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in the Category of the Sciences *Chemistry*

From polymer chemotherapeutics to polymer radiopharmaceutics

Od polymerních chemoterapeutik k polymerním radiofarmakům

Commission for the Doctoral Dissertation Defence in the Field
Macromolecular Chemistry

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Summary of DSc thesis:

In this DSc thesis is presented the contribution of our work to the opening of possibilities of the use of medically important radionuclides as active components of polymer delivery systems that is based on a transfer of our experience with drug delivery systems for chemotherapeuticals into this newly emerging area. In the *Introduction* chapter the area of polymer carrier systems for radiopharmaceuticals is critically delimited including the advantages and disadvantages. In the chapter *Polymers as carriers of anticancer drugs and radiopharmaceuticals* we describe our contribution to the area of water-soluble and self-assembled supramolecular polymer systems sensitive to external stimuli, especially to the pH- and temperature change. We also state our contribution to the theranostic (i.e., combining diagnostic and therapeutic approaches enabling therapy with simultaneous tracking of the fate of the system in the patient´s organism) polymer systems for delivery of Auger electron emitters, to the hybrid polymer carriers on the basis of body´s own polysaccharide glycogen and to the use of nanodiamonds as advantageous fluorescent probe for following the fate of the polymer conjugates. Micellar self-assembled systems enable efficient accumulation in solid tumors due to Enhanced Permeability and Retention (EPR) effect with retained ability of the system to be eliminated from the body after fulfilling its task. Spontaneous assembly of polymers into micelles upon heating of the polymer solution to body temperature significantly simplifies preparation of the formulation. The systems were studied as radiotheranostics. Similar polymers of, e.g., polyacrylamide and poly(2-alkyl-2-oxazoline) could also be exploited for local radiotherapy of prostate cancer, as we proved on an *in vivo* murine model. Multistage-targeted systems are intended for the delivery of Auger electron emitters into nuclei of the tumor cells. Auger electrons emitted during the decay of certain radionuclides possess extremely high biological efficacy but have very close range in living tissue. The system is based on an intercalator labeled with Auger electron emitter, which is bound by hydrolytically labile spacer to a polymer carrier. The polymer carrier ensures accumulation of the system in solid tumor tissue, where the drop of pH causes cleavage of the linker and release of the radiolabeled intercalator in its active form. The latter subsequently delivers the radionuclide into DNA of the target cell. Such approach enables efficient destruction of tumor cells with low radiation burden to healthy tissues due to short range of Auger electrons (which are effective only if delivered to a very close proximity of DNA). The accompanying γ-rays possess much lower biological efficiency, but enable tracking of the biological fate of the system . Glycogen is a natural high-molecular-weight fully biodegradable poly (D-glucose) dendrimer (typical molecular weight several MDa) with properties highly advantageous for targeted delivery and controlled release of anticancer agents and multimodal diagnostic imaging of solid tumors. In our publications we described several such conjugates based on glycogen and glycogen-*graft*-poly(2-alkyl-2-oxazolines). We also described biocompatible polymer-coated nanodiamonds with radiolabelable peptide as analogous, although non-biodegradable multimodal nanoprobe. The specifics of radiohalogenation of nanocarriers with iodine and astatine radionuclides including special techniques we developed for this purpose is described in the chapter *Radiohalogenation of polymer and nanoparticle carriers – iodine and astatine radionuclides*. Although the use of iodine and astatine radionuclides as active components possesses numerous advantages especially in the development stage, we have demonstrated that in certain cases (e.g., hydrazone conjugates) their use is problematic. Therefore in chapter *Polymer chelates for nuclear medicine* we focus on macroporous chelating microparticles intended for radioembolization of hepatocellular carcinoma solid tumors and for scavenging copper for the therapy of Wilson´s disease and on metal ion-assembled nanoparticles for cancer theranostics. Microparticles with size 20-50 micrometers

after administration into the hepatic artery spontaneously accumulate in liver tumors. Polymer chelating microparticles of such size containing suitable ligands enabling radiolabeling with lutetium 177 and holmium 188 have, as we have proven, advantageous properties for radioembolization of liver tumors in this way. Wilson´s disease is a genetically caused pathological accumulation of copper in organism. Polymer particles with highly selective copper chelators could be potentially used as dietary supplements lowering copper uptake from diet, which is a concept that we proved using radioactive copper 64. Graft copolymers with chelating backbone and biocompatible hydrophilic grafts self-assemble upon addition of iron(III) ions into nanoparticles with metal chelate core and hydrophilic corona with the size in tens of nanometers, which is a suitable size for the EPR effect-based solid tumor accumulation. The residual chelating groups are useable for the labeling with the theranostic radionuclide copper 64. These nanoparticles are biodegradable in the cell by reductive iron rechelation into polymers eliminable by renal filtration. *These research works opened the field of use of polymers as carriers of radionuclides for nuclear medicine that is currently an actively emerging area of research.*

Contents

1. Introduction

Nanotechnologies have recently become very popular and widely studied for various applications. The size of nanometers to hundreds of nanometers brings many useful features, such as extremely high surface area, specific biological behavior or fluorescent properties (quantum dots), which may be beneficial for medicinal use. There is no wonder these properties, opening new perspectives for pharmaceutical market, attracted tremendous attention of scientists in last years.

To tell the truth, the use of nano-sized devices in medicine is not completely new although it is sometimes presented this way. Gelatin-stabilized silver nanoparticles have been used as strong disinfection agents for more than one century (large surface area of silver causes partial dissolution to Ag^+ ions, which are potent bactericides with very low toxicity to human or animal organism[1,2]). Cigarettes from *Datura stramonium* were in pre-world war II pharmacopoieas indicated as efficient antiasthmatic agents. Aerosol of smoke nanoparticles/tar nanodroplets serves as carrier system to lower parts of respiration tract[3] for bronchodilatators atropine and scopolamine that have otherwise low volatility.

In nuclear medicine, ^{99m}Tc-labeled colloids have been used as radiodiagnostics since the sixties[4-7]. However, only recently it was widely recognized that much more may be mined from nanometer-size devices in medicine.

There are two principal questions involving nanotechnology and nuclear medicine: 1. "*What can bring the use of radionuclides and nuclear medicine approach to nanoscience?*" and 2. "*What can nanoscience bring to nuclear medicine?*"

The answer to the first question lies especially in the use of suitable radionuclides to noninvasively track the fate and biodistribution of nanosized devices in organism in biological behavior studies. For instance, labeling with ^{99m}Tc and subsequent scintigraphy became the main method of choice to follow the fate of aerosols and pulmonary drug delivery devices in lungs and airways in general[8]. Although fluorescent labels are now generally preferred to other methods for this use, radiolabeling with γ-emitters with suitable properties (e.g., ^{111}In or ^{99m}Tc) has numerous advantages compared to any other method[9-11]. The main advantages are:

● Radioatom has far lower size than common organic dye fluorophores or even quantum dots, so its influence on the physicochemical properties and biological behavior of the studied nanosystem is lower. In addition, the necessary amount of radionuclide for imaging is orders of magnitude lower than the necessary amount of the organic dye.

● Exact quantitative determination of the label concentration in the target tissue is significantly more accurate compared to fluorescence and is very easy to measure. If a radionuclide with sufficiently high γ-photon energy is used, self-absorption in tissue is negligible (unlike in the case of light used for fluorescent studies).

● Photobleaching/fluorophore photolysis does not exist for radionuclide studies (autoradiolysis is negligible in activities used for biological studies).

However, the important odds are the production of radioactive waste and relative difficulties in following in high spatial resolution intracellular organelle distribution with radiolabeled compounds compared to fluorescence microscopy. The often mentioned health risk of radiation is not statistically significant for the doses used for research purposes if safety precautions are fulfilled. In addition, the fact that the radioactivity is easier to measure even in extremely low (and from the health protection scope of view insignificant) amounts compared to the concentration of some highly carcinogenic organic fluorophores or heavy

metals containing quantum dots may lead to underestimation of the health risk of fluorescence studies.

The answer to the second question ("What can nanoscience bring to nuclear medicine?") is more challenging. Most currently studied nanosystems designed for medicinal purposes are chemical drug or nucleic acid (gene) delivery systems. Radionuclides however have certain advantages compared to chemical drugs for the nanosized drug delivery systems[10-14], in particular:

● In most cases it is not necessary to cleave the radionuclide from the carrier for imaging or therapy due to sufficient effective range of the radiation in the tissue.

● The effective amount of a radionuclide (typically in nanogram scale for common carrier-free therapeutical radionuclides for human patients) is much lower than the effective amount of any chemical drug (typically in milligram scale).

● Because internalization of the radionuclide carrier into the cell is usually not necessary, multidrug resistance, common against chemical drugs, does not apply (however, some drug delivery devices circumvent multidrug resistance by inhibiting P-glycoprotein and related ABC-transporters[15-18]).

● Biodistribution may be followed during therapy to customize care for the individual patient (theranostics).

The main disadvantage of the radionuclides for medicinal use is that certain radiation burden of healthy tissues cannot be avoided, but may be minimized by rapid radionuclide deposition kinetics or in some cases by use of radiation with extremely short effective range (such as Auger electrons). For radiotherapy, one must also consider that larger tumors are usually hypoxic in the middle[9,19,20], which decreases radiosensitivity[21], since the free oxygen-dependent formation of reactive oxygen species is the main mechanism of action of γ and partly also β⁻ radiation. On the other hand, the main mechanism of biological effects of α-radiation and Auger electrons is the free oxygen independent formation of DNA double strand breaks[9].

Polymers and supramolecular polymer assemblies such as micelles may take advantage f Enhanced Permeability and Retention (EPR) effect to accumulate in solid tumor tissue. The EPR effect is a property of most solid tumors and means that the tumor filters off and concentrates in tumor tissue large molecules or supramolecular assemblies as micelles or nanoparticles of tens of thousands to millions kDa mass[22-24]. The accumulation principle is that the endothelium of blood vessels in normal tissue is impermeable for macromolecules, while the fast grown endothelium of neovasculature in solid tumors is generally permeable for macromolecules up to ca 200 nm in diameter depending on the tumor type and animal species[25] (see **Figure 1**). More to that, solid tumors have poor or even completely missing lymphatic drainage, which further hampers elimination of macromolecules from tumor interstitial space.

Solid tumors generally cannot grow above several millimeters in size without forcing the organism to form neovasculature, so the EPR effect works even for relatively small metastases. The EPR effect-based targeting may also be effectively combined with ligand-based targeting with ligands to receptors overexpressed on target.

While these targeting principles are well-established for nanocarriers of anticancer chemotherapeutics, they may therefore bring also significant benefits to polymer radiopharmaceutics for cancer applications. Despite advances in drug delivery systems in the past two decades, polymer nuclear medicine applications still remain well underestimated

compared to more than 100 000 citations to date for delivery systems for chemical drugs (see **Figure 2**). Therefore, the aim of our research was to transfer experience from polymer carriers of chemical drugs to polymer carriers of radionuclides.
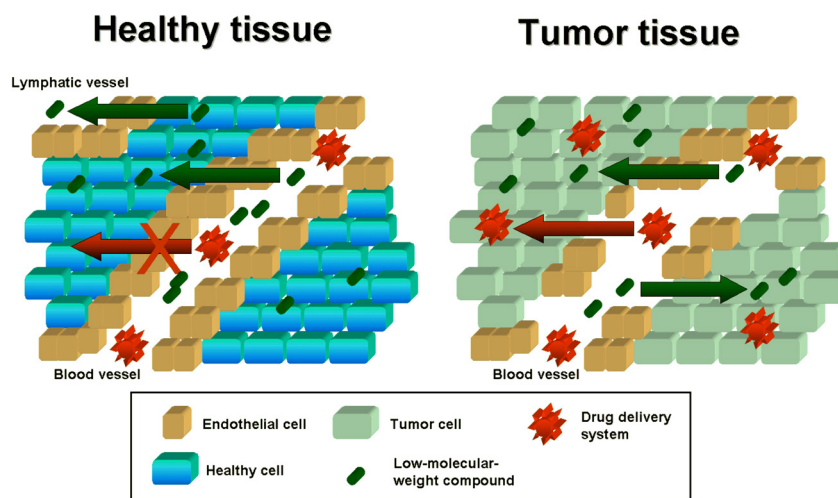
Figure 1. The enhanced permeation and retention (EPR) effect.
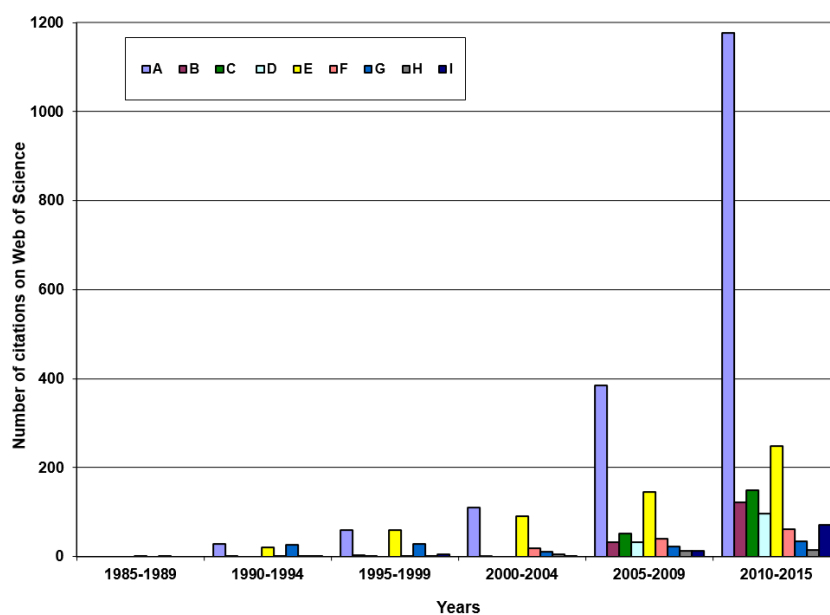
Figure 2. Number of citations in the course of time on the main topics of application of nanoscience in nuclear medicine (according to Web of Science). A: (nanoparticles or colloid) AND (tumor OR tumour) AND (radio* OR PET OR SPECT OR scintigraphy); B: micelle* AND (tumor OR tumour) AND (radio* OR PET OR SPECT OR scintigraphy); C: quantum dot* AND (tumor OR tumour) AND (radio* OR PET OR SPECT OR scintigraphy); D: (nanotubes or nanodiamonds) AND (tumor OR tumour) AND (radio* OR PET OR SPECT OR scintigraphy); E: liposomes AND (tumor OR tumour) AND (radio* OR PET OR SPECT OR scintigraphy); F: dendrimer* AND (tumor OR tumour) AND (radio* OR PET OR SPECT OR scintigraphy); G: (reticuloendothelial OR (bone marrow AND liver AND spleen)) AND (colloid* OR nanoparticles) AND (radio* OR PET OR SPECT OR scintigraphy); H: (nanoparticles OR colloid) AND radiosynovectomy; I: (nanoparticles OR colloid) AND brachytherapy.

2. Polymer carriers of anticancer drugs and radionuclides

2.1 The pH-activated water-soluble systems

The pH-value of blood plasma and interstitial body fluid is maintained to be exactly 7.4[26]. However, there exist compartments with significantly different acidity in the organism. The endosome and lysosome content is also more acidic than blood plasma (pH 5.5 – 6.5 for early endosomes and > 5.0 for late endosomes and lysosomes[27,28]). Pathological phenomena connected with high metabolic activity of certain (especially of solid tumor[29,30]) tissues generally lower the local pH, because rapidly metabolising cells deplete oxygen supply and thus make such tissue hypoxic. Under hypoxic metabolism the cells switch to anaerobic lactic acid-producing metabolism and the lactic acid formed acidifies the tissue to pH typically < 7 and sometimes as low as 6.0 – 6.5[29]. Therefore systems that are relatively stable in bloodstream (pH 7.4), but release the active cargo under mildly acidic conditions of solid tumor tissue possess significant benefits for targeted delivery to cancer tissue. We therefore decided to utilize this physiological specifity of cancer tissue for controlled release of chemical drugs and radiolabeled compounds in the target tumor tissue.

Radioactive decay of some radionuclides produces shower of Auger electrons, which are potent ionizing radiation within their very short range in living tissue (typically tens of nanometers). Therefore they must be brought to DNA-containing cell compartments and preferentially directly to DNA to be fully biologically effective.[10,11] We used them for triple-targeting approach (first targeting: passive accumulation of the polymer-based system targeting into tumor tissue due to EPR effect; second targeting: pH-controlled release of intercalator-bound Auger electron emitter in slightly acidic tumor tissue or endosome; third targeting: into DNA in cell nucleus and mitochondria by the intercalator) minimizing radiation burden of healthy tissues (see **Figure 3**).
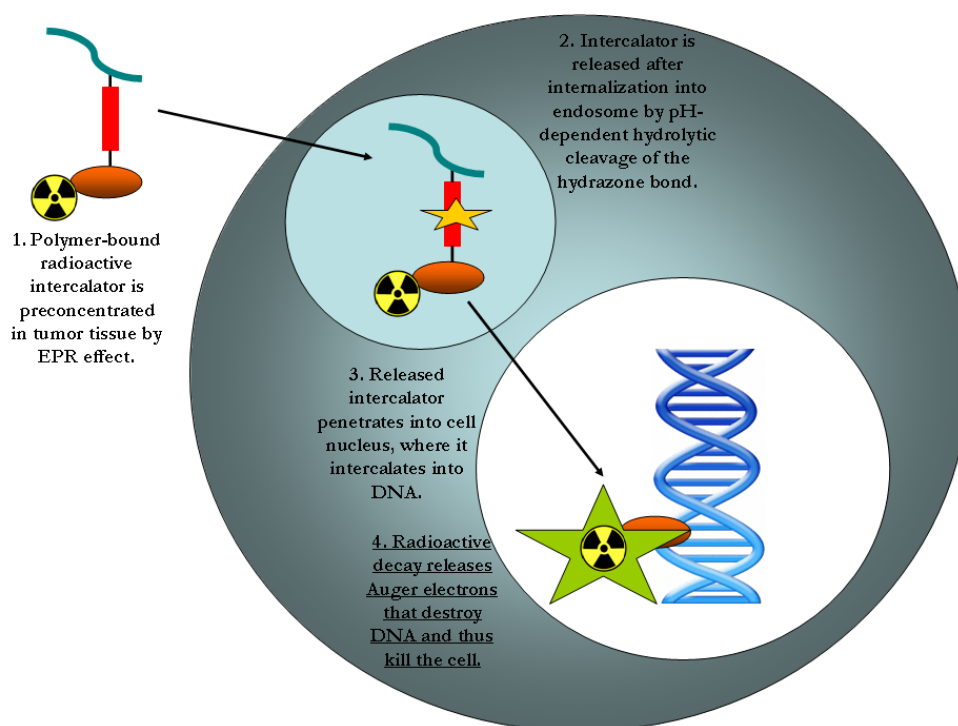
Figure 3. Scheme of the triple-targeted Auger electron emitter delivery.

To optimize the second two targeting steps, we first prepared two model systems having intercalator (either acridine-type[31] or ellipticine-type[32-34]) bound by acid-cleavable bond to polymer.

Acridines are potent DNA-intercalating anticancer agents with high *in vivo* anticancer effectiveness, but also severe side effects. We synthesized five 9-anilinoacridine-type drugs and their conjugates with biocompatible water-soluble hydrazide polymer carrier.[31] All of the synthesized acridine drugs retained their *in vitro* antiproliferative properties. Their polymer conjugates were sufficiently stable at pH 7.4 (model of pH in blood plasma) while releasing free drugs at pH 5.0 (model of pH in endosomes) (see **Figure 4** for the optimized case).
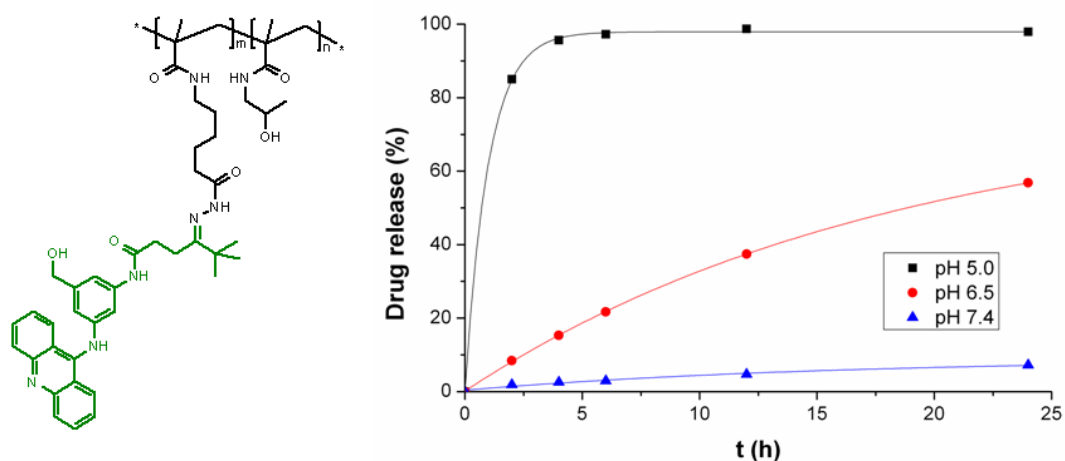

Figure 4. The optimized acridine-type conjugate: structure (left) and pH-dependent release rate (right).

After internalization of the conjugates, the free drugs were released and were visible in cell nuclei by fluorescence microscopy. Their intercalation ability was proven using a competitive ethidium bromide displacement assay. We then synthesized and characterized model drug conjugates with hydrazide bond-containing poly[*N*-(2-hydroxypropyl)methacrylamide] differing in the chemical surrounding of the hydrazone bond-containing spacer to find structure-drug release rate relationships (see **Figure 5** and **6**)[34]. The conjugate selected for further studies was the ellipticine-type **5a** (see **Figure 6**) and shows negligible drug release in a pH 7.4 buffer but releases 44 % of the ellipticinium drug within 24 h in a pH 5.0 phosphate saline buffer. The ellipticinium drug retained the antiproliferative activity of the ellipticine. We then optimized the triple-targeted polymer radionuclide delivery system for the ellipticine-derived intercalator, which contains radioisotope ^{125}I with high specific radioactivity (63.2 GBq/mg).[33] The active compound is a potent intercalator, as shown with direct titration with a DNA solution, and readily penetrates into cell nuclei, as observed by confocal microscopy. Its polymer conjugate is internalized into endosomes and releases the radioactive intercalator, which accumulates in the cell nuclei. *In vivo* experiments on mice with 4T1 murine breast cancer resulted in a statistically significant increase in the survival of mice treated with the polymer radioconjugate (see **Figure 7**). The free radiolabeled intercalator was also shown to be effective, but it was less potent than the polymer conjugate.
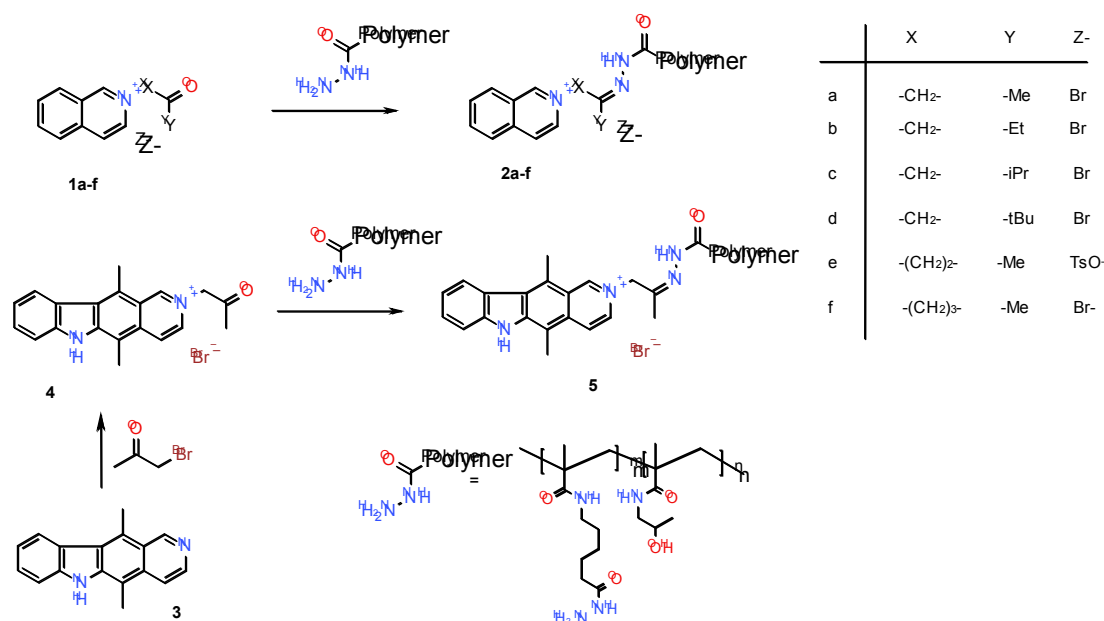
Figure 5. Syntheses and structures of the ellipticine-type polymer conjugates.
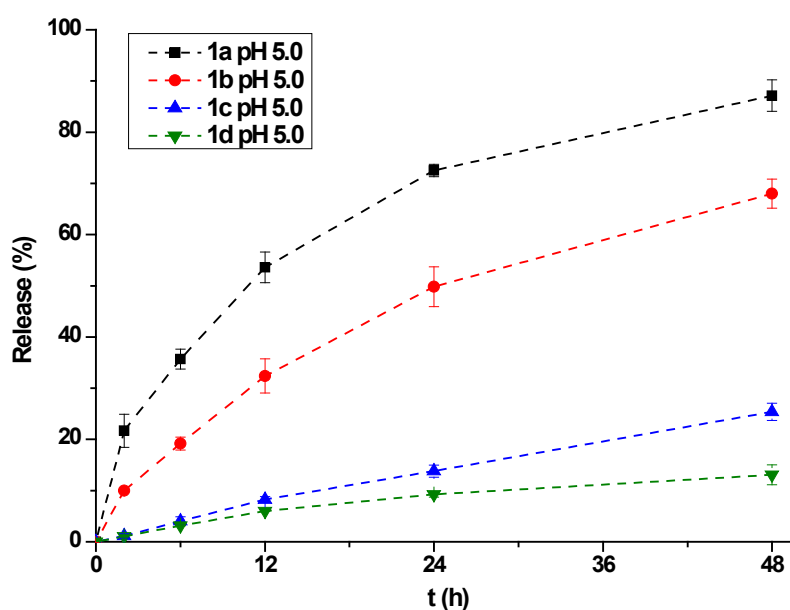
Figure 6. The *in vitro* release rate of derivatives **1a-d** from their conjugates **2a-d** in phosphate buffered media at 37°C. The release in the pH 7.4 phosphate buffer was less than 2% after 24 h (data not shown).

In an other area of applications, we have shown that water-soluble polymer carriers with radionuclides may be targeted into bone metastases by attachment hydroxybisphosphonate moieties onto polymer, which has high affinity to hydroxyapatite, the main mineral component of bone[35]. This is a promising system for applications in theranostics of both primary bone cancer and bone metastases.
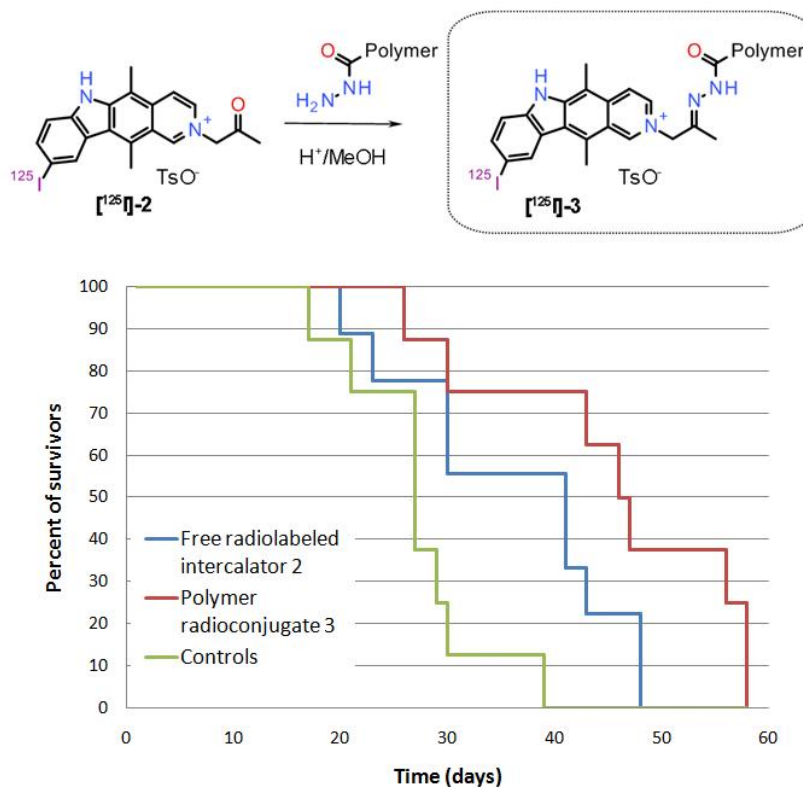
Figure 7. The optimized triple-targeted system for the delivery of Auger electron emitters and its biological efficacy *in vivo* on an 4T1 murine breast cancer model.

2.2 Micellar pH-responsive delivery systems

Polymer micelles of amphiphilic block or graft copolymers have several advantages for the construction of anticancer drug delivery systems[25,36]. Above all, the hydrophobic micelle core is protected against unwanted interactions in organism, which may lead to, e.g., deposition in reticuloendothelial system. Micelles may thus in the simplest application serve just as solubilizers of poorly water soluble hydrophobic drugs, which are dissolved in the micelle core. However, micellar drug delivery systems with core-dissolved drugs have the principal disadvantage that the drug release rate is very hard to control, because it is determined by numerous factors, such as, partition coefficient of the drug between the core and surrounding aqueous milieu, critical micelle concentration (see below), core crystallinity, drug diffusion rate or whether the glass point temperature of the micelle core is below or above body temperature (37 °C), etc.[36-38] Unfortunately, in many cases the drug release rate under non-equilibrium conditions is very fast.[38] One possibility to solve the problem with fast drug release is the use of micelles with the drug covalently bound to the micelle core by a biodegradable bond, so the drug release rate is driven by the degradation of the bond between the carrier and the drug. This allows fine-tuning the drug release rate; however it requires the presence of suitable functional groups both on the drug and on the polymer carrier. We applied this approach to construct micelles with doxorubicin[39-42] and ellipticine[43], respectively, covalently bound to the micelle core by a pH-sensitive hydrolytically labile bond.

We reported on synthesis, physico-chemical properties and biological and pharmacological properties of the polymeric micellar conjugate containing doxorubicin (DOX) covalently bound *via* hydrolytically cleavable hydrazone bond to the micelle core (see **Figure 8**).[39-42] We started the synthesis from the well-defined precursor poly(ethylene oxide)-*block*-poly(allyl glycidyl ether).[44] Drug loading 17 wt. % was achieved and the system may be prepared in freeze-dried lactose-stabilized form. The unimers after DOX release possess molecular weight below the renal threshold and are therefore eliminable from organism by renal filtration. The system had a very low systemic toxicity (almost 20 times lower than free DOX) and slow blood clearance ($T_{1/2}$ = 46 h) as measured using ^{111}In radiolabeling. Significant accumulation of tested micelles within the tumor was confirmed by fluorescent whole body imaging (see **Figure 8**, where is confirmed the colocalization of doxorubicin fluorescence with the fluorescence of fluorescent protein-transfected tumor cells) and *ex vivo* measurements after ^{111}In radiolabeling. Our micellar system showed promising therapeutical activity against established murine EL-4 T-cell lymphoma. It was found that it is able to completely cure about 75 % of tumor bearing mice (with either doses of 1 x 150 mg DOX kg^{-1} or 2 x 75 mg DOX kg^{-1}, administered *i.v.*) (see **Figure 9**). Moreover, the treatment with micelles induced in cured mice tumor-specific resistance. Up to 80 % of these mice survived re-challenge with original tumor cells (but not with distinct tumor cells). We applied this approach also for the ellipticine-based system using the chemistry developed for the triple-targeted Auger electron emitter delivery system (see above).
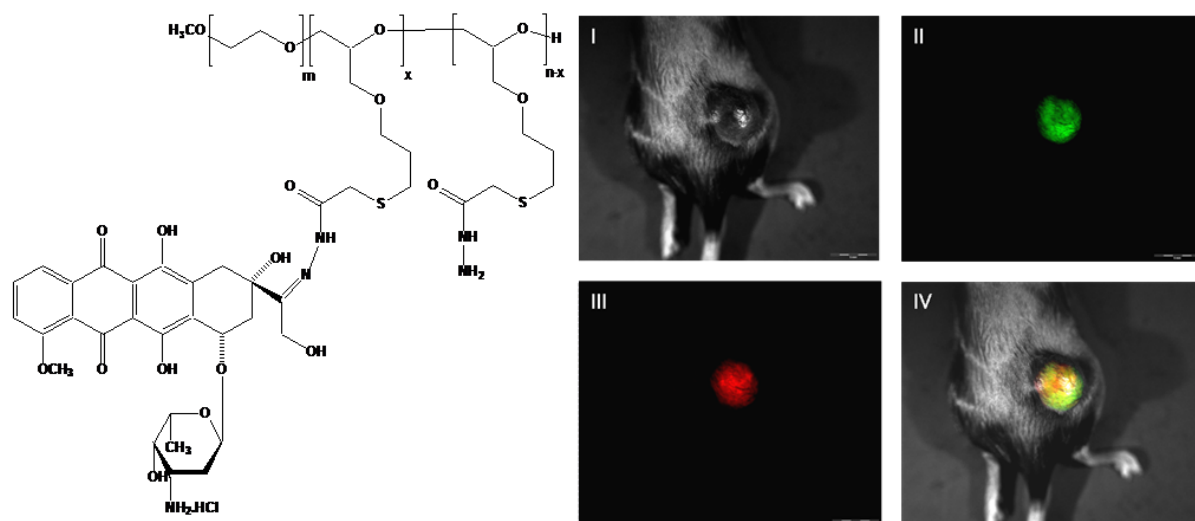
Figure 8. Structure and tumor accumulation of micellar doxorubicin conjugate; I - brightfield, II - fluorescence of the EGFP protein – transfected tumor, III - fluorescence of doxorubicin, IV - overlap II + III.

Also the micelle core may be constructed from a pH-responsive polymer. Therefore, we synthesized and studied in detail the system based on hydrophobic poly(*N*-methacryloyl amino acids) bearing the carboxyl group on every monomeric unit. These polymers form surfactant-stabilized nanoparticles upon a pH-change. Namely, the behavior of pH-responsive polymers poly(*N*-methacryloyl-L-valine) and poly(*N*-methacryloyl-L-phenylalanine) in the presence of non-ionic surfactant Brij98 have been studied.[45-47]
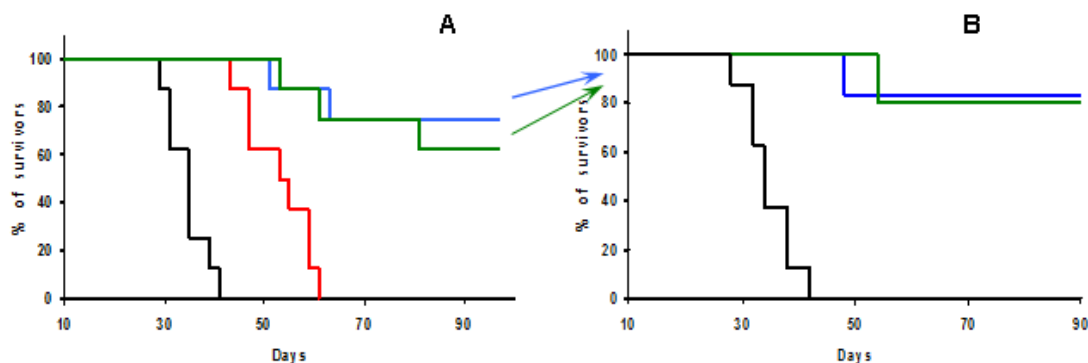
Figure 9. **A)** Survival of mice bearing EL-4 T-cell lymphoma and treated with micellar DOX (black line - Controls; red line – 1 x 75 mg (DOX eq.) kg^{-1}; blue line – 2 x 75 mg (DOX eq.) kg^{-1}; green line – 1 x 150 mg (DOX eq.) kg^{-1}). **B)** Cured mice were re-transplanted with a lethal dose of the same cancer cells and left without treatment (black line - Controls; blue line - mice previously cured with 2 x 75 mg (DOX eq.) kg^{-1}; green line - mice previously cured with 1 x 150 mg (DOX eq.) kg^{-1}). Displayed data represent a typical result of independent experiments (P < 0.05).

Pure polymers phase separate in an acidic medium at critical pH 3.7, 5.5 and 3.4, correspondingly. The addition of the surfactant prevents phase separation and promotes rearrangement of polymer molecules. It was established, that the nature of interaction between polymer and surfactant depends on the amino acid structure in the side chain of a polymer. This effect has been studied by dynamic light scattering, isothermal titration calorimetry, electrophoretic measurement and small-angle neutron scattering. Thermodynamic analysis reveals the endothermic effect of reaction in poly(*N*-methacryloyl-L-valine)/Brij98 system, whereas the strong exothermic effect was observed for poly(*N*-methacryloyl-L-phenylalanine)/Brij98 system. Application of regular solution theory for the analysis of experimental enthalpograms indicates the presence of dominant hydrophobic interaction between the poly(*N*-methacryloyl-L-valine) and Brij98 and specific interactions for the poly(*N*-methacryloyl-L-phenylalanine)/Brij98 system. Electrophoretic and dynamic light scattering measurements justify the applicability of the theory in this case. The specific interactions can be determined as a hydrogen bonds, that were formed between the carboxylic groups of the polymer and oligo(ethylene oxide) head-groups of the surfactant. It was established that the difference in polymer-surfactant interactions for poly(*N*-methacryloyl-L-valine) and poly(*N*-methacryloyl-L-phenylalanine) polymers leads to the different structure of polymer-surfactant complexes. The pearl-necklace complexes and "core-shell" structure were revealed by small-angle neutron scattering for poly(*N*-methacryloyl-L-valine)/Brij98 and poly(*N*-methacryloyl-L-phenylalanine)/Brij98 systems, respectively as a manifestation of different types of interactions in polymer-surfactant systems. The revealed results may help to construct, e.g., new pH responsive site-specific micellar drug delivery systems or pH-responsive membrane disrupting agents. This idea was further extended with the use more hydrophobic and sterically hindered poly(ω-*N*-methacryloyl dipeptides). These copolymers are easily radiolabelable after copolymerization of *N*-methacryloyl-L-tyrosinamide allowing tracking fate of the system by noninvasive imaging.

Numerous polymers show interesting temperature dependence of solubility in aqueous milieu – they are not soluble at low temperature and become soluble above certain critical temperature, or are soluble at low temperature and above certain temperature phase separation occurs.[48] The second case (the polymer is soluble at low temperature and precipitates at higher temperature) is more commonly exploited in micellar systems proposed for drug delivery.[13,48-50] This behaviour is caused by competition of hydrogen bonding between the polymer and water and chain solvation (forcing the polymer to dissolve) and hydrophobic interactions, forcing the polymer to aggregate.[13,48-50] Such partly hydrophilic-partly hydrophobic polymers are thus soluble at low temperature where solvation prevails. Above the lower critical solubility temperature (LCST), when solvation, decreasing with increasing temperature, cannot compensate hydrophobic interactions, the polymer chain is dehydrated[51], the polymer coil collapses (coil-to-globule transition[51]), the polymer becomes hydrophobic and then phase separation occurs within a relatively narrow temperature range. The LCST is the minimum in the dependence of phase separation temperature on concentration, so always phase separation temperature $\geq$ LCST. The phase separation temperature is often expressed as „cloud point temperature" (CPT), the temperature at which the solution becomes visibly turbid.

The important feature of polymers with LCST is that their CPT may be predictably adjusted to the needed value by increasing/decreasing the hydrophobicity of the polymer chain by copolymerization of suitable monomers.[13,48] The increase of hydrophobicity increases hydrophobic interactions among the polymer chain and decreases the CPT; on the other hand, monomers more hydrophilic than the main monomer increase the CPT by the increase of chain solvation and suppression of hydrophobic interactions. In many cases, this decrease/increase in CPT is a linear function of monomeric unit composition of the polymer.[13,49,50]

Block and graft copolymers with one thermoresponsive block and one or more hydrophilic blocks, which are readily soluble below CPT of the thermoresponsive block as molecular solutions. When the temperature is raised up above CPT of the thermoresponsive block, core-shell micelles are formed with core consisting of the phase separated thermoresponsive blocks surrounded by a corona of the hydrophilic blocks.[52-55] A similar type of thermoresponsive nanoparticles can be prepared by heating of solution of thermoresponsive polymer in the presence of surfactant.[56] Temperature-caused self-assembly of these micelles is diffusion driven and these micelles have, due to thermodynamic reasons, very narrow size distribution.

A very important advantage compared to other types of nanoparticles is that these are much easier to prepare (just heating of an aqueous solution of the polymer), no dialysis or other additional treatments are required. Another advantage is that the micelle dissolution into individual polymer chains may be controlled by the increase in polarity of the thermoresponsive block, e.g., by hydrolysis of relatively hydrophobic hydrolytically labile moieties in the thermoresponsive block into a more polar functionality.[53,54,56,57] This increase in polarity increases the CPT and if the original thermoresponsive block had the CPT below body temperature and after degradation its CPT is shifted above body temperature, the micelles disintegrate.

Therefore, we prepared novel polymer micelles by self-assembling thermoresponsive poly[*N*-isopropylacrylamide–*graft*–*N*-(2-hydroxypropyl)methacrylamide] copolymers with

hydrolytically degradable *N*-glycosylamine groups between the polymer blocks (see **Figure 10**) as carriers to deliver diagnostic and therapeutical radionuclides into solid tumors.[12,58]
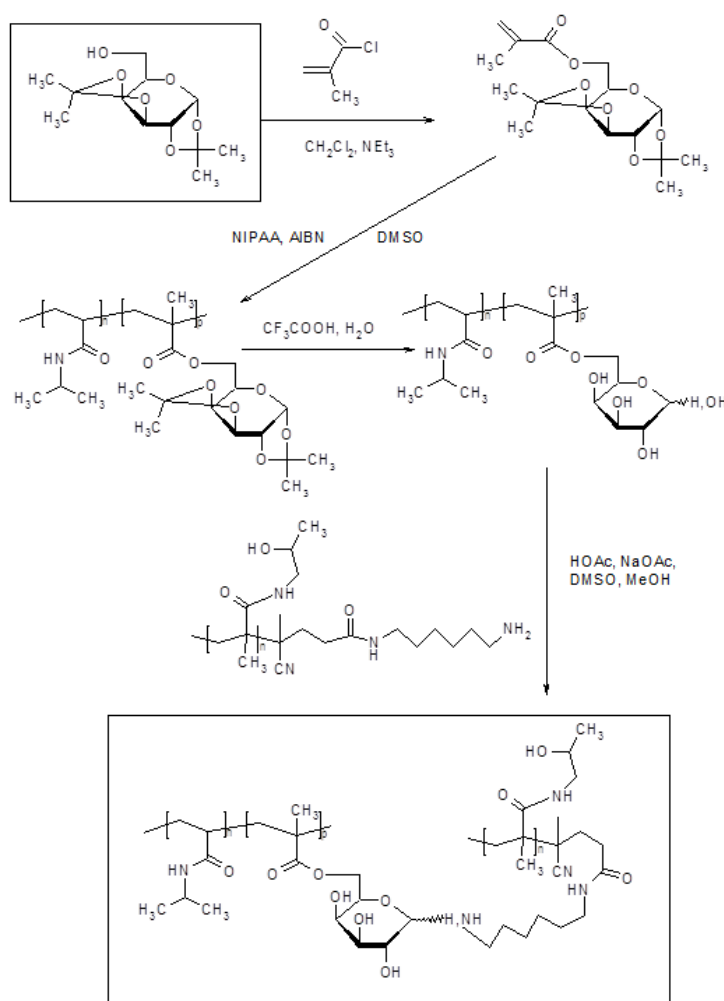

Figure 10. Synthesis of poly[*N*-isopropylacrylamide–*graft*–*N*-(2-hydroxypropyl) methacrylamide].

The micelles were formed by fast heating of an aqueous solution of the copolymer to 37 °C. They had the hydrodynamic diameter 128 nm (obtained by dynamic light scattering) and slowly degraded during incubation in aqueous buffer pH 7.4. Labeling with both ^{131}I and ^{90}Y proceeded with high yields (> 85 %). The nonlabeled polymers were not cytotoxic for any of the tested murine and human cell lines and showed significant tumor accumulation (> 7 tumor to muscle ratio after 24 h) due to the EPR effect. However, we observed some liver accumulation probably due to the galactosyl moiety on the thermoresponsive block. Therefore, we decided to prepare the next generation of micelles that would not contain the galactosyl moiety and would be poly(2-alkyl-2-oxazoline)-based.[59]

We have thus synthesized ABA triblock copolymers poly[2-methyl-2-oxazoline – *block* – (2-isopropyl-2-oxazoline – *co* – 2-butyl-2-oxazoline) – *block* – 2-methyl-2-oxazoline], where the poly(2-methyl-2-oxazoline) terminal blocks are hydrophilic and the central block is thermoresponsive. These polymers are molecularly dissolved in aqueous milieu below the cloud point temperature of the thermoresponsive central block and above CPT they form nanoparticles with diameter ~ 200 nm (see **Figure 11**).
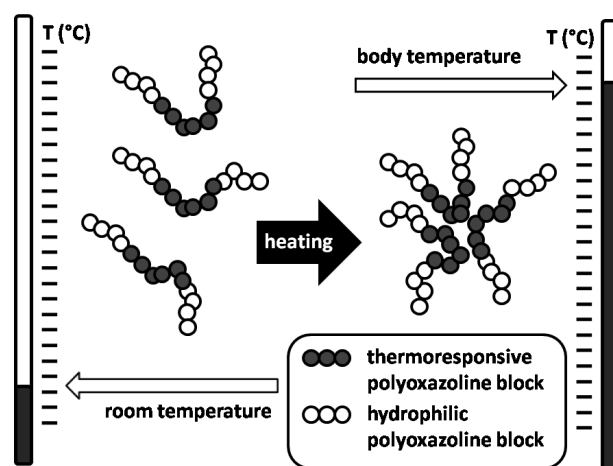
Figure 11. Temperature-controlled self-assembly of poly[2-methyl-2-oxazoline – *block* – (2-isopropyl-2-oxazoline – *co* – 2-butyl-2-oxazoline) – *block* – 2-methyl-2-oxazoline] ABA triblock copolymers into micelles.

Phenolic moiety introduced into the copolymer by thiol-click chemistry (after small part of 2-butyl-2-oxazoline was replaced with 2-(3-butenyl)-2-oxazoline) allowed radionuclide labeling with iodine-125 with good yield with sufficient *in vitro* stability under model conditions (phosphate buffered saline, 37 °C).

Easier way to thermoresponsive nanoparticles than self-assembly of (multi) block or graft copolymers in aqueous milieu is to dissolve a thermoresponsive copolymer in the presence of a biologically acceptable surfactant (e.g., Pluronic F 127) and heat up the solution. Well-defined nanoparticles with thermoresponsive polymer core and hydrophilic surfactant corona are then formed. We therefore also studied the poly(*N*-isopropyl acrylamide) and poly(2-alkyl-2-oxazoline)-based surfactant-stabilized nanoparticles of this type as carriers for iodine radionuclides, where we have demonstrated potential of this concept for variety of construction materials.[60]

2.4 Thermoresponsive polymers for local brachytherapy

Most currently studied drug delivery systems for radiotherapy are based on an active seeking agent, e.g. an antibody[61,62], which acts at the same time as a radionuclide carrier. Sometimes, e.g. in the case of radiosynovectomy or brachyradiotherapy, positioning of a radionuclide carrier in well-defined and limited body volume is the method of choice. These radionuclide applications concern mainly treatment of joints damaged by inflammation or arthrosis by radiosynovectomy[63], which reduces pain and suppresses inflammation, but also intratumoral applications like brachytherapy[64,65] or local adjuvant radiotherapy after surgical removal of malignant tissues.

Brachytherapy is most often carried out with closed emitters which are surgically implanted into the target site. After decay of the radionuclide the emitter is left in the application site or surgically removed. The advantage of brachytherapy is in high radiation doses to the tissue in the nearest neighbourhood while keeping the whole-body doses low, however, surgery is essential in such cases.[64-67] Alternatively, microspheres or nanoparticles, which are labeled, are applied by injection.[63] Their size itself prevents them from escaping

from the application site. There is another approach possible. Thermoresponsive polymers allow for labeling of the carrier in a real solution at usual laboratory temperatures (e.g. < 25 °C). The carrier is then converted to insoluble form only after application to body of higher temperature, usually above 36 °C.

Combining radionuclides as curing agents with thermoresponsive polymers as their carriers, one can get a new promising therapeutic tool. Namely, such a polymer labeled with suitable beta emitter with the CPT closely below the body temperatures allows for depositing rather high doses directly in the application site, because the precipitation occurs almost immediately and with high efficiency. The solution of the polymer may be applied by injection, which is significantly easier than surgical implantation. Since the precipitate re-dissolves very slowly and (meth)acrylamide polymers usually do not cause any significant immune response[68], the radioactivity stays on the application site for enough long time with respect to the radionuclide half-life. Moreover, the size of the polymer molecules can be adjusted below the renal threshold [ca 45 kDa for methacrylamide polymers][69,70], so that the re-dissolved polymer is rapidly excreted via urine. One of the key factors is then the stability of the radioactive labeling.

Drug delivery systems based on thermoresponsive polymers may therefore serve as suitable carriers for local radiotherapy. We have designed and synthesized a radioiodine labelable thermoresponsive polymer for such use.[50,71,72] The polymer was synthesized by copolymerization of *N*-isopropylacrylamide with *N*-methacryloyl tyrosinamide in tetrahydrofuran, and then labeled by [131]I. The solution of this labeled polymer in dimethylsulfoxide (4.4 MBq/ml; 1.8 wt % polymer) was applied to femoral muscle of male Balb/C mice (50 µl per animal). The biodistribution and excretion of radioactivity was followed in 2 h and 1, 7, 14, 28 and 42 d post injection (n = 6 per time point).

As expected, the labeled polymer was left on the application site (ca 90 % 2 h post injection), decreasing slowly to ca 80 % within 14 days (**Figure 12**). At 28 days post injection, ca 70 % of the injected activity was still found on the application site, decreasing to ca 60 % at 42 days.
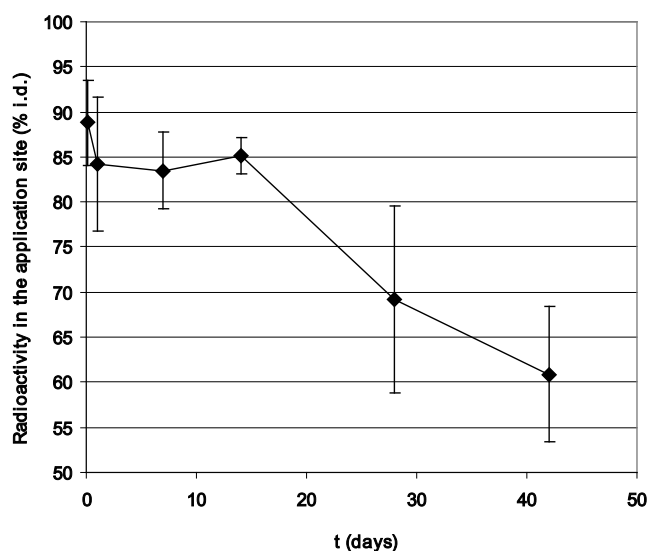
Figure 12. Retention of the ^{131}I-labelled thermoresponsive polymer in the femoral muscle of the Balb/C mice (number of animals per group *n*= 6); error bars mean standard deviation, % i.d. = percent of the injected dose. All the presented data are decay-corrected.

No organ specific accumulation of the radioactivity released from the application site, including thyroid, was observed. The majority of the released radioactivity was excreted via urine and faeces. This preliminary study[71] suggests that thermoresponsive polymers could be used as an effective delivery system for localized radiotherapy.

Pronounced dose-dependent tumor growth reduction was achieved by single dose of injectable intratumoral brachytherapy with ^{131}I-labeled thermoresponsive polymer [poly(N-isopropyl acrylamide)] in murine xenograft model (PC3 human prostate adenocarcinoma) (see **Figure 13**).[72] Two doses the radionuclide were used, 2 MBq/mouse and 25 MBq/mouse. The higher dose caused gradual tumor volume reduction and 2 of 6 mice from this group were cured. The lower dose caused tumor growth retardation only. In both cases there were no signs of inflammation. The effects of both doses were statistically significant compared to untreated controls. Such injectable system should keep advantages of brachytherapy while making the drug administration easier and less invasive (injection instead of implantation), patient-tailored (splitting of doses into several depots) and bioerodable in the course of time.
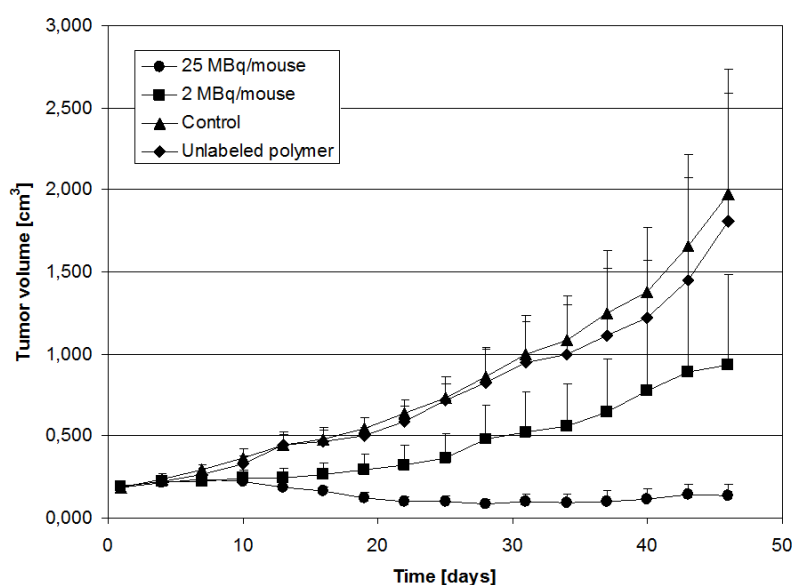
Figure 13. Tumor volume as a function of time after application of the radiolabeled polymer; n = 6.

However, there is a need to fine-tune depot degradation rate to the half-life of the radionuclide used. Therefore, an advanced version of such system was developed.[13] In this system a radionuclide complex is entrapped in a thermoresponsive polymer locally precipitated at body temperature after injection of a polymer - complex solution into the tissue where a therapeutic effect is required. The lifetime of the system is controlled by the rate of polymer hydrolysis, its dissolution and elimination from the body. The thermoresponsive polymer with the cloud temperature below body temperature is based on copolymers of N-isopropylmethacrylamide with a methacrylamide-type comonomer containing hydrophobic n-alkyls of three different sizes (C_3, C_6 and C_{12}) bonded by a hydrolytically labile hydrazone bond.[13] Hydrolysis of hydrazone bond results in a copolymer soluble at body temperature. The copolymer containing 27.5 mole-% of the comonomer with the C_6 moiety, which was chosen for further study, has the CPT 22 °C and its phase separation is complete at 34 °C.

Polymer dissolution is complete within 48 h at both pH 5.0 and 7.4. The model therapeutic radionuclide, ^{64}Cu, in the form of its hydrophobic chelate bis(quinolin-8-olato-N,O) [^{64}Cu]copper, is efficiently kept hydrophobically entrapped in the phase-separated polymer until the dissolution by hydrolytic degradation is completed.

Some antiproliferative drugs show significant synergic effect with ionizing radiation. We prepared a polymer thermoresponsive system for synergic chemoradiotherapy exploiting this phenomenon. The system is based on injectable thermoresponsive polymer bearing the radionuclide and the hydrophobic moiety doxorubicin.[14,73] In the system DOX serves as an antiproliferative agent with known synergic effects with ionizing radiation and the hydrophobic moiety controlling bioerosion and elimination of the system at the same time. DOX is bound to the polymer carrier by a hydrolytically labile N-glycosylamine bond. Hydrolysis of the N-glycosylamine bond thus controls the DOX release and dissolution of the system in model aqueous milieu. DOX is slowly released during incubation in aqueous milieu at 37 °C causing complete dissolution of the bioerodable polymer within ca 2 weeks. The model radionuclide ^{125}I, bound to a small amount of poly(N-isopropylacrylamide-co-N-methacryloyl tyrosinamide), was retained in the separated phase and also slowly dissolved during the incubation.

2.5 Hybrid glycogen-based carriers

Glycogen (GG) is the main animal D-glucose storage polysaccharide.[74] It is present mainly in liver[74] and muscles[75], the average content in adult human organism is highly dependent on nutritional status and is roughly around 100 g.[76] GG is a hyperbranched poly(D-glucose)[74] with D-glucose units connected with each other by α(1→4) bonds, branching is *via* the 6 position on the D-glucose unit in the main chain.[77] In fact GG is a natural high-molecular-weight (typical M_w several MDa, hydrodynamic diameter ca 50 nm, i.e. suitable size for the EPR effect-based solid tumor accumulation) fully biodegradable dendrimer structurally related to dextrin, but significantly more branched. GG, in contrast to dextrin, is relatively inert to amylases (which are omnipresent in bloodstream), but is intracellularly degraded[74,78] by glycogen debranching enzymes. This means that it should be more stable after intravenous administration in bloodstream than dextrin, which is degraded too rapidly. Glycogen´s molecular weight is well-above renal threshold, so it cannot be directly eliminated by kidneys without previous biodegradation, but it should be rapidly degraded into D-glucose after internalization into cells. This would be an advantageous behavior for a carrier for numerous applications such as in magnetic resonance imaging (MRI), single photon emission tomography (SPECT), positron emission tomography (PET), drug delivery.[79]

We described chemical modifications, physico-chemical and biological behavior of glycogen from oyster directed towards functionalization with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid gadolinium(III) complex (DOTA-Gd, a MRI contrast agent) and Dyomics 615, a fluorescent label (see **Figure 14**).[80,81]

The conjugate would thus be suitable for multimodal (MRI + fluorescence) imaging. As proven in *in vitro* cell culture by fluorescence microscopy, the conjugate is uptaken by cells and the cells handle it as their own glycogen including typical intracellular localization in grana in cytoplasm. The fate of the conjugate intravenously applied in healthy mice was followed by MRI. There was no significant accumulation of the contrast agent in reticuloendothelial system or kidneys. A gradual accumulation of degradation fragments was

clearly seen in urine bladder. To the best of our knowledge this is the first such use of glycogen.

Hybrid copolymers comprising natural and synthetic building blocks are becoming an increasingly important class of functional materials.[82-84] They combine the sustainable environment-friendly nature, biodegradability and structure of natural polymers with the processability, tailorability and economy of synthetic polymers. These materials are homogeneous on a macroscopic scale but can be designed to undergo micro- or nanophase separation / self-assembly, that generates materials that can be useful as, inter alia, delivery systems for bioactive species or tissue engineering scaffolds.[85-87]
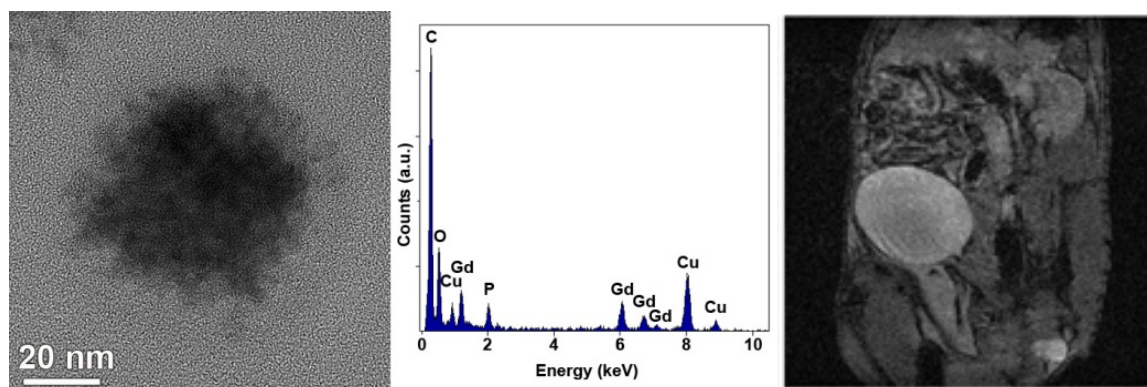

Figure 14. (Left) Bright field TEM image of a typical glycogen nanoprobe; (center) energy-dispersive X-ray spectroscopy (EDX) spectrum taken from several particles simultaneously showing a large Gd content. The Cu signal arises from the sample holder; (right) The MRI image of healthy mouse after administration of the glycogen nanoprobe, accumulation of degradation fragments in urine bladder is clearly visible as proof of biodegradability.

We have developed a completely new concept of biocompatible hybrid copolymers based on glycogen-*graft*-poly(2-alkyl-2-oxazolines).[88] Both glycogen and poly(2-alkyl-2-oxazolines) are biocompatible and therefore the biocompatibility of their hybrids is expected. The poly(2-alkyl-2-oxazolines) were polymerized in a living manner with initiating alkylating agent enabling to choose one end and molecular weight adjustable by the initiator: monomer ratio. The living cationic end after this polymerization was used to alkylate alcoholate of glycogen forming glycogen-graft-poly(2-alkyl-2-oxazolines) (see **Figure 15** for scheme).

This easy two-step one-pot synthesis creates a conceptually new versatile nanostructured platform of hybrid sphere-shaped biodegradable polysaccharide dendrimer-based copolymers with thermoresponsive behavior adjustable in a wide range by poly(2-alkyl-2-oxazolines) chain length, grafting density and reactive chain ends. Special focus is therefore given to the *in vitro* temperature-dependent behavior of these materials in solution. Such thermoresponsive polymers are potentially suitable as drug releasing depots, self-organized nanostructured tissue engineering scaffolds or materials for injectable brachytherapy, where widely adjustable properties, temperature-responsivity, biodegradability and biocompatibility are advantageous. As confirmed by light and X-ray scattering as well as by cryo-transmission electron microscopy, the grafted dendrimer structure allows easy adjustment of the cloud point temperature, its concentration dependence and of the nanostructure of the self-assembled phase separated polymer by crosstalk among graft composition, graft length and grafting density in a very wide range.

These nanoprobes were compared to the non-biodegradable fluorescent nanodiamonds covered by biocompatible *N*-(2-hydroxypropyl)methacrylamide copolymer corona synthesized by radical „grafting from" polymerization containing glioma-targeting cyclic RGD (Arg-Gly-Asp motif) peptide.[89] We have shown selective and highly effective targeting of glioblastoma cells (U87MG) expressing integrin $\alpha_v\beta_3$ using polymer-modified fluorescent nanodiamond particles bearing cyclic RGD peptide.
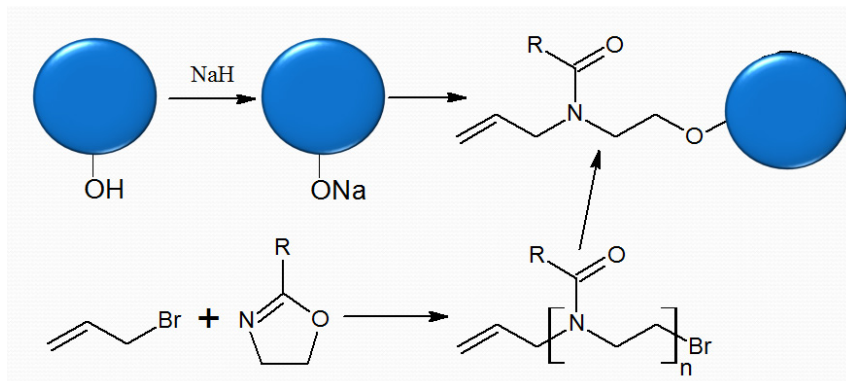
Figure 15. Scheme of synthesis of glycogen-*graft*-poly(2-alkyl-2-oxazolines).

## 3 Radiohalogenation of polymer and nanoparticle carriers – iodine and astatine radionuclides

The easiest and most accurate way to study the biodistribution of compounds (including polymer drug conjugates) *in vivo* in real time is the use of radiolabeled systems.[90,91] However, for polymer prodrugs, it is especially important to note that radioimaging essentially allows the fate of the radiotracer, which may be attached to the drug, the carrier or both, to be followed; double-labeled systems using radionuclides with different spectral nuclear properties allow the fates of the drug and carrier to be simultaneously followed.[92] Radiolabeling may be performed through either chelating approaches (e.g., metal radionuclides, e.g., ^{99m}Tc and ^{177}Lu)[93] or covalent approaches (e.g., ^{18}F and iodine radionuclides)[94]. In particular, iodine radionuclides are advantageous due to the wide selection of available radionuclides with various half-life times $T_{1/2}$ and decay modes.[94] Namely, ^{131}I ($T_{1/2}$ = 8.04 days) is a theranostic β^- and γ-emitter that is suitable for both therapy. SPECT ^{125}I ($T_{1/2}$ = 60.14 days) decays by electron-capture and can be utilized in biological assays, SPECT and endoradionuclide therapy.[32] ^{124}I ($T_{1/2}$ = 4.18 days) is a relatively long-lived positron emitter that is suitable for PET[95], and ^{123}I ($T_{1/2}$ = 13.13 h) is an electron capture emitter well suited for low-radiation-burden SPECT diagnostics.[96,97]

One difficulty in the synthesis may be that direct electrophilic radioiodination may be tricky for polymer systems because it is not fully compatible with several groups, including hydrazones, hydrazides, other reducing groups and DOX.[98]

We described for the first time a robust radioiodination protocol for the high-yield chemically stable radiolabeling of a hydrazone-type drug delivery system using DOX (which has synergic biological effects with ionizing radiation) as a model drug. This system also accounts for all structural and chemical obstacles to radioiodination that are common in such polymer drug delivery systems, making it an ideal model for the development of a universal protocol for the radioiodination of hydrazone-containing polymer conjugates. In particular, it

is crucial that the polymer radioiodination step be performed before the deprotection of the hydrazide and doxorubicin binding.

An alpha emitter [211]At is a prospective radionuclide for therapy of smaller tumors and metastases.[99-101] Nevertheless, its chemical properties together with the fact that it is always present in trace amounts make the labeling of its prospective carriers rather complicated. We studied a new class of possible astatine carriers—nanoparticle systems. Silver nanoparticles were chosen as carriers due to extremely high affinity of astatide to silver, which is even higher than that of iodide.[102] In order to reach high labeling yields and to protect the nanoparticles from being quickly scavenged by the immunity system, silver containing particles covered by poly (ethylene oxide) were developed and tested. An effect of the different reducing and oxidizing agents on the labeling yield was determined. It was found that the yields were almost quantitative and well reproducible under reducing conditions, but also in absence of any redox agent. The labeled nanoparticles were stable even in high surplus of competing chloride ions. Therefore, we developed a simple mix-and-use method of astatination of nanoparticulate carriers.
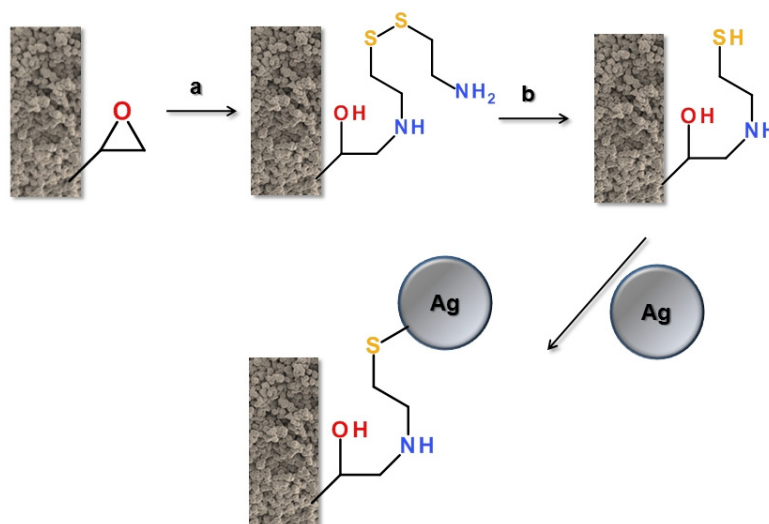
Figure 16. Preparation of monolithic column modified with silver nanoparticles. Reagents: (a) cystamine/NaOH; (b) NaBH$_4$/MeOH.

High affinity of heavy halogens iodine and astatine towards silver may be used in another way – to simply remove excess of radioiodide and astatide after radiolabeling of the system if silver nanoparticles, possessing inherently high active surface area, are immobilized on insoluble carrier. The insoluble carrier is then removed from the reaction mixture with the excess of radiohalogenides. We have developed two such materials, a monolithic high performance liquid chromatography (HPLC) column with immobilized silver nanoparticles[103] and polymer microparticles with silver nanoparticle coating[104] which may me removed after scavenging excess radioiodide by centrifugation.

We developed a macroporous monolithic column containing anchored silver nanoparticles and its use for the elimination of excess of radiodiodine from the radiolabeled pharmaceutical.[103] Poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) monolith was first functionalized with cystamine and the free thiol groups liberated by reaction with borohydride. In-house prepared silver nanoparticles were then attached via interaction with

the surface thiols (see **Figure 16**). The de-iodization process was demonstrated with the commonly used radiopharmaceutical *m*-iodobenzylguanidine labeled with radionuclide [125]I.

The work devoted to microparticles[104] is related to the design of anionic and cationic macroporous polymer microspheres based on poly(glycidyl methacrylate) (PGMA) obtained using a multistep swelling polymerisation. According to scanning electron microscopy, the microspheres were monodisperse in size and 4.2 µm in diameter. The presence of the carboxyl and amino groups in the PGMA-COOH and PGMA-NH$_2$ microspheres was confirmed by FT-IR spectroscopy. Capillary electrophoresis (CE) and pressure-assisted capillary electrophoresis (PACE) were used to study the electrophoretic behaviour of both types of microparticles and determination of their ζ potential using Smoluchowski modeling. Finally, silver-containing microspheres were prepared by reducing silver nitrate in the presence of the microspheres, and they proved effective for scavenging radioiodide ions from a model medium.

4   *Polymer chelates for nuclear medicine*

Although the use of iodine and astatine radionuclides as active component possesses numerous advantages especially in the development stage, we have proven that in certain cases their use is problematic, so we further concentrated on the use of chelates.

4.1 Polymer microparticles for radioembolization of liver tumors

Liver may be damaged and its function hampered by numerous malignant tumors, of primarily hepatic origin (mainly relatively common hepatocellular carcinoma, HCC) or by metastases of tumors originated elsewhere (colorectal carcinoma etc.), both with poor survival prognosis.[105-107] Normal liver tissue is supplied mainly from portal vein (which drains blood from digestive tract and contains nutrients uptaken from food), while typically strongly vascularized liver tumors take up nutrients and oxygen only from the hepatic artery. If 20 - 50 micron microparticles are injected into hepatic artery which supplies blood to cancer lesion, these particles embolize vessels predominantly in tumor tissue.[108-111] The microparticles may carry an active therapeutical, either a chemotherapeutic agent (chemoembolization) or a radiotherapeutical (radioembolization , the latter radiotherapeutical method gives especially promising results)[106-108,112-114].

We described the synthesis, characterization and radiochemical studies of macroporous chelating polymer beads as carriers of beta-emitters ^{177}Lu and ^{131}I intended for radioembolization of liver tumors.[115] The starting poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) (bead size 20 - 40 microns) was reacted with ammonia or methylamine to introduce primary and secondary amino groups, respectively. The primary amino groups containing polymer was used for the attachment of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) moieties, while quinoline-8-ol or 8-hydroxyquinoline-5-sulfonic acid moieties, respectively, were introduced onto the secondary amino groups containing polymer.

All the polymers were labeled quantitatively by ^{177}Lu in ammonium acetate buffer. DOTA containing beads required heating to 80 °C while the quinoline-8-ol or 8-hydroxyquinoline-5-sulfonic acid moieties containing polymers were quantitatively radiolabeled within 1 h at room temperature. The quinoline-8-ol groups containing polymer

was radioiodinated in 95 % yield by a chloramine method. Both ^{177}Lu and ^{131}I radiolabels were stable in an *in vitro* study in rat blood plasma. Quinoline-8-ol or 8-hydroxyquinoline-5-sulfonic acid moieties are thus more suitable for the radiolabeling of macroporous beads with ^{177}Lu for radioembolization purposes than well-established DOTA moieties and in addition, quinoline-8-ol also allows radiolabeling with [131]I.

4.2 Polymer copper chelators for the therapy of Wilson´s disease

Wilson's disease is a genetic disorder that leads to a high accumulation of copper in multiple organs with subsequent toxic effects especially on liver and neural system. The average prevalence of this disease in the general population is 1:30 000 and it is fatal unless treated Current first line therapy is based on a decrease in the amounts of copper by an administration of low-molecular-weight copper(II) chelators (penicillamine, triethylenetetramine or tetrathiomolybdate)[116,117], leading to a decreased absorption of copper from food with a subsequent increase in biological elimination. As an adjuvant therapy, high doses of zinc(II) salts are administered as these ions competitively block copper uptake from the gastrointestinal tract.

Suitable forms of zinc are not always available worldwide. Current therapies also suffer from serious side effects, such as myelosuppression, lupus and penicillamine-associated myasthenia as a consequence of the re-formation of a complex of essential elements after the gastrointestinal absorption of the chelating agent.[116,117] Zinc therapy is typically accompanied with strong gastrointestinal adverse effects, with typical doses of zinc of up to 1200 mg / day[118], which is approximately one hundred-fold more than the daily intake of zinc (ca 8 – 15 mg).

The average uptake of copper from food ranges from 0.6 – 1.6 mg per day. At the beginning of the therapy for Wilson's disease, a diet with low quantities of copper is often recommended (*i.e.,* avoiding food with high copper content, such as liver, nuts or mushrooms).[116] Considering the omnipresence of copper in food, a copper-less diet is nearly impossible.[119] A significant amount of copper is eliminated through the alimentary tract followed by a subsequent re-uptake, representing higher amounts than the quantities obtained from food (ca 4.4 – 5.3 mg of copper is secreted daily, with 7 % from saliva, 20 % from gastric juices, 50 % from bile, 18 % from pancreatic juices and 5 % from duodenal secretion).[119]

We proposed a gentle therapy to eliminate harmful copper concentrations in patients with Wilson's disease using an oral administration of insoluble polymeric sorbents containing selective chelating groups for copper(II), which will lower copper uptake from diet and shift copper balance towards elimination (see **Figure 17**).[120,121] The sorbents contain triethylenetetramine, *N,N*-di(2-pyridylmethyl)amine, 8-hydroxyquinoline or 8-hydroxyquinoline-5-sulfonic acid chelating groups bound to a methacrylate-based macroporous support. Nearly quantitative copper(II) uptake within minutes was achieved in buffers modeling the pH range present in the gastric environment (pH 2.0 and 4.0). The sorbents demonstrated chelating selectivity for copper(II) against zinc(II) with ratios of up to 940 (for 8-hydroxyquinoline).[121] The sorbents demonstrated sufficient stability of the copper complexes against rechelation using studies in a model environment for the small intestine (the presence of chelating amino acids, pH 6.8).
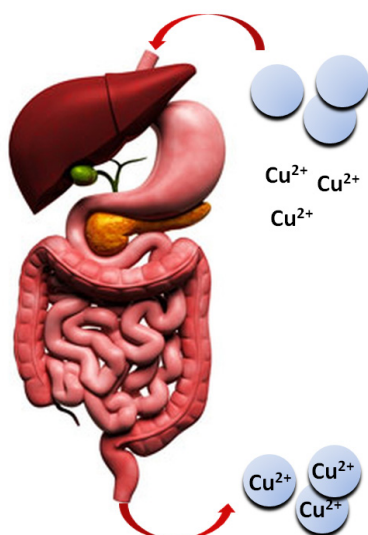
Figure 17. Scheme of the use of copper-chelating macroporous polymers for the treatment of Wilson´s disease.

In preliminary copper uptake experiments we have found that especially the polymer with 8-hydroxyquinoline significantly reduced copper uptake after oral administration in Wistar rats (see **Figure 18**).[120] [64]Cu was used as radiotracer, liver radioactivity was measured to evaluate copper uptake because liver is the main copper-storing organ in organism) Further, we have proven that the polymer with 8-hydroxyquinoline radiolabeled with ^{125}I is not absorbed from gastrointestinal tract after oral administration by determining the radioactivity in organs of rats. Non-resorbability and blocking of copper uptake was also supported with small animal imaging (PET/CT) in mice (see **Figure 19** and **20**), while for [^{64}Cu]-CuCl$_2$ alone there is visible accumulation of radiocopper in liver, with polymer all radiocopper remains in gastrointestinal tract. In a long-term experiment with Wistar rats fed with diet containing the polymers we have found that there were no signs of polymer toxicity and the addition of polymers to the diet led to significant reduction of the copper contents in the kidneys, brains and livers of the rats. With these experiments we have shown that polymers containing specific ligands have a potential as novel therapeutics for Wilson's disease.

For this application as well as for, e.g., separation of ^{64}Cu from target after irradiation during preparation technology it is necessary to have as ligand with the highest copper-selectivity.[122] Macrocyclic ligands are an obvious choice here. Two novel macrocyclic ligands based on *trans*-substituted cyclam with *N*-methyl and *N*-(4-aminobenzyl) groups as well as with two methylphosphinic (H$_2$L1) or methylphosphonic (H$_4$L2) acid pendant arms were synthesised and investigated in solutions. The ligands form stable complexes with transition metal ions. Both ligands show a high thermodynamic selectivity for divalent copper over nickel(II) and zinc(II) (K(CuL) is higher than K(Ni/ZnL) by about 7 orders of magnitude). Complexation is significantly faster for the phosphonate ligand H$_4$L2 probably due to stronger coordination ability of more basic phosphonate groups which efficiently binds the metal ion in an *out-of-cage* complex and, thus, accelerates its *in-cage* binding.
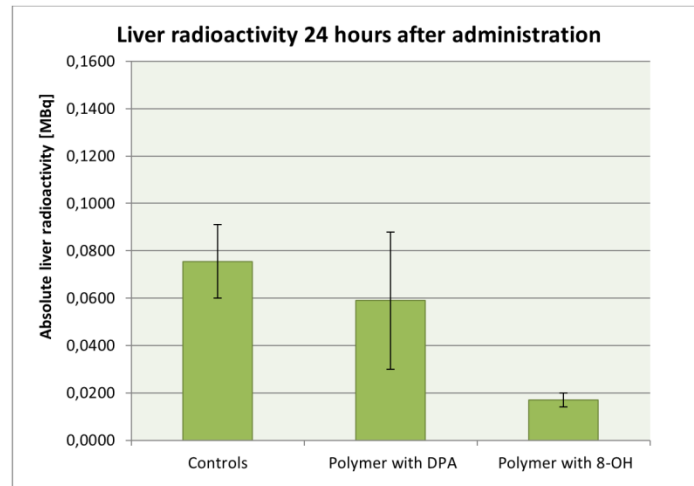
Figure 18: Liver radioactivity measured 24 hours after administration. Especially polymer with 8-hydroxyquinoline is apparently very efficient for uptake of copper from gastrointestinal tract. DPA – *N,N*-di(2-pyridylmethyl)amine; 8-OH – 8-hydroxyquinoline.
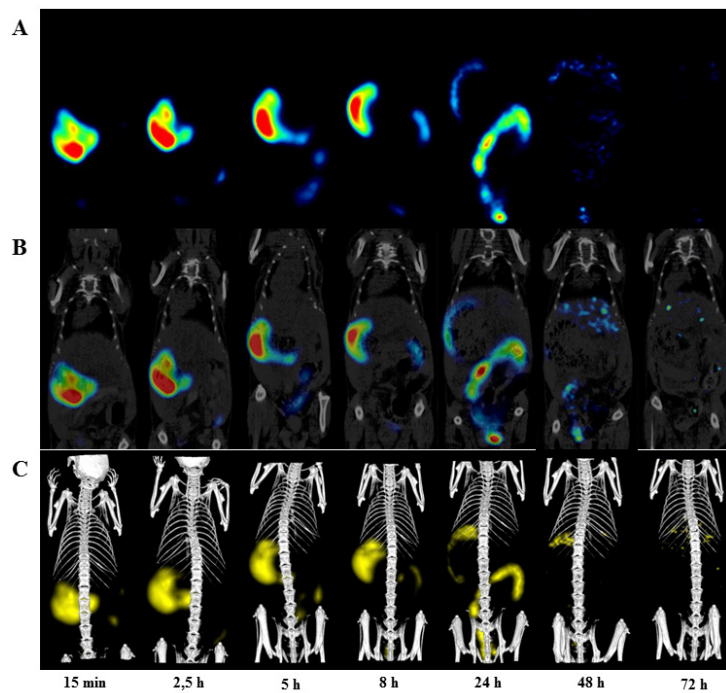
Figure 19. Biodistribution of ^{64}CuCl$_2$ in mouse at selected time intervals. PET (A) and fused PET/CT (B) images (coronal slices) of a mouse scanned 15 min, 2.5 h, 5 h, 8 h, 24 h, 48 h and 72 h after p.o. administration of ^{64}Cu-chloride displayed in PMOD software. (C) Fused PET/CT 3D images of the same mouse displayed in VolView software.
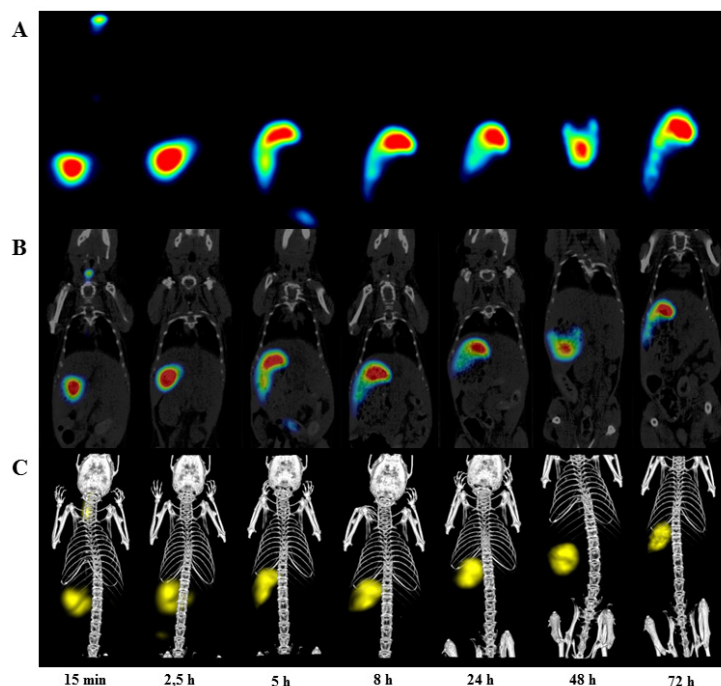
Figure 20. An example of biodistribution of ^{64}Cu-8HQB in mouse at selected time intervals. PET (A) and fused PET/CT (B) images (coronal slices) of a mouse scanned 15 min, 2,5 h, 5 h, 8 h, 24 h, 48 h and 72 h after p.o. administration displayed in PMOD software. (C) Fused PET/CT 3D images of the same mouse displayed in VolView software.

Rate of Cu(II) complexation by the phosphinate ligand H$_2$L1 is comparable to that of cyclam itself and its derivatives with non-coordinating substituents. Acid-assisted decomplexation of the copper(II) complexes is relatively fast ($\tau_{1/2}$ 44 and 42 s in 1 M aq. HCl at 25 °C for H$_2$L1 and H$_4$L2, respectively). Combination of the properties is convenient for selective copper removal/purification. Thus, these macrocyclic ligands were employed in preparation of ion-selective resins for radiocopper(II) separation. Glycidyl-methacrylate copolymer beads were modified with the ligands through diazotation reaction. The separation ability of the modified polymers was tested with nonradioactive copper(II) or non-carrier-added (NCA) ^{64}Cu in the presence of a high excess of both nickel(II) and zinc(II). The experiments exhibited high overall separation efficiency leading to 60–70 % recovery of radiocopper with high selectivity over the other metal ions originally present in 900-times molar excess. The results showed the concept of chelating resins with properly tuned selectivity of complexing moieties can be employed for radiocopper separation.

4.3 Metal ion-assembled nanoparticles

Metal ion chelation may not only serve for radionuclide attachment to the polymer carrier, but may also serve for the controlled assembly of the nanoparticles. We described the synthesis and study of properties of novel graft biodegradable polymer nanoparticles assembled by the addition of metal ions.[123,124] The polymer structures are composed of a graft polymer consisting of backbone chelating polymeric chain poly(*N*-methyl methacryloyl hydroxamic acid) with hydrophilic poly(ethylene oxide) grafts. The backbone polymer chain is able to bind essential metal cations (Fe^{3+} or Cu^{2+}) that serve as bioreversible crosslinkers (see **Figure**

28

21), since these metals should be directly or reductively transchelated inside the cells after internalization. At the same time the chelate bond is sufficiently stable during the transport in blood.
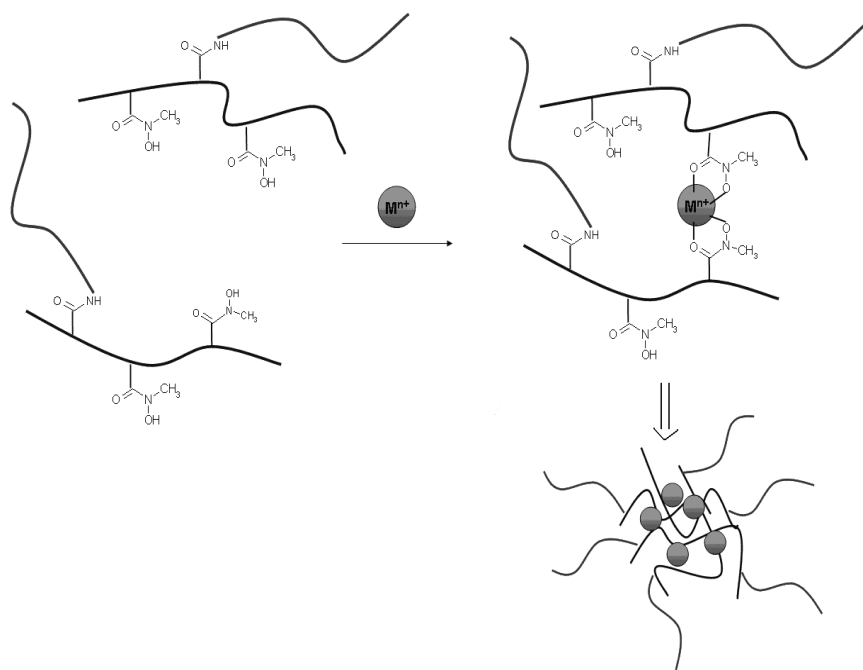
Figure 21. Reaction of ready graft-polymer (PMMHA-PEG) with metal cation (Fe^{3+} or Cu^{2+}) and formation of the "micelle-like" structure.
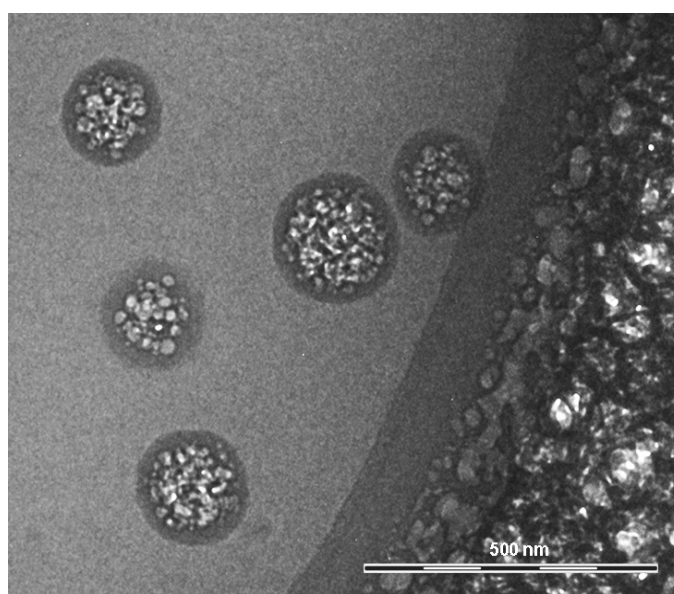
Figure 22. Cryo-TEM image of the nanoparticles with Fe^{3+}.

With Cu^{2+} ions, we obtained only small particles of several nanometers and relatively slow kinetics of formation. On the other hand, with Fe^{3+} the nanoparticles were of convenient size for passive tumor targeting (hydrodynamic diameter below 200 nm, see **Figure 22**) with

kinetics of formation within milliseconds. *In vitro* degradation of these particles was achieved with deferoxamine as model of *in vivo* transchelation capacity.

5 Conclusions

In this DSc thesis is accented contribution of our work to opening possibilities of the use of medically important radionuclides as active components of polymer delivery systems with transfer of experience from the drug delivery systems for chemotherapeuticals into this newly emerging area. Both approaches "What can bring the use of radionuclides and nuclear medicine approach to nanoscience?" and "What can nanoscience bring to nuclear medicine?" were actively implemented and critically evaluated. We studied chemical, physico-chemical and biological aspects of several newly developed systems and have selected candidates with vivid potential for further use. We also developed specific radiolabeling methods tailored to polymer radionuclide delivery systems.

Considering outlook and future development, we will particularly focus on self-assembled nanosystems for cancer radiotheranostics, especially those based on hybrid copolymers natural polysaccharide-poly(2-alkyl-2-oxazoline), and on systems for radiation-assisted cancer immunotherapy.

References:

(1)   Chaloupka, K.; Malam, Y.; Seifalian, A. M. *Trends Biotechnol.* 2010, *28*, 580.
(2)   Sharma, V. K.; Yngard, R. A.; Lin, Y. *Adv. Colloid Interface Sci.* 2009, *145*, 83.
(3)   Charpin, D.; Orehek, J.; Velardocchio, J. M. *Thorax* 1979, *34*, 259.
(4)   Harper, P. V.; Lathrop, K. A.; Richards, P. *J. Nucl. Med.* 1964, *5*, 382.
(5)   Palestro, C. J.; Love, C.; Tronco, G. G.; Tomas, M. B.; Rini, J. N. *Radiographics* 2006, *26*, 859.
(6)   Steigman, J.; Solomon, N. A.; Hwang, L. L. Y. *Appl. Radiat. Isot.* 1986, *37*, 223.
(7)   Wilhelm, A. J.; Mijnhout, G. S.; Franssen, E. J. F. *Eur. J. Nucl. Med.* 1999, *26*, S36.
(8)   Scheuch, G.; Bennett, W.; Borgstrom, L.; Clark, A.; Dalby, R.; Dolovich, M.; Fleming, J.; Gehr, P.; Gonda, I.; O'Callaghan, C.; Taylor, G.; Newman, S. *J. Aerosol Med. Pulmonary Drug Delivery* 2010, *23*, S39.
(9)   Hong, H.; Gao, T.; Cai, W. B. *Nano Today* 2009, *4*, 252.
(10)   Ting, G.; Chang, C. H.; Wang, H. E. *Anticancer Res.* 2009, *29*, 4107.
(11)   Ting, G.; Chang, C. H.; Wang, H. E.; Lee, T. W. *J. Biomed. Biotechnol.* 2010, 2010: 953537.
(12)   Hruby, M.; Konak, C.; Kucka, J.; Vetrik, M.; Filippov, S. K.; Vetvicka, D.; Mackova, H.; Karlsson, G.; Edwards, K.; Rihova, B.; Ulbrich, K. *Macromol. Biosci.* 2009, *9*, 1016.
(13)   Hruby, M.; Kucka, J.; Lebeda, O.; Mackova, H.; Babic, M.; Konak, C.; Studenovsky, M.; Sikora, A.; Kozempel, J.; Ulbrich, K. *J. Controlled Release* 2007, *119*, 25.
(14)   Hruby, M.; Kucka, J.; Mackova, H.; Konak, C.; Vetrik, M.; Kozempel, J.; Lebeda, O. *J. Appl. Polym. Sci.* 2009, *111*, 2220.
(15)   Colabufo, N. A.; Berardi, F.; Perrone, M. G.; Capparelli, E.; Cantore, M.; Inglese, C.; Perrone, R. *Curr. Top. Med. Chem.* 2010, *10*, 1703.
(16)   Dong, X. W.; Mumper, R. J. *Nanomedicine* 2010, *5*, 597.
(17)   Hanke, U.; May, K.; Rozehnal, V.; Nagel, S.; Siegmund, W.; Weitschies, W. *Eur. J. Pharm. Biopharm.* 2010, *76*, 260.

(18)   Minko, T. *Adv. Drug Deliv. Rev.* 2010, *62*, 192.
(19)   Feige, J. J. *Bull. Cancer* 2010, *97*, 1305.
(20)   Matsumoto, S.; Yasui, H.; Mitchell, J. B.; Krishna, M. C. *Cancer Res.*, *70*, 10019.
(21)   Moyal, E. C. J. *Targeted Oncology* 2008, *3*, 51.
(22)   Maeda, H. *Adv. Drug Delivery Rev.* 2001, *46*, 169.
(23)   Maeda, H. *J. Controlled Release* 2012, *164*, 138.
(24)   Maeda, H.; Nakamura, H.; Fang, J. *Adv. Drug Delivery Rev.* 2013, *65*, 71.
(25)   Torchilin, V. P. *Pharm. Res.* 2007, *24*, 1.
(26)   Gillies, E. R.; Frechet, J. M. J. *Bioconjugate Chem.* 2005, *16*, 361.
(27)   Bae, Y.; Nishiyama, N.; Fukushima, S.; Koyama, H.; Yasuhiro, M.; Kataoka, K. *Bioconjugate Chem.* 2005, *16*, 122.
(28)   Gillies, E. R.; Goodwin, A. P.; Frechet, J. M. J. *Bioconjugate Chem.* 2004, *15*, 1254.
(29)   Fang, J. S.; Gillies, R. D.; Gatenby, R. A. *Semin. Cancer Biol.* 2008, *18*, 330.
(30)   Lee, E. S.; Gao, Z. G.; Bae, Y. H. *J. Controlled Release* 2008, *132*, 164.
(31)   Sedlacek, O.; Hruby, M.; Studenovsky, M.; Vetvicka, D.; Svoboda, J.; Kankova, D.; Kovar, J.; Ulbrich, K. *Bioorg. Med. Chem.* 2012, *20*, 4056.
(32)   Sedlacek, O.; Hruby, M.; Studenovsky, M.; Kucka, J.; Vetvicka, D.; Kovar, L.; Rihova, B.; Ulbrich, K. *Bioconjugate Chem.* 2011, *22*, 1194.
(33)   Sedlacek, O.; Kucka, J.; Mattova, J.; Parizek, M.; Studenovsky, M.; Zadinova, M.; Pouckova, P.; Hruby, M. *Eur. J. Pharm. Sci.* 2014, *63*, 216.
(34)   Sedlacek, O.; Studenovsky, M.; Vetvicka, D.; Ulbrich, K.; Hruby, M. *Bioorg. Med. Chem.* 2013, *21*, 5669.
(35)   Hruby, M.; Etrych, T.; Kucka, J.; Forsterova, M.; Ulbrich, K. *J. Appl. Polym. Sci.* 2006, *101*, 3192.
(36)   Torchilin, V. P. *J. Controlled Release* 2001, *73*, 137.
(37)   Liu, J. B.; Xiao, Y. H.; Allen, C. *J. Pharm. Sci.* 2004, *93*, 132.
(38)   Teng, Y.; Morrison, M. E.; Munk, P.; Webber, S. E.; Prochazka, K. *Macromolecules* 1998, *31*, 3578.
(39)   Hruby, M.; Konak, C.; Ulbrich, K. *J. Controlled Release* 2005, *103*, 137.
(40)   Vetvicka, D.; Hovorka, O.; Hruby, M.; Mrkvan, T.; Ulbrich, K.; Rihova, B. *Tumor Biol.* 2007, *28*, 88.
(41)   Vetvicka, D.; Hruby, M.; Hovorka, O.; Etrych, T.; Vetrik, M.; Kovar, L.; Kovar, M.; Ulbrich, K.; Rihova, B. *Bioconjugate Chem.* 2009, *20*, 2090.
(42)   Vetvicka, D.; Hruby, M.; Ulbrich, K.; Rihova, B. *Mol. Cancer Ther.* 2007, *6*, 3562S.
(43)   Studenovsky, M.; Sedlacek, O.; Hruby, M.; Panek, J.; Ulbrich, K. *Anticancer Res.* 2015, *35*, 753.
(44)   Hruby, M.; Konak, C.; Ulbrich, K. *J. Appl. Polym. Sci.* 2005, *95*, 201.
(45)   Bogomolova, A.; Keller, S.; Klingler, J.; Sedlak, M.; Rak, D.; Sturcova, A.; Hruby, M.; Stepanek, P.; Filippov, S. K. *Langmuir* 2014, *30*, 11307.
(46)   Filippov, S.; Hruby, M.; Konak, C.; Mackova, H.; Spirkova, M.; Stepanek, P. *Langmuir* 2008, *24*, 9295.
(47)   Filippov, S. K.; Starovoytova, L.; Konak, C.; Hruby, M.; Mackova, H.; Karlsson, G.; Stepanek, P. *Langmuir* 2010, *26*, 14450.
(48)   Gil, E. S.; Hudson, S. A. *Prog. Polym. Sci.* 2004, *29*, 1173.
(49)   Hruby, M.; Kucka, J.; Mackova, H.; Lebeda, O.; Ulbrich, K. *Chem. Listy* 2008, *102*, 21.
(50)   Hruby, M.; Subr, V.; Kucka, J.; Kozempel, J.; Lebeda, O.; Sikora, A. *Appl. Radiat. Isot.* 2005, *63*, 423.
(51)   Annaka, M.; Yahiro, C.; Nagase, K.; Kikuchi, A.; Okano, T. *Polymer* 2007, *48*, 5713.

(52)   Konak, C.; Oupicky, D.; Chytry, V.; Ulbrich, K.; Helmstedt, M. *Macromolecules* 2000, *33*, 5318.

(53)   Neradovic, D.; van Nostrum, C. F.; Hennink, W. E. *Macromolecules* 2001, *34*, 7589.

(54)   Neradovic, D.; van Steenbergen, M. J.; Vansteelant, L.; Meijer, Y. J.; van Nostrum, C. F.; Hennink, W. E. *Macromolecules* 2003, *36*, 7491.

(55)   Virtanen, J.; Holappa, S.; Lemmetyinen, H.; Tenhu, H. *Macromolecules* 2002, *35*, 4763.

(56)   Konak, C.; Panek, J.; Hruby, M. *Coll. Polym. Sci.* 2007, *285*, 1433.

(57)   Soga, O.; van Nostrum, C. F.; Hennink, W. E. *Biomacromolecules* 2004, *5*, 818.

(58)   Hruby, M.; Filippov, S. K.; Panek, J.; Novakova, M.; Mackova, H.; Kucka, J.; Ulbrich, K. *J. Controlled Release* 2010, *148*, E60.

(59)   Hruby, M.; Filippov, S. K.; Panek, J.; Novakova, M.; Mackova, H.; Kucka, J.; Vetvicka, D.; Ulbrich, K. *Macromol. Biosci.* 2010, *10*, 916.

(60)   Panek, J.; Filippov, S. K.; Hruby, M.; Rabyk, M.; Bogomolova, A.; Kucka, J.; Stepanek, P. *Macromol. Rapid Commun.* 2012, *33*, 1683.

(61)   Murdoch, D.; Sager, J. *Curr. Opin. Oncol.* 2008, *20*, 104.

(62)   Nimmagadda, S.; Ford, E. C.; Wong, J. W.; Pomper, M. G. *Semin. Radiat. Oncol.* 2008, *18*, 136.

(63)   Chrapko, B.; Zwolak, R.; Nocun, A.; Golebiewska, R.; Majdan, M. *Rheumatol. Int.* 2007, *27*, 729.

(64)   Aronowitz, J. N. *Brachytherapy* 2008, *7*, 55.

(65)   Butler, W. M.; Merrick, G. S. *Int. J. Radiat. Oncol. Biol. Phys.* 2008, *71*, S142.

(66)   Beitsch, P. D.; Hodge, C. W.; Dowlat, K.; Francescatti, D.; Gittleman, M. A.; Israel, P.; Nelson, J. C.; Potruch, T.; Snider, H. C., Jr.; Whitworth, P.; Zannis, V. J.; Patel, R. R. *Breast J.* 2009, *15*, 93.

(67)   Saibishkumar, E. P.; Borg, J.; Yeung, I.; Cummins-Holder, C.; Landon, A.; Crook, J. *Int. J. Radiat. Oncol. Biol. Phys.* 2009, *73*, 61.

(68)   Liu, R.; Fraylich, M.; Saunders, B. R. *Coll. Polym. Sci.* 2009, *287*, 627.

(69)   Kissel, M.; Peschke, P.; Subr, V.; Ulbrich, K.; Schuhmacher, J.; Debus, J.; Friedrich, E. *PDA J. Pharm. Sci. Technol.* 2001, *55*, 191.

(70)   Kissel, M.; Peschke, P.; Subr, V.; Ulbrich, K.; Strunz, A. M.; Kuhnlein, R.; Debus, J.; Friedrich, E. *Eur. J. Nucl. Med. Mol. Imaging* 2002, *29*, 1055.

(71)   Kucka, J.; Hruby, M.; Lebeda, O. *Appl. Radiat. Isot.* 2010, *68*, 1073.

(72)   Hruby, M.; Pouckova, P.; Zadinova, M.; Kucka, J.; Lebeda, O. *Eur. J. Pharm. Sci.* 2011, *42*, 484.

(73)   Hruby, M.; Kucka, J.; Konak, C.; Vetrik, M.; Lebeda, O.; Ulbrich, K. *J. Controlled Release* 2008, *132*, E40.

(74)   Tirone, T. A.; Brunicardi, F. C. *World J. Surgery* 2001, *25*, 461.

(75)   Shearer, J.; Graham, T. E. *Can. J. Appl. Physiol.* 2002, *27*, 179.

(76)   Campbell, N. A. B. W. R. J. H. *Biology: Exploring Life*; Pearson Prentice Hall: Boston, Massachusetts, 2006.

(77)   Manners, D. J. *Carbohydrate Polym.* 1991, *16*, 37.

(78)   Rybicka, K. K. *Tissue Cell* 1996, *28*, 253.

(79)   Hoffman, A. S. *J. Controlled Release* 2008, *132*, 153.

(80)   Aasen, S. N.; Pospisilova, A.; Eichler, T. W.; Panek, J.; Hruby, M.; Stepanek, P.; Spriet, E.; Jirak, D.; Skaftnesmo, K. O.; Thorsen, F. *Int. J. Mol. Sci.* 2015, *16*, 21658.

(81)   Filippov, S. K.; Sedlacek, O.; Bogomolova, A.; Vetrik, M.; Jirak, D.; Kovar, J.; Kucka, J.; Bals, S.; Turner, S.; Stepanek, P.; Hruby, M. *Macromol. Biosci.* 2012, *12*, 1731.

(82)   Andre, R.; Tahir, M. N.; Natalio, F.; Tremel, W. *FEBS J.* 2012, *279*, 1737.

(83)   Aoi, K.; Takasu, A.; Okada, M.; Imae, T. *Macromol. Chem. Phys.* 1999, *200*, 1112.

(84)   Nakamura, R.; Aoi, K.; Okada, M. *Macromolecul. Biosci.* 2004, *4*, 610.
(85)   Cheng, J.; Park, M.; Hyun, J. *Cellulose* 2014, *21*, 1699.
(86)   Karaaslan, M. A.; Gao, G. Z.; Kadla, J. F. *Cellulose* 2013, *20*, 2655.
(87)   Madry, H.; Rey-Rico, A.; Venkatesan, J. K.; Johnstone, B.; Cucchiarini, M. *Tissue Eng. B Rev.* 2014, *20*, 106.
(88)   Pospisilova, A.; Filippov, S. K.; Bogomolova, A.; Turner, S.; Sedlacek, O.; Matushkin, N.; Cernochova, Z.; Stepanek, P.; Hruby, M. *RSC Advances* 2014, *4*, 61580.
(89)   Slegerova, J.; Hajek, M.; Rehor, I.; Sedlak, F.; Stursa, J.; Hruby, M.; Cigler, P. *Nanoscale* 2015, *7*, 415.
(90)   Jung, K.-H.; Park, J. W.; Paik, J.-Y.; Cung Hoa Thien, Q.; Choe, Y. S.; Lee, K.-H. *Nucl. Med. Biol.* 2012, *39*, 1122.
(91)   Rosebrough, S. F. *Nucl. Med. Biol.* 1993, *20*, 663.
(92)   Nori, A.; Kopecek, J. *Adv. Drug Delivery Rev.* 2005, *57*, 609.
(93)   Liu, S. *Chemical Society Reviews* 2004, *33*, 445.
(94)   Seevers, R. H.; Counsell, R. E. *Chemical Reviews* 1982, *82*, 575.
(95)   Simone, E. A.; Zern, B. J.; Chacko, A.-M.; Mikitsh, J. L.; Blankemeyer, E. R.; Muro, S.; Stan, R. V.; Muzykantov, V. R. *Biomaterials* 2012, *33*, 5406.
(96)   Culbert, P. A.; Hunter, D. H. *React. Polym.* 1993, *19*, 247.
(97)   Hunter, D. H.; Zhu, X. Z. *J. Labelled Compd. Radiopharm.* 1999, *42*, 653.
(98)   Yang, F.-L.; Tian, S.-K. *Angew. Chem. Int. Ed.* 2013, *52*, 4929.
(99)   Vaidyanathan, G.; Zalutsky, M. R. *Phys. Med. Biol.* 1996, *41*, 1915.
(100) Guerard, F.; Barbet, J.; Chatal, J. F.; Kraeber-Bodere, F.; Cherel, M.; Haddad, F. *Q. J. Nucl. Med. Mol. Imaging* 2015, *59*, 161.
(101) Kim, G.; Chun, K.; Park, S. H.; Kim, B. *Phys. Med. Biol.* 2014, *59*, 2849.
(102) Kucka, J.; Hruby, M.; Konak, C.; Kozempel, J.; Lebeda, O. *Appl. Radiat. Isot.* 2006, *64*, 201.
(103) Sedlacek, O.; Kucka, J.; Svec, F.; Hruby, M. *J. Sep. Sci.* 2014, *37*, 798.
(104) Mackova, H.; Oukacine, F.; Plichta, Z.; Hruby, M.; Kucka, J.; Taverna, M.; Horak, D. *J. Colloid Interface Sci.* 2014, *421*, 146.
(105) Bosch, F. X.; Ribes, J.; Diaz, M.; Cleries, R. *Gastroenterology* 2004, *127*, S5.
(106) Llovet, J. M.; Bruix, J. *Hepatology* 2003, *37*, 429.
(107) Llovet, J. M.; Burroughs, A.; Bruix, J. *Lancet* 2003, *362*, 1907.
(108) Raoul, J. L.; Boucher, E.; Rolland, Y.; Garin, E. *Nat. Rev. Gastroenterol. Hepatol.* 2010, *7*, 41.
(109) Salem, R.; Lewandowski, R. J.; Atassi, B.; Gordon, S. C.; Gates, V. L.; Barakat, O.; Sergie, Z.; Wong, C. Y. O.; Thurston, K. G. *J. Vasc. Interv. Radiol.* 2005, *16*, 1627.
(110) Zielhuis, S. W.; Nijsen, J. F. W.; Krijger, G. C.; van het Schip, A. D.; Hennink, W. E. *Biomacromolecules* 2006, *7*, 2217.
(111) Zielhuis, S. W.; Seppenwoolde, J. H.; Bakker, C. J. G.; Jahnz, U.; Zonnenberg, B. A.; van het Schip, A. D.; Hermink, W. E.; Nijsen, J. F. W. *J. Biomed. Mater. Res. Part A* 2007, *82A*, 892.
(112) Raoul, J. L.; Boucher, E.; Roland, Y.; Garin, E. *Q. J. Nucl. Med. Mol. Imag.* 2009, *53*, 348.
(113) Raoul, J. L.; Bourguet, P.; Bretagne, J. F.; Duvauferrier, R.; Coornaert, S.; Darnault, P.; Ramee, A.; Herry, J. Y.; Gastard, J. *Radiology* 1988, *168*, 541.
(114) Riaz, A.; Lewandowski, R. J.; Kulik, L. M.; Mulcahy, M. F.; Sato, K. T.; Ryu, R. K.; Omary, R. A.; Salem, R. *J. Vasc. Interv. Radiol.* 2009, *20*, 1121.
(115) Hruby, M.; Skodova, M.; Mackova, H.; Skopal, J.; Tomes, M.; Kropacek, M.; Zimova, J.; Kucka, J. *React. Funct. Polym.* 2011, *71*, 1155.

(116)   Ala, A.; Walker, A. P.; Ashkan, K.; Dooley, J. S.; Schilsky, M. L. *Lancet* 2007, *369*, 397.

(117)   Merle, U.; Schaefer, M.; Ferenci, P.; Stremmel, W. *Gut* 2007, *56*, 115.

(118)   Brewer, G. J.; Yuzbasiyangurkan, V.; Johnson, V.; Dick, R. D.; Wang, Y. X. *Am. J. Med. Sci.* 1993, *305*, 199.

(119)   van den Berghe, P. V. E.; Klomp, L. W. J. *Nutr. Rev.* 2009, *67*, 658.

(120)   Mattova, J.; Pouckova, P.; Kucka, J.; Skodova, M.; Vetrik, M.; Stepanek, P.; Urbanek, P.; Petrik, M.; Novy, Z.; Hruby, M. *Eur. J. Pharm. Sci.* 2014, *62*, 1.

(121)   Skodova, M.; Kucka, J.; Vetrik, M.; Skopal, J.; Walterova, Z.; Sedlacek, O.; Stepanek, P.; Mattova, J.; Pouckova, P.; Urbanek, P.; Hruby, M. *React. Funct. Polym.* 2013, *73*, 1426.

(122)   Paurova, M.; Havlickova, J.; Pospisilova, A.; Vetrik, M.; Cisarova, I.; Stephan, H.; Pietzsch, H.-J.; Hruby, M.; Hermann, P.; Kotek, J. *Chem. Eur. J.* 2015, *21*, 4671.

(123)   Skodova, M.; Cernoch, P.; Stepanek, P.; Chanova, E.; Kucka, J.; Kalalova, Z.; Kankova, D.; Hruby, M. *ChemPhysChem* 2012, *13*, 4244.

(124)   Skodova, M.; Hruby, M.; Filippov, S. K.; Karlsson, G.; Mackova, H.; Spirkova, M.; Kankova, D.; Steinhart, M.; Stepanek, P.; Ulbrich, K. *Macromol. Chem. Phys.* 2011, *212*, 2339.

Publications included in the dissertation thesis:

Corresponding author marked with asterix (*).

1.   Šlegerová, J., Hájek, M., Řehoř, I., Sedlák, F., Štursa, J., **Hrubý, M.**, Cígler, P.* Designing the nanobiointerface of fluorescent nanodiamonds: highly selective targeting of glioma cancer cells, Nanoscale. Roč. 7, č. 2 (2015), s. 415-420, impact factor (IF) 7,394, references with/without autocitations (CIT) 6/6.

2.   Aasen, S. N., Pospíšilová, A., Eichler, T. W., Pánek, J., **Hrubý, M.**, Štěpánek, P., Spriet, E., Jirák, D., Skaftnesmo, K.O., Thorsen, F.* A novel nanoprobe for multimodal imaging is effectively incorporated into human melanoma metastatic cell lines,International Journal of Molecular Sciences. Roč. 16, č. 9 (2015), s. 21658-21680, IF 2,862, CIT 0/0.

3.   Sedláček, O., Kučka, J., **Hrubý, M.*** Optimized protocol for the radioiodination of hydrazone-type polymer drug delivery systems, Applied Radiation and Isotopes. Roč. 95, 2015, s. 129-134, IF 1,231, CIT 0/0.

4.   **Hrubý, M.**, Filippov, S., Štěpánek, P.* Smart polymers in drug delivery systems on crossroads: which way deserves following? European Polymer Journal. Roč. 65, 2015, s. 82-97, IF 3,005, CIT 1/1.

5.   Paúrová, M., Havlíčková, J., Pospíšilová, A., Vetrík, M., Císařová, I., Stephan, H., Pietzsch, H.-J., **Hrubý, M.**, Hermann, P., Kotek, J.* Bifunctional cyclam-based ligands with phosphorus acid pendant moieties for radiocopper separation: thermodynamic and kinetic studies, Chemistry-A European Journal. Roč. 21, č. 12 (2015), s. 4671-4687, IF 5,731, CIT 1/1.

6.   Macková, H., Oukacine, F., Plichta, Z., **Hrubý, M.**, Kučka, J., Taverna, M., Horák, D.* Poly(glycidyl methacrylate)/silver nanocomposite microspheres as a radioiodine scavenger: electrophoretic characterization of carboxyl- and amine-modified particles, Journal of Colloid and Interface Science. Roč. 421, 2014, s. 146-153, IF 3,368, CIT 3/3.

7.   Mattová, J., Poučková, P., Kučka, J., Škodová, M., Vetrík, M., Štěpánek, P., Urbánek, P., Petřík, M., Nový, Z., **Hrubý, M.*** Chelating polymeric beads as potential therapeutics for Wilson's disease, European Journal of Pharmaceutical Sciences. Roč. 62, 2014, s. 1-7, IF 3,350, CIT 1/1.

8.   Sedláček, O., Kučka, J., Švec, F., **Hrubý, M.*** Silver-coated monolithic columns for separation in radiopharmaceutical applications, Journal of Separation Science. Roč. 37, č. 7 (2014), s. 798-802, IF 2,737, CIT 6/5.

9.   Sedláček, O., Kučka, J., Mattová, J., Pařízek, M., Studenovský, M., Zadinová, M., Poučková, P., **Hrubý, M.*** Multistage-targeted pH-responsive polymer conjugate of Auger electron emitter: optimized design and in vivo activity, European Journal of Pharmaceutical Sciences. Roč. 63, 2014, s. 216-225, IF 3,350, CIT 2/1.

10.   Bogomolova, A., Keller, S., Klingler, J., Sedlák, M., Rak, D., Šturcová, A., **Hrubý, M.**, Štěpánek, P., Filippov, S. * Self-assembly thermodynamics of pH-responsive amino-acid-based polymers with a nonionic surfactant, Langmuir. Roč. 30, č. 38 (2014), s. 11307-11318, IF 4,457, CIT 0/0.

11.   Pospíšilová, A., Filippov, S.K., Bogomolova, A., Turner, S., Sedláček, O., Matushkin, N., Černochová, Z., Štěpánek, P., **Hrubý, M.*** Glycogen-graft-poly(2-alkyl-2-oxazolines) -

the new versatile biopolymer-based thermoresponsive macromolecular toolbox, RSC Advances. Roč. 4, č. 106 (2014), s. 61580-61588. IF 3,840, CIT 0/0.

12.   Škodová, M., Kučka, J., Vetrík, M., Skopal, J., Walterová, Z., Sedláček, O., Štěpánek, P., Mattová, J., Poučková, P., Urbánek, P., **Hrubý, M.*** Chelating polymeric particles intended for the therapy of Wilson's disease, Reactive and Functional Polymers. Roč. 73, č. 11 (2013), s. 1426-1431, IF 2,515, CIT 2/1.

13.   Sedláček, O., Studenovský, M., Větvička, D., Ulbrich, K., **Hrubý, M.*** Fine tuning of the pH-dependent drug release rate from polyHPMA-ellipticinium conjugates, Bioorganic & Medicinal Chemistry. Roč. 21, č. 18 (2013), s. 5669-5672, IF 2,793, CIT 5/2.

14.   Sedláček, O., **Hrubý, M.***, Studenovský, M., Větvička, D., Svoboda, J., Kaňková, D., Kovář, J., Ulbrich, K. Polymer conjugates of acridine-type anticancer drugs with pH-controlled activation, Bioorganic & Medicinal Chemistry. Roč. 20, č. 13 (2012), s. 4056-4063, IF 2,793, CIT 17/14.

15.   Filippov, S., Sedláček, O., Bogomolova, A., Vetrík, M., Jirák, D., Kovář, J., Kučka, J., Bals, S., Turner, S., Štěpánek, P., **Hrubý, M.***, Macromolecular Bioscience. Roč. 12, č. 12 (2012), s 1731-1738, IF 3,851, CIT 5/2.

16.   Sedláček, O., Monnery B., D., Filippov, S., Hoogenboom, R.*, **Hrubý, M.*** Poly(2-oxazoline)s - are they more advantageous for biomedical applications than other polymers?, Macromolecular Rapid Communications. Roč. 33, č. 19 (2012), s. 1648-1662, IF 4,941, CIT 54/46.

17.   Pánek, J., Filippov, S., **Hrubý, M.***, Rabyk, M., Bogomolova, A., Kučka, J., Štěpánek, P. Thermoresponsive nanoparticles based on poly(2-alkyl-2-oxazolines) and pluronic F127, Macromolecular Rapid Communications. Roč. 33, č. 19 (2012), s. 1683-1689, IF 4,941, CIT 9/6.

18.   Škodová, M.*, Černoch, P., Štěpánek, P., Mázl Chánová, E., Kučka, J., Kálalová, Z., Kaňková, D., **Hrubý, M.** Self-assembled polymeric chelate nanoparticles as potential theranostic agents, ChemPhysChem. Roč. 13, č. 18 (2012), s. 4244-4250, IF 3,419, CIT 0/0.

19.   **Hrubý, M.***, Poučková, P., Zadinová, M., Kučka, J., Lebeda, O. Thermoresponsive polymeric radionuclide delivery system - an injectable brachytherapy, European Journal of Pharmaceutical Sciences. Roč. 42, č. 5 (2011), s. 484-488, IF 3,350, CIT 7/4.

20.   Škodová, M., **Hrubý, M.***, Filippov, S., Karlsson, G., Macková, H., Špírková, M., Kaňková, D., Steinhart, M., Štěpánek, P., Ulbrich, K. Novel polymeric nanoparticles assembled by metal ion addition, Macromolecular Chemistry and Physics. Roč. 212, č. 21 (2011), s. 2339-2348, IF 2,616, CIT 5/3.

21.   Sedláček, O., **Hrubý, M.***, Studenovský, M., Kučka, J., Větvička, D., Kovář, L.,Říhová, B., Ulbrich, K. Ellipticine-aimed polymer-conjugated Auger electron emitter: multistage organelle targeting approach, Bioconjugate Chemistry. Roč. 22, č. 6 (2011), s. 1194-1201, IF 4,513, CIT 11/6.

22.   **Hrubý, M.***, Škodová, M., Macková, H., Skopal, J., Kropáček, M., Zimová, J., Kučka, J., Tomeš, M. Lutetium-177 and iodine-131 loaded chelating polymer microparticles intended for radioembolization of liver malignancies, Reactive and Functional Polymers. Roč. 71, č. 12 (2011), s. 1155-1159, IF2,515, CIT 3/1.

23.   **Hrubý, M.***, Filippov, S., Pánek, J., Škodová, M., Macková, H., Kučka, J., Větvička, D., Ulbrich, K. Polyoxazoline thermoresponsive micelles as radionuclide delivery systems, Macromolecular Bioscience. Roč. 10, č. 8 (2010), s. 916-924, IF 3,851, CIT 30/23.

24.   Kučka, J., **Hrubý, M.**, Lebeda, O.* Biodistribution of a radiolabelled thermoresponsive polymer in mice, Applied Radiation and Isotopes. Roč. 68, č. 6 (2010) s. 1073-1078, IF 1,231, CIT 6/2.

25.   **Hrubý, M.***, Kučka, J., Škodová, M., Macková, H., Vetrík, M. New coupling strategy for radionuclide labeling of synthetic polymers, Applied Radiation and Isotopes. Roč. 68, č. 2 (2010), s. 334-339, IF 1,231, CIT 2/1.

26.   **Hrubý, M.***, Filippov, S., Pánek, J., Škodová, M., Macková, H., Kučka, J., Ulbrich, K. Thermoresponsive micelles for radionuclide delivery, Journal of Controlled Release. Roč. 148, č. 1 (2010), s. e60-e62, IF 7,705, CIT 4/2.

27.   **Hrubý M.***, Kučka J., Macková H., Koňák Č., Vetrík M., Kozempel J., Lebeda O. New binary thermoresponsive polymeric system for local chemoradiotherapy, Journal of Applied Polymer Science 111, (2009),2220-2222, IF 1,768, CIT 10/2.

28.   **Hrubý M.***, Koňák Č., Kučka J., Vetrík M., Filippov S. K., Větvička D., Macková H., Karlsson G., Edwards K., Říhová B., Ulbrich K. Thermoresponsive hydrolytically degradable polymer micelles intended for radionuclide delivery, Macromolecular Bioscience. Roč. 9, č. 10 (2009), s. 1016-1027, IF 3,851, CIT 21/15.

29.   Větvička, D.*,**Hrubý, M.**,Hovorka, O.,Etrych, T.,Větřík, M.,Kovář, L.,Ulbrich, K.,Říhová, B. Biological evaluation of polymeric micelles with covalently bound doxorubicin, Bioconjugate Chemistry 20, č. 1, (2009), 2090-2097, IF 4,513, CIT 36/36.

30.   Kozempel, J.*,**Hrubý, M.**,Škodová, M.,Kučka, J.,Lešetický, L.,Lebeda, O. Novel polymer vectors of Cu-64, Radiochim. Acta 97, (2009) s. 747-752, IF 1,014, CIT 4/3.

31.   **Hrubý, M.***, Kučka, J., Lebeda, O., Macková, H., Babič, M., Koňák, Č., Studenovský, M., Sikora, A., Kozempel, J., Ulbrich, K. New bioerodable thermoresponsive polymers for possible radiotherapeutic applications, Journal of Controlled Release, 2007, Roč. 119, č. 1, s. 25-33, IF.7,705, CIT 24/14.

32.   Kučka, J., **Hrubý, M.**, Koňák, Č., Kozempel, J., Lebeda, O.* Astatination of nanoparticles containing silver as possible carriers of 211At, Applied Radiation and Isotopes, 2006, Roč. 64, 2, s. 201-206, IF 1,231, CIT 11/9.

33.   **Hrubý, M.***, Etrych, T., Kučka, J., Forsterová, M., Ulbrich, K. Hydroxybis phosphonate-containing polymeric drug-delivery systems designed for targeting into bone tissue, Journal of Applied Polymer Science, 2006, Roč. 101, č. 5, s. 3192-3201, IF 1,768, CIT 23/18.

34.   **Hrubý, M.**, Koňák, Č., Ulbrich, K.* Poly(allyl glycidyl ether)-block-poly(ethylene oxide): a novel promising polymeric intermediate for the preparation of micellar drug delivery systems, Journal of Applied Polymer Science, 2005, Roč. 95, č. 2, s. 201-211, IF 1,768, CIT 42/38.

35.   **Hrubý, M.***, Koňák, Č., Ulbrich, K. Polymeric micellar pH-sensitive drug delivery system for doxorubicin, Journal of Controlled Release, 2005, Roč. 103, č. 1, s. 137-148, IF 7,705, CIT 218/209.

36.   **Hrubý, M.***, Šubr, V., Kučka, J., Kozempel, J., Lebeda, O., Sikora, A. Thermoresponsive polymers as promising new materials for local radiotherapy, Applied Radiation and Isotopes, 2005, Roč. 63, č. 4, s. 423-431, IF 1,231, CIT 25/11.

Statement about the authorship of the presented research results:

In the majority of the presented research results the contribution of the author of this Thesis was pivotal, designing concepts of the described systems, performing syntheses and experimental measurements, data processing and interpretation, suggesting and developing novel experimental methodologies as well as supervising of master degree and Ph.D. students during their research work (Ondřej Sedláček, Miroslav Vetrík, Michaela Škodová, Aneta Pospíšilová, Jan Skopal).

Scientometric data for M. Hrubý

ResearcherID: H-6479-2014, URL: http://www.researcherid.com/rid/H-6479-2014

Number of publications: 78
Number of papers (applicant first author): 20
Number of papers (applicant corresponding author): 29
Number of citations (with autocitations): 1052
Number of citations (without autocitations): 639
H-index: 17
Sum of impact factors: 243.094
Average impact factor per paper: 3.116