ABSTRACT

The doctoral thesis concerns the synthesis, characterization and evaluation of multilayer hydrogel nano- and microcarriers loaded with active substances of natural origin and coated with functional polyelectrolyte films. The designed systems were formed using emulsification under normal pressure, emulsification under high pressure - high pressure homogenization (HPH) and extrusion with ionic gelation. The design of experiments (DoE) and the selection of appropriate process parameters allowed to obtain the products with specific functional features, i.e. desired size, low polydispersity, high encapsulation efficiency and loading capacity, proper half-life of active substance and biological properties (anticancer or antimicrobial activity). The prepared hydrogel nano- and microcarriers were stable systems intended for the simultaneous or subsequent delivery of plant-derived active substances with anticancer or antibacterial properties in the combination chemotherapy.

At each stage of this work, the design of experiment was applied to select the most favorable parameters of particle formation process and to choose the appropriate components of carrier systems. The selected parameters enabled to obtain products that fulfill both physicochemical and biological criteria required for the modern drug delivery systems (DDS). Biocompatible and biodegradable materials such as natural polyelectrolytes (sodium alginate, chitosan) and synthetic polyelectrolytes (polyacrylic acid, polyallylamine chloride) were used for the synthesis of hydrogel nano- and microparticles. Multilayer nano- and microcarriers were obtained in two stages. In the first stage, chemotherapeutic (chemopreventive) compounds including curcumin (CUR), resveratrol (RES) and epigallocatechin gallate (EGCG) or a antimicrobial substance such as cranberry fruit extract (EOZ) were encapsulated in a hydrogel core. In the second step, the prepared carriers were functionalized with biocompatible polyelectrolyte films using layer-by-layer (LbL) technique to form multifunctional systems. The obtained nano- and microcarriers are modern formulations connecting several functions, which means that they can be applied in combination therapies.

The characterization of hydrogel nano- and microparticles included the determination of their sizes and polydispersity index (PdI) as well as imaging of their morphology using several techniques, such as Dynamic Light Scattering (DLS), Optical Microscopy (OM), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Confocal Laser Scanning Microscopy (CLSM), and Atomic Force Microscopy (AFM). The conducted research allowed to select carriers with the most favorable physicochemical parameters. The obtained microcarriers had sizes in the range of 30-70 μm or 600-1000 μm, depending on the used methodology and they were characterized by a polydispersity coefficient - PdI<1. In turn, the obtained nanocarriers had a diameter in the range of 100-200 nm and a polydispersity index - PdI<0.3. In addition, both the hydrogel nano- and microparticles had a spherical shape. Fourier Transformed Infrared Spectroscopy (FTIR) and Ultraviolet/Visible Spectroscopy (UV-Vis) were used to confirm the effective encapsulation of active substances in carriers. The prepared systems were characterized by a high and long-term colloidal stability. Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) and Spectroscopic Ellipsometry (ES) allowed to study the adsorption kinetic and viscoelastic properties of polyelectrolyte films. The mechanical properties of the microcarriers were determined using the parallel plates compression test, which confirmed the high strength and mechanical resistance of the

microparticles. The study of the release rate of active substances from hydrogel particles confirmed their ability to release the encapsulated compounds in a controlled manner.

The last stage of this work was the biological evaluation of selected products. The cytotoxic activity - *in vitro* interaction of nano- and microcarriers with human cancer cell lines (bladder cancer *5637*) or antimicrobial activity of microcarriers against a Gram-positive bacteria (*Staphylococcus aureus* PCM 2054) and Gram-negative bacteria (*Serratia marcescens* PCM 549) was assessed. The cytotoxicity of the obtained particles was tested using the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay (MTT) and the sulforhodamine B assay (SRB). The antimicrobial properties of microcarriers were tested using the disc diffusion test (DDT). The biological studies confirmed the efficient cytotoxic effect of the tested nano- and microsystems against bladder cancer cells. The evaluation of antimicrobial properties of microparticles showed that the obtained carriers possess good antibacterial activity against *Staphylococcus aureus* and *Serratia marcescens*.

The obtained results indicate that the produced hydrogel nano- and microparticles are effective carrier systems capable of encapsulating both hydrophilic and hydrophobic biologically active compounds. The usage of design of experiments simplified the methodology of nano- and microcarriers formation, reduced production costs as well as increased the effectiveness of the process. The selection of the key control parameters of the carrier fabrication process allowed to achieve an effective technological solution based on the formation of a product with the desired functional properties. The presented results are the basis for further research concerning the application of obtained structures as effective drug delivery systems.