA, J., MUSILOVA, L., STUPAVSKA, M., LEHOCKY, M. The effect of plasma pretreatment and cross-linking degree on the physical and antimicrobial properties of nisin-coated PVA films. *Materials* 2018, 11(8), 1451.

P10 SEDLARIKOVA, J., JANALIKOVA, M., RUDOLF, O., PAVLACKOVA, J., EGNER, P., PEER, P., VARADOVA, V., KREJCI, J. Chitosan/thyme oil systems as affected by stabilizing agent: physical and antimicrobial properties. *Coatings* 2019, 9, 165.

P11 KOUSAL, J., KRTOUS, Z., KOLAROVA-RASKOVA, Z., SEDLARIKOVA, J., SCHAFER, J., KUCEROVA, L., SHELEMIN, A., SOLAR, P., HURAJOVA, A., BIEDERMAN, H., LEHOCKY, M. Degradable plasma polymer films with tailored hydrolysis behavior. *Vacuum* 2019, 173, 109032.

P12 KOUSAL, J., SEDLARIKOVA, J., KOLAROVA-RASKOVA, Z., KRTOUS, Z., KUCEROVA, L., HURAJOVA, A., VAIDULYCH, M., HANUS, J., LEHOCKY, M. Degradable poly(ethylene oxide)-like plasma polymer films used for the controlled release of nisin. *Polymers* 2020, 12, 1263.

P13 PEER, P., SEDLARIKOVA, J., JANALIKOVA, M., KUCEROVA, L., PLEVA, P. Novel poly vinylbutyral/monoacylglycerol nanofibrous membrane with antifouling activity. *Materials* 2020, 13, 3662.

2. THEORETICAL BACKGROUND

2.1 Types of carriers

In the area of polymer active systems, types of carriers play an important role for subsequent applicability. Carriers for active molecules can be prepared from many types of materials and can exist in different forms, such as micro/nanoparticles, emulsion systems, polymer films, fibers, micelles, liposomes etc. A number of factors have to be taken into consideration, especially the character of active substance, potential mutual interactions with the carrier, and/or required properties and application. Optimum supporting system should be stable, safe, and economically, as well as ecologically favourable.

2.1.1 Micelles

Micelles are colloid self-assembled aggregates formed by amphiphilic surface active compounds, called surfactants, above the critical micelle concentration (CMC). Surfactant molecules contain two parts, hydrophilic head and hydrophobic tail. Micellar aggregates can exist in different forms, some types are shown in Fig. 1 [1].

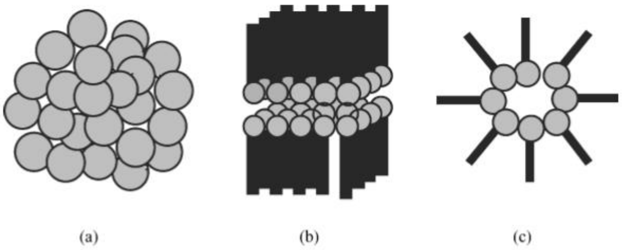

Fig. 1. Types of aggregates: a) spherical, b) lamellar, c) reversed micelle [2]

The standard Gibbs free energy of non-ionic micelle formation per mole of surfactant is given by Equation 1.1:

$$\Delta G_m^0 = RT\ln(CMC) \tag{1.1}$$

Solubilization capacity of surfactant micelles is determined by the molecular structure of amphiphile, as well as solubilized molecule, mechanism of entrapment and physicochemical interactions of present constituents [1]. In aqueous solutions, hydrophobic agents, e.g. drugs, are incorporated into the hydrophobic core of micelles enhancing their solubility. On the other hand, polar materials are commonly located in so-called palisade layer of the micelle (Fig. 2) [2].

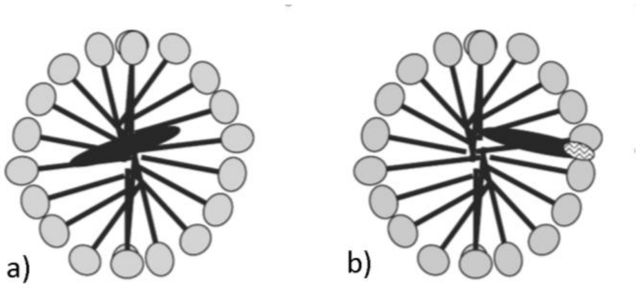

Fig. 2 Solubilization in micelle a) hydrophobic substance in core, b) hydrophilic substance in palisade layer [2]

Through the solubilization into micelles, quite large quantities of poorly soluble active agents can be incorporated in the polar solution. Moreover, due to low water concentration in the micellar core, hydrolytic degradation is suppressed, which increases the chemical stability of the solubilized molecule. Release kinetics can be controlled, thanks to the partitioning of the encapsulated agent toward the micelle, and the effect of the active molecule in the desired target is enhanced [3].

Block copolymer micelles

A special type of aggregates is represented by polymer micelles based on the self-assembly of block copolymers above the critical micelle concentration. These diblock or triblock copolymers may form not only micelles in dilute solutions, but also a number of liquid crystalline phases. In polar media, these nanostructures contain external hydrophilic corona and hydrophobic core, whereas in an opposite arrangement exists in nonpolar solvents (Fig. 3).

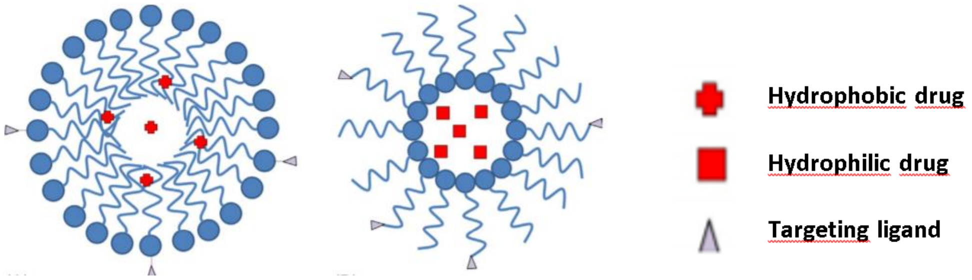

Fig. 3 Structure of polymer micelle in a) polar and b) nonpolar media [4]

Particularly, poly(ethylene oxide)/poly(propylene oxide) copolymers (PEO/PPO) have been intensively studied during the last decade for potential applications as drug delivery systems, especially for their commercial accessibility and variability of molecular weights. Similarly to low molecular weight non-ionic surface active agents, micelle formation of block copolymers is supported by an increasing length of hydrophobic part (PPO) and decreasing proportion of hydrophilic part (PEO) of a molecule. Micellization of PEO/PPO block copolymers is significantly affected by temperature. Because of the lower solubility of PEO blocks at a higher temperature, the process of micelle formation is promoted. As in case of common low molecular weight aggregates, the solubilizate location is primarily affected by its properties. More hydrophobic molecule will tend to organize into the micelle core, whereas more amphiphilic agents will preferably locate in the palisade layer.

Triblock copolymers of A-B-A type, known under the trade name Pluronic or Lutrol, represent an example of polymer surfactant suitable for drug delivery system. Such structure has a hydrophobic PEO based core ended with hydrophilic blocks of PEO. In the work of Chaudhari et al. [5], solubilization of poorly water soluble drug Lamotrigine into Pluronic based aggregates was investigated. High entrapment efficiency was achieved as well slower release rate of a model drug from Pluronic mixed micelles.

2.1.2 Emulsion/dispersion based systems

Emulsions are dispersion systems composed of two immiscible liquids, of which one represent the internal or discontinuous phase that is distributed in the second continuous phase. Emulsions are widely applied in the food industry, pharmacy and cosmetics. They can be classified according to the polarity to oil in water (O/W) and water in oil (W/O) emulsions, however, even multiple emulsion types (e.g. O/W/O or W/O/W) exist. Based on the size of droplets, there are macroemulsions (1 to 100 μm), nanoemulsions (20 to 200 nm) and microemulsions (10 to 100 nm). Under homogenization procedure, the concentration of an emulsifier, as well as a co-surfactant play an important role here, too [6].

Microemulsions and nanoemulsions

Microemulsions are transparent, thermodynamic stable isotropic dispersion systems containing particles ranged from 10 to 100 nm. They are formed spontaneously with a higher portion of co-surfactant that causes a further decrease of interphase tension. Nanoemulsions usually contain particles in size from 20 to 200 nm with the high interfacial area and exhibit a high solubilization capacity and low viscosity. Compared to microemulsions, they are not thermodynamically stable but they can possess a high portion of kinetic stability against sedimentation, flocculation and/or coalescence when prepared under optimum conditions [7]. The formation of nanoemulsions can occur by high or low energy homogenization methods. The latter mentioned group includes techniques utilizing the phase inversion during emulsification, such as emulsion inversion point (EIP) or phase inversion temperature (PIT) method [6].

Surfactants play important roles in emulsion formation, as well as their stability. They decrease the interfacial tension γ, which leads to the reduction of droplet size in emulsions. In practical applications, the mixtures of surfactants are commonly used because of their synergetic effects leading to a more significant decrease of the interfacial tension γ. It is known that different components provide varying surface activity, and the compounds with the lowest γ are preferentially located at the interface. Even the mixtures of polymers and surfactants exhibit some synergetic effects in the surface activity [8].

2.1.3 Liposomes, niosomes, ethosomes

Liposomes are spherical vesicles with a hydrophilic core surrounded by at least one phospholipid-based bilayer. Liposomes can entrap hydrophilic, hydrophobic and even amphiphilic molecules, despite the fact that their core is hydrophilic [9, 10]. These vesicles represent perspective candidates for delivery systems due to their biodegradability, biocompatibility, low toxicity and easy preparation methods. A rapid release of poorly water-soluble drugs encapsulated in the lipid bilayer is one of the negative aspects. Another drawback consists in low physical and chemical stability caused by the oxidation and hydrolysis of phospholipids [9]. Liposomes can be prepared by the dispersion of lipid molecules in an aqueous phase carried out by mechanical methods, such as film hydration method, sonication, microfluidization, extrusion, or by the replacement of organic solvents, such as proliposome-liposome method, ethanol injection, and lyophilisation [11].

Niosomes are biodegradable and biocompatible vesicles based primarily on non-ionic surfactants and might contain cholesterol or its derivatives, too. Cholesterol serves as an additive substance able to interact with hydrophilic part of non-ionic surface active agent. Some of the niosome properties, such as entrapment efficiency, stability (storage time) and release conditions, can be affected by its incorporation [12]. The stability of niosomes might also be enhanced by the addition of charged molecules that prevent aggregation mechanisms and improve skin permeation properties. Non-ionic molecules based vesicles are resistant to oxidation and high temperatures. Niosomes might be easily prepared by thin layer hydration technique, the organic solvent injection method or e.g. the reverse-phase evaporation method [9].

Ethosomes are phospholipid-based vesicles with high ethanol content (20–25 %) developed in 1997. These vesicles are significantly deformable with high ability to permeate into the skin. Included ethanol serves as an agent for enhancing the permeation due to its ability to affect the bilayer structure of the *stratum corneum*. Ethosomal vesicles enable to deliver the encapsulated molecule deeper in the skin in comparison to liposomes. Additionally, ethosomes are smaller with better entrapment efficiency and stability. On the other hand, they are unstable because of oxidative degradation. Ethosomes can be prepared by the convenient hot and cold methods, or e.g. by the classic mechanical dispersion method [13]. The scheme of selected structures is shown in Fig. 4.

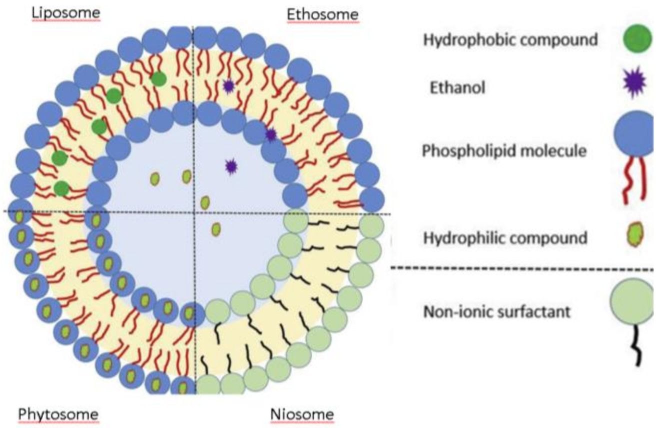

Fig. 4 Scheme of selected vesicle-based systems [14]

2.2 Polymer systems

Polymer materials have found applications in a wide range of disciplines including pharmaceutical, agricultural, food and cosmetic industry. Polymers can be classified according to the structure to linear, branched and cross-linked types. Based on their origin, they are divided into natural and synthetic materials. The latter mentioned group comprises of the polymers prepared in the laboratory, such as polyethylene, polystyrene, polyvinyl chloride, synthetic rubber etc. Natural polymers are isolated from natural materials (plant and/or animal sources) and are of great interest nowadays due to the general trend of eliminating synthetic substances in the environment. The representatives include polysaccharides, proteins, nucleic acids and natural rubber.

2.2.1 Antimicrobial polymers – mechanisms of action

Active polymer materials have been under extensive research to reduce the risk of microbial contamination that represent serious problems especially in healthcare products, food applications or water treatment systems [15]. Hence, several methods have been developed to obtain polymers with desired antimicrobial properties. Polymer systems with bioactive compounds can be found in the form of coatings, films, micro- or nanospheres, nanogels, or e.g. capsules [16]. Considering the function of antimicrobial polymers, they can work either in a passive or active way. Passive action includes a material passively preventing adhesion of bacteria, not actively interacting with them. Passive polymers are mostly hydrophilic, having low surface free energy, or negatively charged. Polyphenol or poly(ethylene glycol) based polymers have been extensively studied as passive polymers preventing the growth of Gram negative and Gram positive bacteria [18,19]. Active polymers are based on the active action when bacteria adhering to the polymer surface are killed. In this case, functional active agents play a predominant role. Examples of active polymers are poly(iminoethylene), poly(guanidine) and positively charged quaternary ammonium types [21].

Antimicrobial polymers can be classified into polymer biocides, biocidal polymers and biocide-releasing polymers consisting of polymers enriched with active molecules (Fig. 5).

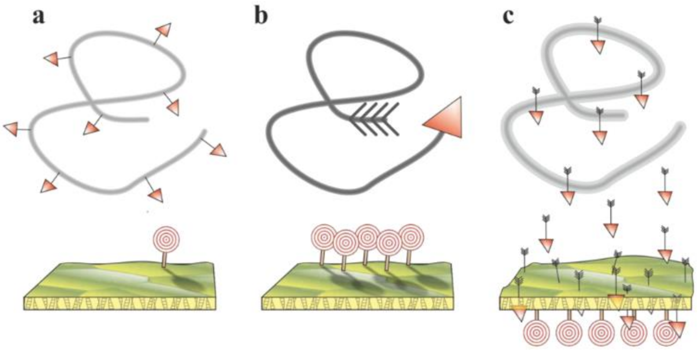

Fig. 5 Types of antimicrobial polymers: a) polymer biocides, b) biocidal polymers, c) biocide-releasing polymers [20]

Polymer biocides include materials consisting of repeating bioactive molecules, such as hydroxyl, carboxyl or amino groups that are covalently linked. However, the process of polymerization of biocidal monomer units does not always lead to the production of active polymer, caused either by an insufficient solubility in water or because the active substances are not able to reach the target site. Examples of polymer biocides include polymers with quaternary ammonium and phosphonium salts or benzimidazole derivatives [21].

In the case of biocidal polymers, the active site is represented by the macromolecule itself and no repeating bioactive units are required. Since many microbial organisms carry a negative charge, polycations are attracted to their surface, which results in the disruption of their outer membrane leading to cell lysis and death. Therefore, many types of biocidal polymers contain cationic groups, such as quaternary ammonium, phosphonium, tertiary sulfonium, or guanidinium. Chitosan and its derivatives have been studied, mainly due to their advantageous properties including biodegradability, biocompatibility and absence of toxicity. Chitosan’s antimicrobial properties are strongly governed by its pH value. Generally, when the pH value is less than pKa, amino groups are in protonated form and can interact with the cell wall. On the other hand, when pH is above pKa, chitosan can operate as antimicrobial due to hydrophobic interactions.

The third group comprises so called biocide-releasing polymers that can proceed through either polymerization of biocide-releasing molecules to polymeric backbone, or the polymer/biocide-releasing molecules composites. The polymer material serves as a carrier system for active molecules, such as antibiotic or antiseptic compounds. Systems for controlled release have been intensively studied during the last years as the innovative principle in reducing the risk of microbial infections by the targeted delivery. A great effort has been devoted to the study of the release kinetics and influential factors [21].

2.2.2 Modification of polymers with antimicrobial substances

Antimicrobial polymers can be prepared by several methods, including deposition of active agents on the substrate surface, such as vapour, sputter or ion beam coating, or plasma treatment. The last mentioned technique was applied in the author's publications **P9**, **P11** and **P12**. Also, dip or spin coating can be utilized as an alternative approach to minimize bacterial contamination. However, coating or immersion of the material in a solution of an antibacterial substance suffers from many limitations, including the rapid release of active substance in a short time. Another possibility includes incorporating antimicrobial substance into polymer matrix, e.g. be melt blending or solvent casting technique. Melt compounding has been investigated in **P5** and **P6** using polyvinyl chloride as the base matrix and organic or inorganic active molecules to get the systems with antibacterial activity. The latter method, solvent casting, has been utilized in most of the author's publications (**P1**–**P4**, **P7**, **P8, P10**) investigating both synthetic and natural polymers as potential carriers of active agents.

2.2.3 Antifouling surfaces

Antifouling surfaces are used to eliminate the consequences of biofouling mechanisms occurring through the colonization by microorganisms resulting in the formation of biofilms on the exposed surface. To enhance the antifouling properties, the surface modification, either by coating a thin film, or by grafting of polymer chains on the surface can be carried out (Fig. 6).

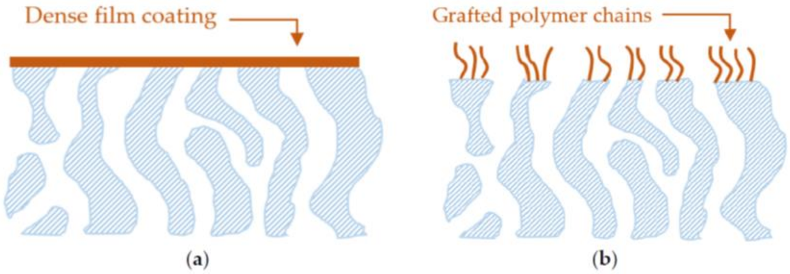

Fig. 6 Modification of polymer surface by: a) thin film coating, b) grafting of polymer chains [22]

It is necessary to realize that materials selected for these processes (coating or grafting) are not affine to potential foulants, which leads to the limitation of any undesirable interactions between them and the substrate. The antifouling properties are also affected by the surface roughness, wettability and charge. Rough surfaces are more prone to spontaneous fouling because of the increased deposition of the foulants in the “pockets” of the surface. If the surface is charged, it can attract the foulants with counter ions due to the electrostatic interactions [22]. Therefore, the knowledge on the surface properties, such as wettability and contact angle (reported on in chapter 2.2.4), is crucial to understand this topic in a broader context. Antifouling membranes based on poly(vinyl butyral) enriched with monocaprin were designed in the author’s publication **P13**.

2.2.4 Characterization methods of active polymer systems

When active polymer systems, both film forming polymer solutions, and films or thin layers, are prepared, various techniques for their characterization might be used. A range of these is employed for evaluation of the short or long-term stability, so that the products fulfil the requirements of practical applications. Stability can be understood as the ability to resist external factors leading to the structural changes of the product. It is known that all the systems tend to retain the most energetically advantageous state. The following chapters include the selection of methods used within the author’s research for the characterization of active polymer systems.

Particle size

Particle size belongs among the important characteristics giving evidence of the physical stability of the solution, as well as affecting the texture and appearance of the resultant system [23]. A dynamic light scattering (DLS) technique can be utilized to measure the size of particles, which is based on the analysis of the intensity of light scattered from solution or suspension. DLS (also known as photon correlation spectroscopy – PCS) measures so called Brownian motion (random thermal motion of fine particles and molecules). Since smaller particles move faster than larger ones, there is a relationship between the particle size and its speed defined by the Stokes-Einstein equation (1.2) [24]:

$$D = \frac{kT}{6\pi\eta R_H} \tag{1.2}$$

Where D is the translational diffusion coefficient, η is the viscosity of dispersion medium, k is Boltzmann’s constant, T is the absolute temperature, R_H is the hydrodynamic particle radius.

Electrokinetic measurement

Zeta (ζ) potential of liquid dispersions informs on an electrostatic charge present on the slipping plane between fixed and diffuse region of the electric double layer. It depends on the physicochemical properties of systems and can affect the processes of dispergation, adsorption and/or aggregation. Generally, particles possessing a sufficiently positive (> +30 mV) or negative (< –30 mV) zeta potential, repel each other, which results in an increase of stability. On the contrary, a significant decrease in stability is monitored when zeta potential value ranges from 0 to ± 10 mV due to a higher potential to aggregation [25].

The electrokinetic measurement can also be carried out on the solid surfaces and is associated with the dissociation of surface groups, the adsorption of cations or anions, or polyelectrolytes, or electron depletion. This can be measured either by electrophoretic methods, or the streaming potential can be determined when a relative movement of the solid or liquid phases is performed to generate an electrical potential or current. The latter mentioned is often applied for the measurement on planar surfaces [26].

Surface tension measurement

When the surfactant is included in the system, the measurement of surface tension plays a significant role. The surface tension of liquids is the result of attractive forces between the molecules. While the molecules present in bulk liquid exhibit the same attractive forces in all directions, the surface molecules are in asymmetric force field, which leads to so called surface energy and/or surface tension (Fig. 7) [28].

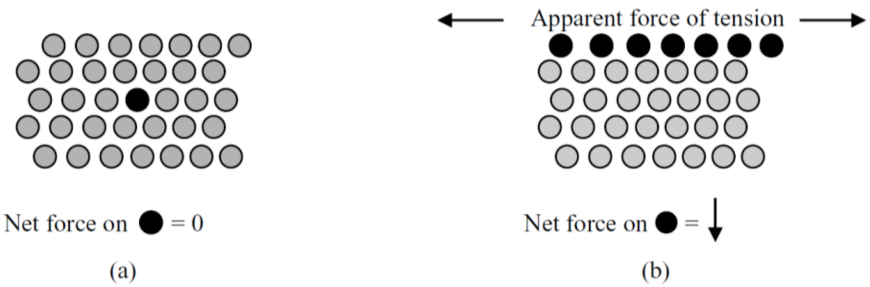

Fig. 7 Attractive forces acting on the molecule (a) in the bulk, (b) at the interface [2]

The surface tension can also be identified as a force acting at the surface related to the length, thus, it is expressed in N/m, or mN/m. It is known that surfactants significantly reduce the surface tension even at very low concentrations, until the critical micelle concentration (CMC) is reached, after which it is more or less constant (Fig. 8). The efficiency of a surfactant is determined to characterize its amount necessary to achieve a certain reduction in surface tension, usually set to 20 mN/m (the corresponding surfactant concentration is indicated as c_{20}). On the other hand, the effectiveness of the surfactant is identified as the reduction of surface tension at the critical micelle concentration (γ_{CMC}) [28].

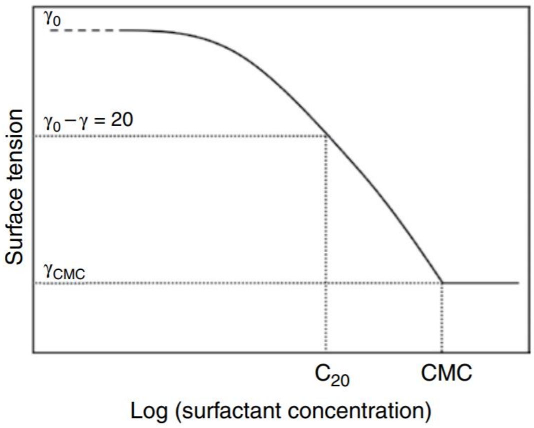

Fig. 8 Surface tension vs. the surfactant concentration logarithm [28]

The surface tension can be measured by different techniques including capillary elevation, stalagmometry, or Wilhelmy plate method, applied in the author's publications (**P8**, **P10**, **P13**). Within this technique, a thin plate with defined dimensions is vertically immersed into the liquid, and the acting force, which is related to the surface/interfacial tension and contact angle, is measured (Equation 1.7).

$$\gamma = \frac{F}{L \cdot cos\theta} \qquad (1.7)$$

Where L is the wetted length of plate, equal to its perimeter (thickness is small that can be neglected), F is the maximum force achieved just before the plate detachment from the liquid surface.

Mechanical properties

Mechanical properties, such as tensile strength, elongation at break, puncture strength, or puncture deformation, belong among the important characteristics of polymer films [29]. For measuring mechanical characteristics, various types of equipment can be applied, depending on the type and size of the sample. Texture analysis machine can be utilized even for the measurement of a large range of parameters providing complex testing applications. For example, in the case of edible polymer films intended as the primary packaging of food products, properties like puncture and tensile strength are significant. The puncture strength is expressed in N/mm as the maximum puncture strength F_{max} divided by the average thickness of the specimen T (Eq. 1.8).

$$PS = \frac{F_{max}}{T} \qquad (1.8)$$

Tensile strength is calculated from the maximum tensile strength F_t divided by the product of the average sample thickness T and width W (Eq. 1.9) [30].

$$TS = \frac{F_t}{TW} \tag{1.9}$$

Due to the incorporation of active molecules into the polymer matrix, the significant changes in mechanical properties can occur, as the consequence of the sample structure disruption [31].

Surface wettability

Surface properties, regarding the hydrophilicity/hydrophobicity can be determined by measuring the contact angle between a solid surface and the tangent leading from the contact position of liquid on the substrate (Fig. 9). When a sample hydrophobicity increases, a higher contact angle is obtained [32]. In case that the contact angle approaches zero, the surface is completely wetted by a liquid. If the contact angle is ≥ 90°, the liquid droplet does not spread easily. When the volume of liquid drop is changed, the different contact angles can be obtained. While the liquid is withdrawn, the angle is lower when compared to the situation when the liquid is slowly added. Then, so called *advancing contact angle* θ_A is obtained as the liquid droplet expansion is finished, or the *receding contact angle* θ_R is acquired, when the liquid has just been withdrawn. The values close to 180° are characteristic for so called completely non wetting liquids [26].

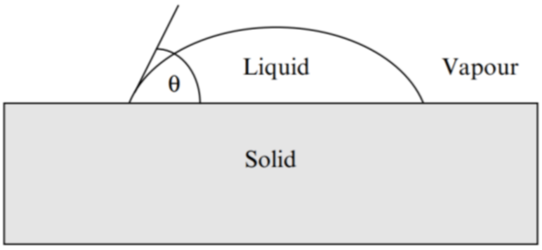

Fig. 9. The contact angle formed by a liquid drop on the solid surface [33]

Contact angles can be measured by different techniques, including sessile drop, captive bubble, or Wilhelmy plate method. Within the author's research, a sessile drop method was applied to get the information on hydrophilicity of polymer surface, which might provide valuable knowledge on e.g. the eventual attachment of active molecules, or the potential antifouling activity useful for many practical applications, such as water treatment [26].

The contact angle depends on the surface tension/energies vectors of individual phase boundaries and can be expressed by the Young equation (1.10):

$$\cos\theta = \frac{\gamma_{SG}-\gamma_{SL}}{\gamma_{LG}} \quad (1.10)$$

Where θ is the contact angle, γ_{SG}, γ_{SL} and γ_{LG} is the surface tension in mN/m at solid/gas boundary, solid/liquid boundary, and liquid/gas boundary, respectively.

A surface energy can be evaluated from the contact angle measurement. Hydrophilic surfaces having lower contact angle exhibit a higher surface energy whereas more hydrophobic substrates have lower water wettability associated with higher contact angles and lower surface energy [34].

Testing of antimicrobial properties

Antimicrobial properties of polymer materials can be determined by several methods, depending on the specific application, external factors and expected microbial species that encounter the tested system, since different microorganisms require varying nutritive media and conditions for their growth. The antimicrobial efficiency is strongly dependant on the potential and rate of migration of active molecules from the polymer, different interfacial phenomena, and the hydrophilicity of the surfaces.

Due to the present problem of increasing antibiotic resistance, the methods for evaluating antibiotic efficiency have been developed. These include a disk diffusion (Kirby Bauer, Stokes test), broth dilution (determination of the minimum inhibition concentration, MIC), diffusion and dilution (E-test) method. During the disk diffusion procedures, the samples are positioned on the agar plates with solid nutritive media containing a microbial suspension of defined concentration. When antibiotics are tested, these are impregnated into a sterile filter disk. In the case of polymer sample, this is cut into an appropriate size and placed onto the agar plate. After the incubation time, the inhibition zones around the samples are recorded (Fig. 10) [35].

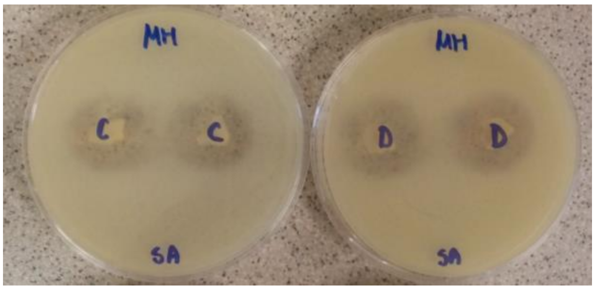

Fig. 10 Inhibition zones around the chitosan samples obtained by an agar diffusion test against Staphylococcus aureus

Antifouling activity is another important characteristic that should be considered for surfaces applied in areas with an excessive tendency to microbial fouling, such as water treatment technologies [35]. Testing of antifouling properties can proceed by different techniques. One of the methods uses the direct measurement of the permeate flux during the filtration process. A decrease in the filtration rate usually indicates membrane fouling. The fouling process can also be analyzed by the microscopic techniques if the membranes with sufficient optical transparency are applied [36]. Bacterial adhesion test and comparison of viable bacterial counts in planktonic and sessile form can also be performed to test the antifouling properties [37].

2.3 Active agents

Various organic or inorganic agents can be used as active agents for incorporation into the polymer matrix, which might have antioxidant, antimicrobial, antiviral or preservation effects. These substances can be classified according to different criteria, such as the chemical structure, source, spectrum of activity, or function [38].

2.3.1 Organic active agents

Organic antimicrobials act at low concentrations offering a favourable cost/performance ratio for polymers processed at temperatures not exceeding 250 °C. Several substances can be included here, such as antibiotics, vitamins, essential oils, antioxidants, quaternary ammonium compounds etc.

Essential oils (EO) are volatile aromatic compounds derived usually from plants. Their main applications include fragrances in the cosmetic industry; some can be used for skin healing and aromatherapy. EO might be incorporated in various formulations to prevent their evaporation or oxidation mechanisms [39]. Within the author’s publications, oregano and marjoram (**P8**) and thyme (**P10**) essential oils were used for incorporation into polymer matrices and will be discussed in appropriate sections (Chapter 3.1.4).

Antibiotics can be classified according to different criteria, such as type of action, source, or chemical structure. Based on the spectrum of activity, narrow, medium and wide spectral antibiotics are known. Within the author’s works (**P4** and **P6**), three antibiotics from the last mentioned group were tested (ampicillin, minocycline, rifampicin). Antibacterial activity of minocycline/rifampicin coatings was investigated within several studies. The results showed the sufficient effect against Gram positive and Gram negative bacteria, as well as *Candida* species [40]. Nisin, belonging to the group of small-peptide antibiotics, was applied in the papers **P9** and **P12**. This natural antimicrobial agent is the only bacteriocin allowed for food industry use as a preservative [21].

2.3.2 Inorganic active agents

The antimicrobial activity of metal ions, such as silver, copper, or zinc has been widely investigated. Their microbicidal effect can be achieved by affecting the electrochemical potential between the internal and external cell components, or by penetrating into the cells, to compete with other essential ions like magnesium, calcium or potassium, and aggregating with thiol groups of enzymes or proteins. The advantage of inorganic antimicrobial agents consists in the negligible impact on the processing mechanisms and the potential use for the polymers processed at high temperatures. They are highly recommended for the applications requiring long-lasting antimicrobial activity and the risk of bacterial resistance is reduced in comparison to other antibacterial agents (such as antibiotics) [41].

Within the author's publications (**P1**, **P2**, **P3**, **P5**, **P7**), silver and zinc ions were investigated for incorporation into polymer matrices by solvent casting or thermoplastic compounding. Discussion on these active agents is included in the appropriate chapters of the thesis (3.1.1 to 3.1.3).

3. DISCUSSION ON PAPERS

Within the following chapters, the main author's research papers will be overviewed. The topics are classified into the sections according to the form of the polymer system carrying the active antimicrobial agent, namely the films, thin layers and nanofibrous membranes.

3.1 Polymer films

Polymer films represent perspective carriers for embedding various active molecules that can serve as "smart" coatings and packaging for preserving foods, or as wound dressings. These systems can control the gas or moisture permeability, prevent oxidation mechanisms, limit the biofilm formation and thus prolong the product shelf life [42]. Synthetic and/or natural polymers might be used for the preparation of such systems.

Synthetic polymers gained a high portion of attention in many industrial fields, especially due to a variability of physical and chemical properties that can be achieved, based on the character of monomer units, type of polymerization reaction and potential formation of copolymers with different components [43]. In bioactive systems, hydrolytically degradable polymers are often applied, whereas the process of enzymatic degradation is preferred for biopolymers.

Concerning author's publications, the following text is divided into the chapters according to the processing technique (the melt compounding and solvent casting), and the base polymer used as the potential carrier of the active substance. Several polymers were investigated, such as vinyl polymers, poly(vinyl alcohol) and poly(vinyl chloride), as the representatives of synthetic materials, and chitosan from the group of biopolymers.

3.1.1 Poly(vinyl alcohol) based films prepared by solvent casting

Poly(vinyl alcohol) (PVA) is a synthetic biocompatible, biodegradable, water-soluble polymer, properties of which are driven by the preparation method and hydrolysis degree [44, 45]. It can be applied in different commercial sectors, e.g. to produce artificial skin replacements, contact lenses, food packaging systems, and/or carriers for the drug release. Combinations of PVA and antibacterial agents, such as zinc or silver, have been studied within the authors' publications (**P1**, **P2**, **P3**). Active compounds were encapsulated into polymer matrix by the casting technique, which represents a promising solution in the development of antibacterial polymer system with a longer-term effect fulfilling the high quality requirements [46, 47, 48]. Films with uniform thickness and low haze can be obtained in this way [49].

Active multilayer materials have been investigated in the work of Tampau et al. [42], in which the polymer solution with an active compound is placed on appropriate polymer support. The efficiency of such prepared multilayers was significantly affected by the wettability of the solution and the spreadability on the supporting layer. Therefore, the contact angle of the polymer-solution system and surface tension of the applied solutions play a crucial role, too.

Silver compounds have been known as broad-spectrum antibacterial agents for many years. Their activity is attributed to the interaction of Ag^+ ions with bacterial DNA, which leads to the loss of its replication ability. In addition, the effect of Ag^+ ions on the membrane-bound enzymes has been reported. Silver nitrate ($AgNO_3$), known for its toxicity to bacteria, can serve as the source of silver ions. Poly(vinyl alcohol) films enriched with silver nitrate were prepared by the solvent casting in **P1**. The thermal and spectral analysis proved the presence of the silver particles in the PVA matrix. Surprisingly, the mechanical properties were positively affected by the addition of active agent up to 1 wt.% and the tensile strain was even enhanced over the whole tested concentration range of Ag (0.5 to 9 wt.%). Prepared systems proved a high activity against both tested Gram negative (*E. coli*) and Gram positive (*S. aureus*) microorganisms, which makes them useful in different applications including the protecting packaging.

Poly(vinyl alcohol) carrier was also used in the papers **P2** and **P3**, however, zinc ions were selected as an active substance for the encapsulation. It is known that its antibacterial activity consists in the formation of zinc ions in an aqueous environment that bind the cell membranes, thus prolonging the lag phase. Specifically, zinc nitrate ($Zn(NO_3)_2$), as one of the most frequently reported zinc source, was applied in **P2** in the concentrations recalculated for Zn from 0.5 to 9 wt.%. Mechanical testing proved an increase of E modulus up to 3 wt.% Zn content, after which a gradual decrease was observed, with further addition of modifier. A similar trend was shown for tensile strength, whereas tensile strain was decreasing within the whole concentration range of Zn. The results revealed that the low zinc concentrations (0.5, 1 and 3 wt.%) enhanced the toughness. On the other hand, due to a higher zinc content, the mechanical properties were generally weakened. Differential scanning calorimetry proved an initial increase (up to 72 °C for 3 wt.% Zn) of the glass transition temperature (T_g) at the lower modifier concentrations followed by a further decrease (up to 55°C for 9 wt.% of Zn). These observations indicated that the changes in the polymeric chains mobility occurred. The structural analysis by Fourier transform infrared spectroscopy (ATR-FTIR) of the PVA/Zn films proved a uniform distribution of zinc nitrate in the polymer matrix. Antibacterial tests confirmed the inhibition activity of prepared samples against the selected bacteria, even from the lowest tested zinc concentration (0.5 wt.%) against *Staphylococcus aureus*.

PVA matrix enriched with zinc sulphate ($ZnSO_4$) was investigated in the publication **P3**. The resultant physico-chemical and antibacterial properties, as well as the effect of PVA hydrolysis degree was investigated. The type of PVA proved to have a significant impact on the distribution of zinc sulphate within the PVA matrix, and mechanical and antibacterial properties, too. The films based on the fully hydrolysed polymer exhibited notable increase in Young's modulus and tensile strength. On the other hand, these samples revealed a hindered release of active agent from the matrix, probably due to the different solubility of partially and fully hydrolysed PVA. From the microorganisms tested, Gram positive *Staphylococcus aureus* proved to be the most sensitive, whereas Gram negative bacteria showed higher resistance to the tested films (*Pseudomonas aeruginosa* exhibited zero inhibition zones for the fully hydrolysed PVA samples). Beside the common agar diffusion test, a dilution and spread plate technique was applied, which is considered as the highly sensitive and accurate method enabling to evaluate the activity in liquid medium. The higher sensitivity of Gram positive bacteria, as well as the effect of PVA hydrolysis degree, was also proved by this procedure, due to the obtained parameters of the effectiveness of antibacterial activity (*EAA*) and the efficiency constant (-*k*), which informs on the antibacterial efficiency of the selected agent under specific conditions.

3.1.2 Poly(vinyl chloride) based films prepared by thermoplastic compounding

Poly(vinyl chloride) (PVC) molecule contains ethylene backbone with covalently bonded chlorine. It is one of the most frequently applied thermoplastics with versatile properties, good mechanical strength, chemical resistance and inertness to biological fluids [50]. Modification of medical grade PVC with organic/inorganic active substances was investigated in the publications (**P5**, **P6**).

Thermoplastic compounding or melt blending technique has been extensively applied for the preparation of polymers with uniformly distributed antimicrobial agents enabling a long-term activity. Various antimicrobial agents can be incorporated into the polymer matrix. From the group of inorganic substances, silver and its derivatives has been intensively studied due to its well-known antimicrobial activity based on the silver cation Ag^+ that strongly binds to electron donor parts of biomolecules containing sulphur, nitrogen, or oxygen. The advantage of silver-based polymer composites prepared by thermoplastic compounding consists not only in the high antimicrobial effect, stability and non-toxicity of silver ions to human cells, but also in the great temperature processibility [51].

In the publication **P5**, a medical-grade PVC was used for thermoplastic compounding with silver nitrate (recalculated to Ag content from 0.5 to 5 wt.%) in a Brabender Plasti-corder kneader. The process was carried out at 160°C and 30 rpm for 10 min, after which the samples were compression molded at 160°C for 5 minutes in the manual press to obtain the thin films. This polymer substrate was selected as a carrier for its good compatibility with a number of additives, its mechanical properties and suitable economic characteristics. The aim of the author’s work was to study the effect of modification on the final physico-chemical and antimicrobial properties. The incorporation of a modifier led to a decrease of tensile characteristics, probably due to an infracted structure caused by silver aggregates. In comparison to the above mentioned results from **P1**, where the improvement of mechanical properties (Young's modulus, tensile strength) was observed up to 1 wt.% of silver concentration in PVA, it can be assumed that the polymer matrix, as well as the processing method, play a crucial role in the final properties of the antibacterial polymer system. Nevertheless, the obtained nonhomogeneous structure of PVC/Ag samples did not negatively affect the antibacterial action. All tested bacterial strains including both the Gram positive and Gram negative ones (*Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) proved to be sensitive to prepared PVC-silver composite, even at the lowest filler concentration (0.5 wt.%).

The method of thermoplastic compounding of PVC was also applied in the further study (**P6**), however, three types of antibiotics were incorporated (0.1 to 1 wt.%) in the polymer matrix to obtain the system with antibacterial properties that could be potentially applied in a big-scale production. The active substances (sodium ampicillin, minocycline, rifampicin) were selected regarding their activity spectrum, functional groups and the thermal stability, too. Regarding the effect of the PVC modification with antibiotics, a key role of their chemical structure as well as physico-chemical properties was confirmed. X-ray diffraction measurement revealed that processing conditions (160°C, 10 minutes) did not have any negative impact on the structural properties of used antibiotics.

It is known that mechanical characteristics of polymer materials have a significant impact on the potential applicability of the product. Although a decrease in tensile strength and strain was observed in all antibiotics, the effect on E modulus was negligible.

Considering the antibacterial properties, all tested samples inhibited the growth of Gram positive *Staphylococcus aureus,* whereas Gram negative *Escherichia coli* proved to be more resistant against lower antibiotic concentrations; and completely inert to rifampicin samples. Microscopic evaluation confirmed a higher degree of incorporation, which definitely let to its limited migration from polymer matrix.

Table 1 Constants achieved by the release studies of antibiotics from PVC systems

Antibiotic (1 wt.%)	**Distilled water**			**Physiological solution**		
	C_{max} (μ/g)	**-k (1/h)**	**R^2**	**C_{max} (μ/g)**	**-k (1/h)**	**R^2**
ampicillin	2006	123	0.99	2359	0.26	0.99
minocycline	720	0.02	0.99	110	1.00	0.99
rifampicin	152	0.04	0.97	206	0.02	0.99

This statement was supported by the kinetic study that proved the impact of different molecular structures of active agents on their release from the matrix. While in the case of ampicillin and minocycline a burst effect was observed within the initial phase of testing, rifampicin release was slower. This different behaviour can be confirmed by the appropriate constants shown in Table 1, where the highest concentrations of all three types of antibiotics (1 wt.%) were compared.

It is necessary to state that the amount of active molecule released into the environment is strongly affected by the nature of the polymer matrix (hydrophobicity) and the type of entrapment. It is clear that the released concentrations differ depending on the type of antibiotic used; from which the maximum released amount (C_{max}) was observed for ampicillin. When the initial antibiotic loading is considered, it takes only 0.2 wt%. However, it is known that the release course is controlled not only by the diffusion, but also by the area of the interface related to the polymer surface/surrounding environment. Therefore, in practical applications, this parameter will be predominant.

3.1.3 Poly(vinyl chloride) based films prepared by solvent casting

To compare the effect of the processing technique on the films' character, medical-grade poly(vinyl chloride) based films enriched with sodium ampicillin prepared by the solvent casting were investigated in the author's publication **P4**. A significant impact of applied solvent (cyclohexanone, CYH, or N,N-dimethylformamide, DMF) was confirmed. Tensile properties were enhanced if the film was cast from CYH, whereas the latter mentioned solvent caused an opposite effect.

The effect of different nature of used solvents on the film properties was proved even in the morphological study. Whereas CYH-based films were smooth with uniform filler distribution, the DMF sample contained ampicillin clusters on the surface (Fig. 11). On the other hand, these samples showed a more significant inhibition effect against all tested bacteria.

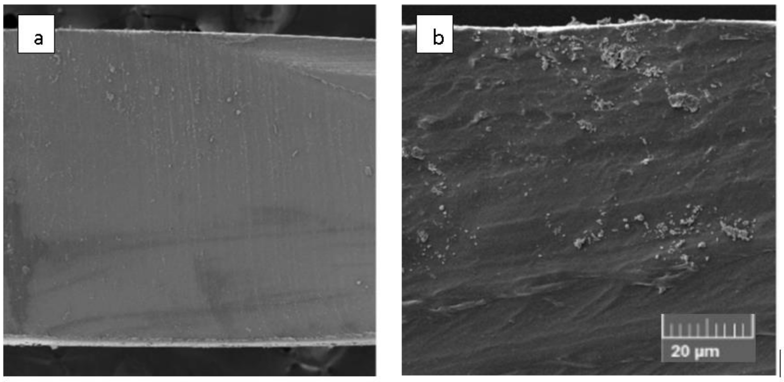

Fig. 11 SEM microghraphs of (a) PVC/CYH/5 wt.% ampicillin and (b) PVC/DMF/5 wt.% ampicillin

A first-order kinetic was successfully applied to the release experiment; slower release of ampicillin was observed from cyclohexanone samples, whereas a burst effect was shown in dimethylformamide solvent. This different behaviour was evidenced by the constants obtained from the release studies, when the maximum theoretical antibiotic concentration released from 1 g of the sample (C_{MAX} in μg/g) was two orders of magnitude higher when compared CYH (41 μg/g) and DMF (1026 μg/g) sample modified with 1 wt.% of sodium ampicillin.

Beside the release of ampicillin into the water, the experiment in the physiological solution was also carried out to evaluate the effect of ionic strength. It was shown that substantially higher antibiotic content was released into physiological solution, both in cyclohexanone and dimethylformamide, probably due to the affinity of ampicillin to isotonic solutions.

The casting technique using the same solvents, CYH and DMF, was applied in the author's publication **P7**, where the addition of silver nitrate (recalculated for the silver content from 0.5 to 5 wt.%) into PVC carrier, concretely the effect on the final structural, mechanical and antimicrobial properties, was investigated,. Prepared samples showed a significant antimicrobial activity even at the lowest tested silver concentration (0.5 wt.%). The antimicrobial activity remained almost unaltered even after 24h immersion in physiological solution. Surface analysis by X-ray photoelectron spectroscopy confirmed that PVC based prepared samples contained the silver mostly in Ag^0 form and the immersion in the physiological solution resulted in the transformation to Ag^+, due to the presence of AgCl. The incorporation of silver nitrate had a negligible impact on mechanical properties. The interesting results came from comparing the tensile characteristic measurements of the PVC samples prepared in CYH and/or DMF, since no significant differences were observed.

3.1.4 Natural polymer/surfactant based films prepared by solvent casting

With increasing demands on environmentally friendly, well degradable materials, a number of present works are oriented more toward natural polymers derived from plant or animal sources. Polysaccharides and proteins are the common representatives of natural polymers that can be applied as suitable carrier materials for various active agents [52].

Chitosan belongs to polysaccharides derived mostly from crustacean cells. From the chemical point of view, it is linear copolymer based on D-glucosamine and *N*-acetyl-D-glucosamine, linked by β-(1→4) bonds. Chitosan is a biocompatible, biodegradable, nontoxic polymer that even possesses antimicrobial properties, which predetermines it for application in many industrial sectors, such as the medical, food or water treatment industry. During the last decade, chitosan and its derivatives were applied either in the form of gels, coatings, food packaging or wound dressings. A solvent casting technique with the following evaporation of solvent represents the standard method for film preparation. Several aspects, as low water vapour permeability, high mechanical strength, sufficient stability and antimicrobial properties, have to be considered [53]. Although chitosan itself offers antimicrobial activity due to interacting of its positively charged amino groups with negative bacterial membranes, this effect can substantially decrease when it is in the form of film. Following this fact, different active substances have been tested for incorporation into chitosan films [54, 55].

Essential oils and their main components belong to attractive candidates, due to their interesting biological activity. Their structure and actually their functional groups play a crucial role in resultant antimicrobial properties. In this respect, phenolic and terpenoic compounds proved to be highly effective [56]. Several studies have been dealt with the combination of chitosan and different essential oils, such as lemon, rosemary, basil, cinnamon or clove [57].

The chitosan films were investigated in the papers **P8** and **P10**, which were aimed at the study of mutual interactions between polymer, active molecule and surfactant. The author's paper **P8** is devoted to a complex study of oregano and marjoram essential oils incorporated into chitosan matrix, regarding both film forming solutions and resulting films. The principle consisted in the addition of a third component, non-ionic surfactant Tween 80, to ensure the enhanced dispersibility and stability of chitosan samples.

Since the properties of individual essential oils are significantly affected by the specific functional groups present in the molecule, the chromatography analysis is recommended to obtain the appropriate data on the molecular structure. Although both oregano and marjoram comes from the same *Origanum* genus, gas chromatography of applied essential oils revealed a different composition of active components that affected physico-chemical and antimicrobial properties of prepared solutions and films. Oregano oil included carvacrol as a predominant substance, known for its strong antioxidant and antimicrobial properties. On the other hand, an alcohol terpinen-4-ol was found as the main constituent in marjoram essential oil. These findings reflected in the antimicrobial properties. While oregano samples proved a high antimicrobial efficiency against all tested strains that was even enhanced after one week in some cases, marjoram films did not show any significant inhibition effect, probably due to a more compact structure limiting diffusion of the active agent into surrounding. The addition of non-ionic surfactant affected the properties of film forming solutions when the particle size significantly decreased with an increasing Tween 80 concentration, regardless the essential oil used. Moreover, a good dispersion stability of both oregano and marjoram film forming solutions was achieved, based on the zeta potential values, which exceeded +30 mV in all samples. For the polymer films intended for use in packaging applications, the barrier properties play a substantial role. Water vapour permeability (WVP) is the parameter indicating the duration of water vapour transmission through the unit area of tested material, evaluating the difference between the unit vapour pressure between two surfaces (under specific temperature and humidity). The results showed that modification of chitosan with essential oil/surfactant mixture led to WVP decrease (e.g. from 16.5×10^{-3} g/Pa.h.m^2 for pure chitosan to 7.8×10^{-3} g/Pa.h.m^2 for chitosan sample with 5 wt.% oregano/Tween 80) and thus, to enhancement of barrier properties. Based on the findings in this publication, chitosan/oregano/Tween 80 films have the potential for the efficient bioactive coatings.

The effects of thyme oil incorporation into chitosan matrix were investigated in the publication **P10**, which was predominantly focused on the type of stabilizer used. Three types of non-ionic surface active agents with different hydrophilic-lipophilic balance (HLB) values belonging to the group of ethoxylated sorbitan esters (polysorbates, Tweens) were selected. A more homogeneous structure was obtained with the emulsifiers of higher HLB (Tween 80 with HLB 15 provided the most stable system). Due to the modification of chitosan with Tween/thyme oil combinations, stable polymer films with sufficient antimicrobial and enhanced water barrier properties were prepared by cost-effective and environmentally favourable way (Fig. 12). In addition, the final physico-chemical properties proved to be tuneable by the surfactant/stabilizer selection.

Fig. 12 Appearance of the chitosan films a) without and b) with thyme essential oil/Tween 80

The interesting results were obtained by the morphological analysis of the films. Fig. 13 showing the SEM photographs of the chitosan films reveals the different structure depending on the added active substance. Samples with thyme and oregano essential oil contain bigger particles (Fig. 13 a, b) compared to the film with marjoram oil (Fig. 13 c). This corresponds to the particle size measurement of the appropriate film forming solutions, where the latter mentioned sample exhibited one order of magnitude smaller particles. Moreover, a more compact structure was observed in marjoram films, probably due to more hydrophilic components. On the other hand, this arrangement hindered the diffusion of active agent from the chitosan matrix, which resulted in a weaker antimicrobial activity in comparison to thyme and oregano oil.

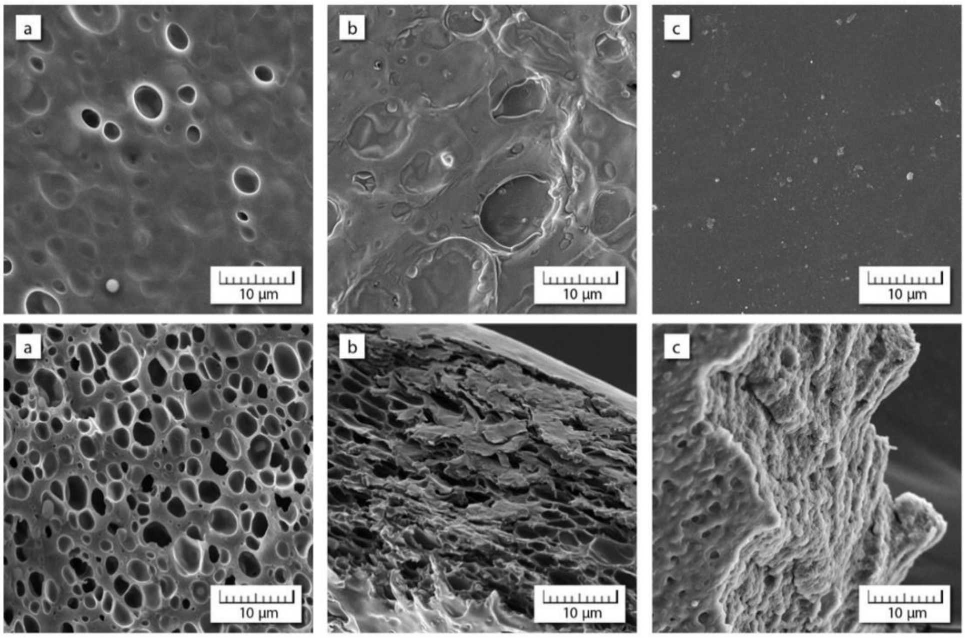

Fig. 13 SEM micrographs of chitosan films with Tween 80 and 2 % essential oil: a) thyme, b) oregano, c) marjoram oil: up (surface), down (cross section)

To compare the effect of polymer matrix, as well as the selected incorporated active molecule, the non-published results of the ongoing research at the author's workplace dealing with zein films are shortly mentioned. Zein belongs to the class of plant proteins known as prolamines that occur specifically in cereals. It is insoluble in water and can dissolve only in the presence of alcohol or high concentrations of anionic detergents. Zein is known for its valuable potential for the formation of films that provide optimum moisture and oxygen barrier properties. Due to this fact, different antibacterial and antioxidant agents, such as salicylic acid and its derivatives, nisin, thymol, as well as cationic surfactant lauroyl arginate, were incorporated in zein matrix to achieve the bioactive protein based biodegradable films [58−63]. Within the author's research, zein films enriched with the combinations of essential oils (thyme or oregano) with monoacylglycerol of lauric acid (monolaurin, MAG 12) were prepared by the solvent casting technique to evaluate the potential synergic effects of these two types of active agents. Comparing the results obtained by the SEM analysis for chitosan and zein (Fig. 13 and Fig. 14) reveals the significant difference in morphology when the crystals of various shapes and size and the key role of added monolaurin was monitored in the zein samples.

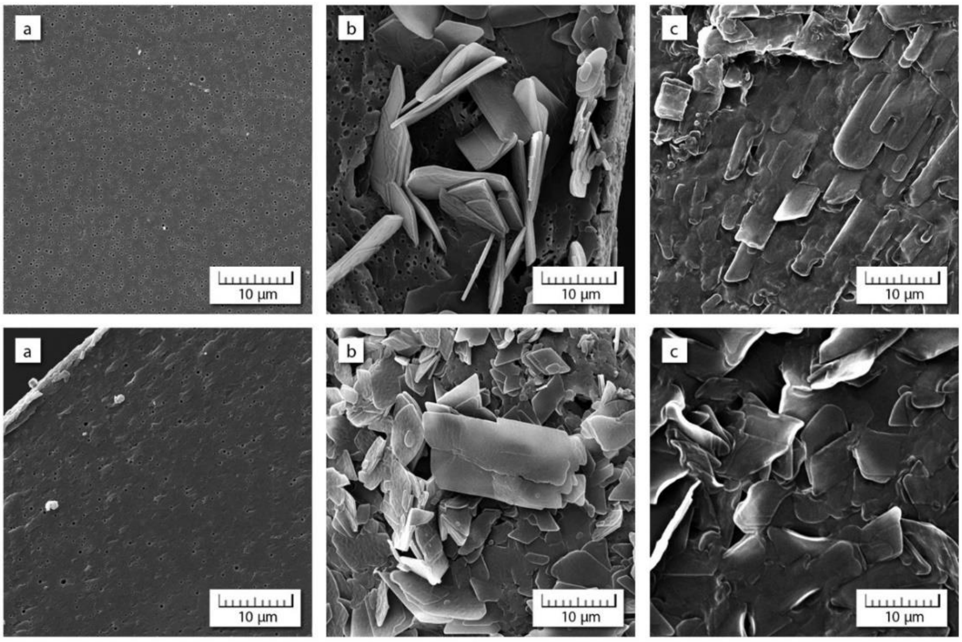

Fig. 14 SEM micrographs of zein films with: a) 4%MAG12/2% thyme oil, b) 5%MAG12/2% oregano oil, up (surface), down (cross section)

3.2 Plasma polymer thin layers

Several approaches are utilized to modify polymer substrate surface properties in order to enhance the consequent attachment of active molecules. Plasma technologies represent clean, environmentally favourable methods of control the chemical and mechanical properties of polymers. The advantage consists in the fact that the material surface can be modified without a significant alteration of bulk properties, due to which original mechanical characteristics are preserved. Ultra-thin films with controllable properties can be obtained by plasma treatment. Moreover, speed, practical scalability of the process and no need for solvents make this technique popular for the production of active substances carriers [64].

Different plasma treatment methods can be used, such as low temperature short term irradiation for material surface modification or high intensity plasma treatment preferred more for surface sterilization. For the preparation of thin films, plasma-enhanced chemical vapour deposition (PECVD) can be applied. The film properties, such as density, thickness and roughness depend on various factors, including sputtered precursor, flow rate and/or type of gas in a plasma chamber. Within the study of controlled active agent release from polymer materials modified by plasma treatment, the majority of experiments are carried out in air, however, atmosphere or other gases (nitrogen, argon) can also be used [64].

Many systems for the controlled release of active molecules prepared by plasma treatment have been investigated, such as films, microparticles, electrospun membranes or tablets [65–67]. It is known that release of active agents from polymeric materials generally occurs via diffusion. However, the degradation process can play an important role, too, especially for easily degradable systems. In this case, a surface wettability has to be considered as the factor affecting a degradation rate as well as a diffusion coefficient of an incorporated drug and consequently its release kinetics. In this context, plasma treatment can be applied as an efficient tool for modifying the hydrophilicity of polymer materials surfaces [64].

The effect of plasma pre-treatment and a crosslinking degree on the poly(vinyl alcohol) (PVA) based films was investigated in the publication **P9** in order to evaluate their potential for carriers of the bioactive molecule, nisin. PVA is known for its biodegradability, good mechanical properties, large swelling capacity and low cost. This polymer contains a number of potential functional groups enhancing the adhesion of various active or preserving agents [68]. Surface plasma dielectric coplanar surface barrier discharge was used to modify the surface hydrophilicity of PVA film so that an enhanced adhesion of nisin is achieved. This technique uses atmospheric plasma surface activation operating in a non-thermal, uniform, glow plasma regime and can be used even for large area surface modifications [69].

The results proved that both crosslinking degree and plasma discharge power are crucial for PVA layer/nisin layer stability. A strong nisin attachment was achieved in the films with 10 and 20% crosslinking degree. In these samples, a significant increase in wettability by the plasma treatment was observed. Consequently, the higher total surface energy was monitored, which enhanced a nisin adhesion. Data from the release kinetics study revealed that the slowest nisin release was obtained at 20% crosslinking degree after the plasma treatment. Based on the antimicrobial assay results, this sample was proved as an efficient and stable system with controllable release conditions.

In the publication **P11**, poly(lactic acid) (PLA) based thin films were prepared by a plasma assisted vacuum thermal deposition (PAVTD) to study the effect of varying radio frequency discharge power on chemical composition, structural properties and hydrolysis rate of prepared thin films. PAVTD technique uses oligomers released during low-pressure thermal decomposition of bulk polymer precursor to form the thin film either directly or after plasma re-polymerization. Due to this fact, various properties of the film can be controlled. Poly(lactic acid) is a synthetic biodegradable polymer widely applied in biomedical field, as well as in food packaging. Its degradation mechanism is based on the hydrolysis of ester bonds [70]. High performance liquid chromatography with UV detection (HPLC-UV) was used for the determination of lactic acid released into the water as a measure of the hydrolysis reaction. It was shown that a faster hydrolysis proceeded in the films prepared at lower power, whereas higher effective power resulted in a slower hydrolysis rate. These samples provided a more cross-linked structure although the PLA-like groups were preserved. This was also proved by structural analysis (ATR-FTIR spectroscopy), where prepared PLA plasma films revealed no significant changes when compared to the original PLA polymer.

The PAVTD method was also applied in the publication **P12** to obtain films based on poly(ethylene oxide) (PEO) precursors. The aim of the study was to investigate the effect of molecular weight (Mw) of precursor on the physical properties of final system, and the influence of plasma power on the biomolecule permeability conditions. The Mw of the precursors showed a negligible effect on the film properties while the process conditions (presence/absence of plasma) had a stronger influence. The rate of thickness loss after water immersion was found to be tuneable in this way. These films were used as cover and permeation layers for bacteriocin nisin dispersed in PVA carrier. A controlled rate of release of biologically active nisin molecules into the water was achieved.

3.3 Polymer nanofibrous membranes

The electrospinning process is another method that can be used for encapsulation of active molecules into the polymer matrix. This technique is based on the application of electric charge enabling the production of nanofibers from polymer solutions or melts. When polymer precursor is placed at the pipette tip, the high voltage is generated leading to the formation of Taylor cones at the polymer material surface [71, 72]. Charged polymer jets pass from the tip to the grounded collector that is accompanied by a reduction of their diameters (Fig. 15).

The resultant polymer membranes are defined as solid sheets porous enough to enable the passage of small particles that can be applied for permeability of gases, vapours and liquids. Incorporation of organic or inorganic substances has been recently studied to obtain materials with enhanced magnetic properties, increased hydrophobicity, and antibacterial or antifouling activity [73, 74].

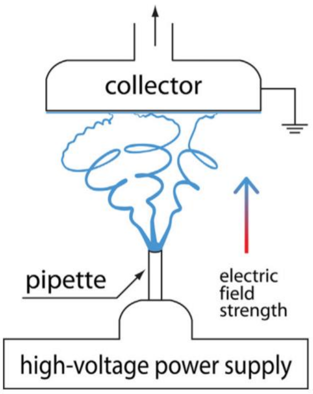

Fig. 15 Scheme of an electrospinning apparatus [71]

Antibacterial nanofibrous membranes find applications in many industrial fields, such as medical and healthcare, food preservation, protecting of textile materials, or water treatment [75–78]. The active agents, which are most widely utilized for the preparation of antibacterial nanofibrous membranes, include silver nanoparticles, antibiotics, quaternary ammonium salts, or essential oils [79–81]. Materials with antifouling properties gain increasing interest in the applications, where biofouling of polymer membranes leading to the biofilm formation represent a serious obstacle. Antifouling surfaces prevent the accumulation of biofilms on their surfaces by either affecting the structure of microbial biofilm (due to the steric repulsion or nanoscale rough topography) or repelling microorganisms [82, 83]. Recent research focuses on novel strategies bringing about new antibacterial and antifouling surfaces that are safe and do not promote bacterial resistance at the same time.

Antifouling membranes based on poly(vinyl butyral) and monocaprin (MAG 10) were prepared and characterized in **P13**. Poly(vinyl butyral) (PVB) is frequently applied in the electrospinning process to enhance the spinnability of polymer solutions [84]. Monocaprin exhibits antibacterial properties against both Gram positive and Gram negative bacteria, due to which it interferes with bacterial cell membranes that might lead to the inhibition of cell metabolism or a cell lysis. Already Kabara [85] revealed some general statements concerning the antimicrobial activities of lipid and their derivatives. Regarding monoacylglycerols, those of medium-chain saturated fatty acids proved to be more effective than the free acids. On the other hand, di- and triacylglycerols do not show a significant antimicrobial activity. Antibacterial properties of short-chain saturated fatty acids (from 6 to 10 carbons), where monocaprin is classified, are associated with the dissociation degree, and the higher activity was proved at pH 6.5. MAGs can be utilized in the pharmaceutical industry or as the protection of textile materials. Since monoacylglycerols have a specific amphiphilic structure, they are applicable as surfactants in the food industry [86]. The positive effects of surfactants on the character of polymer solutions applied for electrospinning was even shown in some of previous studies [87].

Nanofibrous membranes were prepared from various PVB concentrated solutions (8, 10, and 12 wt%) with different amounts of monocaprin (from 0.5 to 3 wt%). Increasing the concentration of monoacylglycerol resulted in different shape of the nanofibers, probably because MAG 10 stayed embedded in the inner fiber structure. From this reason, the diffusion from the sample was hindered, as well as the antibacterial effect tested by the agar-diffusion method. On the other hand, a good antifouling activity of nanofibrous membranes was shown, when bacterial viable counts in planktonic vs. sessile form was investigated. Simultaneously, the level of wettability was heightened, which is favourable in the development of antifouling surfaces, too. The prepared nanofibrous membranes showed the potential as an antifouling filtration membrane in the processes of wastewater treatment. Due to various factors affecting the physico-chemical and antibacterial properties, further research will focus on the investigation of the molecular structure of the applied active agent (monoacylglycerols with different chain length will be tested) and mutual interactions with the polymer.

4. CONTRIBUTION TO SCIENCE/PRACTICE

The habilitation thesis summarises the work related to the research on the formulation and preparation of active polymer systems, carried out from 2008 until the present. Within the author's works, the various preparation techniques ranging from the thermoplastic compounding through the solvent casting to electrospinning or the plasma deposition processes have been used. Similarly, attention was paid to the careful selection of antimicrobial agents, of which inorganic (based on silver or zinc ions) and/or organic (based on antibiotics and phenol) compounds were investigated.

The papers **P1 to P7**, published within the years 2008 to 2013, are dealing with the utilization of synthetic polymers, poly(vinyl chloride) and poly(vinyl alcohol), as carriers for selected organic and/or inorganic antimicrobial substances were incorporated by the melt blending or solvent casting technique. The main contribution of these works to science and practice is the successful preparation of the highly efficient and applicable antibacterial polymer systems by using facile and economically favourable materials and methods.

Along with an increasing trend in searching for environmentally friendly materials, the attention was directed towards natural polymer matrices based primarily on chitosan, an organic active agent and a third component - surfactant, which was included to achieve better incorporation of active substances into the polymer matrix (publications **P8** and **P10**). This research unambiguously documented that it is possible to prepare safe, biodegradable, biocompatible and nontoxic films that could be utilized for preserving and protecting the food or cosmetic products from contamination.

The papers **P9**, **P11**, **P12** (2018–2020) were dealing with the plasma deposition used for the modification of the polymer substrate to study the effect on the polymer surface, structural properties and degradability. The interesting pieces of knowledge on the plasma treatment came out of the study on poly(lactide). Here, the original polymer structure was well preserved after the plasma deposition, while other properties, including the degradability, proved to be tuneable, depending on the deposition conditions.

The remarkable outcomes with high application potential were provided in the last study **P13** dealing with the novel nanofibrous membranes based on poly(vinyl butyral) and monocaprin. The modification with this non-ionic amphiphilic substance significantly enhanced the antifouling properties of the resulting membranes, which is highly required in sustainable technologies focusing on water and wastewater treatment, pollution prevention (detection of micropollutants or capture of microplastics) and environmental safety.

The main contribution of the habitation thesis to science lies in increasing the knowledge on the key approaches for incorporation of active agents into polymer and biopolymer matrices, leading to the design of an efficient active polymer based carrier systems. From the scientific point of view, revealing mutual interactions between polymer matrix and the active or stabilizing agent is crucial, resulting in a deeper understanding of phenomena occurring at surfaces and interfaces of the dispersion polymer systems. The contribution to the practice then consists in the development of functional polymer-based materials providing antimicrobial, and/or antifouling properties, with controllable characteristics, such as degradability, stability and/or release of antimicrobial molecules. These materials show the potential to prevent bacterial colonization, which is one of the major problems in the food, cosmetic or medical industry. The efficient suppression of bacterial growth favourably affects not only public health, but also environment and economy.

CONCLUSIONS

Various strategies how to incorporate the active substances into polymer matrices were investigated to evaluate the effect of such modifications on the final antimicrobial, physicochemical and surface properties of the products. The work documented successful preparation of the active polymer systems that can help to reduce and even eliminate the microbial contamination affecting a broad range of industrial and human activities. Following the results of the papers dealing with the solvent casting technique, it was proved, that this relatively simple and low-cost method can be applied for production of the efficient antimicrobial polymer systems. In the case of poly(vinyl chloride) based films, the crucial role of the solvents used for polymer dissolution prior to casting was observed. Within the current author’s research and educational activities, the incorporation of active and/or stabilization substances with the surface activity and their interactions with the polymers have been studied. The key role of the surfactant type and its hydrophilic-lipophilic balance was revealed in the papers investigating the chitosan-based solutions and films, especially in the analysis of their surface and morphological properties. The studies devoted to the plasma-treated polymer thin films showed that their degradation mechanisms as well as the potential to release selected active molecules could be controlled *via* the applied plasma power. Beside the mentioned techniques, the electrospinning process was successfully utilized to prepare the poly(vinyl butyral) membranes with non-ionic surfactant monocaprin, in which the antifouling properties were observed. To sum up, the introduced polymer-based systems have good potential to serve as the carriers of different active molecules that could be efficiently used in several practical above-mentioned applications.

Future perspective of the work will be directed towards the deeper study of release kinetics of active molecules from the polymer substrates, as well as the factors leading to optimization of the delivery conditions for the actives and their required efficacy. In addition, new strategies of enhancing the stability of active polymer systems will be systematically studied. The second important direction of the future research will concentrate on the investigation of new types of biopolymer-based matrices that can be used as carriers of antibacterial agents, and mainly of the possibility to use recycled polymer wastes and their modification with bioactive molecules by eco-friendly techniques. This is an important approach within the applied polymer research, which considers polymer waste as valuable renewable resource and as an inherent part of sustainable circular economy.

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LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

AgCl	silver chloride
$AgNO_3$	silver nitrate
ATR-FTIR	attenuated total reflectance-Fourier transform infrared spectroscopy
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
CAC	critical aggregation concentration
CFU	colony forming units
CMC	critical micellar concentration
CYH	cyclohexanone
DLS	dynamic light scattering
DMF	N, N-dimethylformamide
DNA	deoxyribonucleic acid
DSS	dioctyl sulfosuccinate
EIP	emulsion inversion point
EO	essential oil
GUV	giant unilamellar vesicles
HLB	hydrophilic-lipophilic balance
HPLC-UV	high performance liquid chromatography with UV detection
LUV	large unilamellar vesicles
MAG C10/C12	monoacylglycerol of capric/lauric acid
MIC	minimum inhibition concentration
MLV	multilamellar vesicles
MRSA	methicillin resistant *Staphylococcus aureus*
NLC	nanostructured lipid carriers
OLV	oligolamellar vesicles
O/W	oil in water emulsion
PAVTD	plasma assisted vacuum thermal deposition
PCS	photon correlation spectroscopy
PECVD	plasma-enhanced chemical vapour deposition
PEO	poly(ethylene oxide)
PIT	phase inversion temperature
PPO	poly(propylene oxide)
PVA	poly(vinyl alcohol)
PVB	poly(vinyl butyral)
PVC	poly(vinyl chloride)
SEM	scanning electron microscopy
SLN	solid lipid nanoparticles
SUV	small unilamellar vesicles
TEM	transmission electron microscpy
W/O	water in oil emulsion
WVP	water vapour permeability

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1. GA17-10813S Novel plasma polymers with tunable stability and permeability, 2017–2019, Grant Agency of the Czech Republic, collaborator.
2. OP VK CZ.1.07_2.2.00_28.0132 Increase of exclusiveness in education of fats, cosmetics and detergents technology (Zvyšování exkluzivity výuky technologie tuků, kosmetiky a detergentů). 2012-2015, collaborator.
3. MEO9072 Study of polylactide based material for packaging applications. 2009-2012, Ministry of Education, Youth and Sports, collaborator.
4. MEB090803 Research on thin polymeric layers for biomedical applications, 2008-2009, Ministry of Education, Youth and Sports, collaborator.
5. 2A-1TP1/126 Innovation of Medical Polymeric Aids. 2006 – 2011, Ministry of Industry and Trade, collaborator.
6. MSM7088352101 Multifunctional Composite Systems Based on Natural and synthetic Polymers. 2005-2010, Ministry of Education, Youth and Sports, collaborator.
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