Summary

The aim of this study was to develop novel, pH-responsive systems for the controlled release of biologically active substances. The subjects of the research were micelles and supramolecular hydrogels based on PEGylated copolymers of poly([R, S]-3-hydroxybutyrate) and/or aliphatic polycarbonates. In order to obtain the pH-sensitive nature of the resulting drug carriers, the chemical bonds that are stable under physiological conditions and hydrolyses in acidic environments were introduced into the structure of the copolymers. The change in the morphology of the carriers caused by the acid-triggered hydrolysis of pH-sensitive chemical bonds results in the release of the encapsulated drugs. In the present dissertation, three types of pH-sensitive drug carriers were designed and prepared.

The first part of the dissertation presents the results of research on micelles with a PEG shell-shedding mechanism prepared from poly(ethylene glycol-hydrazone-b-[R,S]-3-hydroxybutyrate) (mPEG-hyd-aPHB) diblock copolymer. The monomethoxy-poly(ethylene glycol) macroinitiator with a molar mass of 5000 g/mol containing a hydrazone bond in the structure and carboxylate end groups was synthesized. Then the macroinitiator was used in the anionic polymerization of β-butyrolactone, and an amphiphilic copolymer capable of self-assembly into micelles was obtained. The physicochemical studies of the obtained micelles using the dynamic light scattering method indicated that the micelles are stable under physiological conditions (pH 7.4), while the hydrolysis of the hydrazone bond in the acidic environment (pH 6.4 and 5.5) causes the shedding of outer PEG layer, resulting in micelles reorganize into larger structures. The mPEG-hyd-aPHB micelles were encapsulated with 8-hydroxyquinoline glycoconjugates (glucose and galactose) in order to increase anticancer therapy selectivity, due to that both vectors (nanocarrier pH-sensitivity and glycoconjugation) take advantage of the characteristic for cancer cells Warburg effect. The drug release studies showed a pH-dependent drug release. *In vitro* cytotoxicity studies confirmed that blank micelles are non-toxic to the tested tumor cell lines (MCF-7 and HCT-116) and healthy cell lines (NHDF-Neo). The micelles encapsulated with 8-hydroxyquinoline glycoconjugates or doxorubicin effectively inhibit the proliferation of neoplastic cells. It was also shown the encapsulation of active compounds in the pH-responsive micelles increases the selectivity index of the tested prodrugs and drugs. The results of apoptosis and cell cycle analyses confirmed the anticancer effect of the tested prodrugs and micelles encapsulated with prodrugs, showed that they are pro-apoptotic and at the same time do not induce cell necrosis. The performed imaging studies using fluorescence microscopy have shown that the mPEG-hyd-aPHB micelles can effectively transport encapsulated drugs into cancer cells and release intracellularly.

The second part of the dissertation concerns micelles with the swelling mechanism prepared using a diblock copolymer of poly(ethylene glycol-b-9,9-dimethyl-2,4,8,10-tetraoxaspiro[5.5]undecan-3-one) and a triblock copolymer poly(ethylene glycol-b-9,9-dimethyl-2,4,8,10-tetraoxaspiro[5.5]undecane-3-one-b-[R]-3-hydroxybutyrate). Copoly(ether-carbonate) were synthesized *via* anionic polymerization of a six-membered cyclic carbonate functionalized with a ketal moiety (9,9-dimethyl-2,4,8,10-tetraoxaspiro[5.5]undecan-3-one) initiated by monomethoxy-poly(ethylene glycol) using superbase (1,5,7-Triazabicyclo[4.4.0] dec-5-ene) as a catalyst. The triblock copolymer was synthesized by esterification of the hydroxyl end group of the copoly(ether-carbonate) with the carboxyl end group of oligo[R]-3-

hydroxybutyrate. The introduction of the ketal group to the copolymer structure allowed to obtain pH-responsive micelles, due to the acid-dependent hydrolysis of this group led to the formation of two hydroxyl groups, weakening the hydrophobic interactions in the micelle core, as a result, the micelles swelled, releasing the encapsulated active substance. The dynamic light scattering studies confirmed the pH-sensitivity of obtained micelles. It was also shown that the addition of the hydrophobic oligo[R]-3-hydroxybutyrate block as an additional micelles core forming unit resulted in an increase in the stability of the tested nanocarrier. The drug release studies showed a pH-dependent drug release profile and confirmed that the addition of oligo[R]-3-hydroxybutyrate increases the stability of the micelles, which also prolongs drug release. MTT assay showed that the blank micelles were non-toxic to tested cell lines, while glycoconjugates-loaded micelles, showed significantly increased ability to inhibit the proliferation of cancer cells compared to free glycoconjugates.

The last part of the dissertation presents the results of research on a supramolecular hydrogel based on host-guest interactions between α-cyclodextrin and poly(ethylene glycol) chains from the pH-responsive micelles made of the poly(ethylene glycol-b-9,9-dimethyl-2,4,8,10-tetraoxaspiro[5.5]undecan-3-one) for the codelivery of two types of anticancer agents, the hydrophilic 8-hydroxyquinoline glycoconjugate and the hydrophobic doxorubicin. As a result of host-guest interactions, micelle-derived poly(ethylene glycol) chains penetrated the α-cyclodextrin cavity to form poly(pseudo)rotaxanes, which then aggregate into channel-type crystalline complexes to form the supramolecular hydrogel. The formation of poly(pseudo)rotaxanes was confirmed using X-ray diffraction and differential scanning calorimetry methods. Rheological studies have shown the thixotropic nature of the supramolecular hydrogel. The obtained supramolecular hydrogel showed an accelerated drug release profile in an acidic environment. *In vitro* studies have shown that the supramolecular hydrogel is non-toxic to cancer and healthy cell lines. Cytotoxicity studies showed that the combination of doxorubicin and the 8-hydroxyquinoline glycoconjugate was more effective in inhibiting cancer cell proliferation than when the compounds were used alone, which was attributed to a synergistic effect.

Based on the research described, it can be concluded that the use of pH-responsive carriers for anticancer drugs, especially glycoconjugates, increases the selectivity of therapy and at the same time allows the use of much smaller doses of drugs to achieve the same therapeutic effect as in the case of using free drugs, which should lead to reducing the side effects of therapy.