8. Summary

The task of this work was to investigate the use of biocompatible copolymers out of poly(ethylene oxide) (PEO) and poly(glutamic acid) or poly(aspartic acid) for possible medical applications.

Chapter 6 deals with the synthesis and characterization of block copolymers with peptide functional blocks namely PEO-*block*-poly(γ-benzyl-L-glutamate) and PEO-*block*-poly(β-benzyl-L-aspartate) and a defined polymer structure. The ring opening polymerizations of N-carboxy anhydrides (NCA) of γ-benzyl-L-glutamate and β-benzyl-L-aspartate were studied in the presence of an ammonium chloride functional poly(ethylene oxide) macroinitiator, which possibly prevents side reactions such as NCA deprotonation. Well-defined diblock copolymers PEO-*b*-poly(γ-benzyl-L-glutamate) and PEO-*b*- poly(β-benzyl-L-aspartate) with very narrow molecular weight distribution (M_w/M_n < 1.05) were successfully prepared by ring opening polymerization of NCAs initiated by ammonium chloride PEO macroinitiators, which allow reduction of the activated monomer mechanism. Polymerization in the presence of such macroinitiators was found to be quite slow as compared to polymerization initiated by primary amine PEO macroinitiators. However, the reaction times could be significantly reduced by increasing temperature or by using an initiating mixed system PEO-$NH_3^+Cl^-$/PEO-NH_2 1:1. As a main conclusion, NCA polymerization initiated by ammonium chloride PEO macroinitiators allows convenient preparation of PEO-*b*-polypeptide conjugates possessing a precisely controlled molecular structure.

The stabilization of maghemite model particles through homopolymers (made of glutamc acid or aspartic acid) or through block and graft copolymers showed distinct differences. The stability of the particles as well as the influence of the molecule architecture on the particle size and on the zeta potential of the coated particles has been tested under physiological conditions. It has also been investigated whether stable particles with different zeta potentials can be made from these polymers.

The results showed that homopolymers made from glutamic acid and aspartic acid could indeed stabilize maghemite particles. Stable particle dispersions were obtained in 0.154 molar NaCl solutions at pH 7.4. The resulting particles were stable above a concentration of $c_{aminoacid} = 3 \times 10^{-6}$ mol $g^{-1}$and independent of the type of the amino acid and the chain length of the homopolymer. They showed a constant zeta potential of approximately -70 mV even at higher concentrations of amino acids. It was thus not possible to

produce stable particles with different zeta potentials under physiological conditions from these polymers.

The use of block copolymers from PEO and poly(aspartic acid) or poly(glutamic acid) for coating of the maghemite particles resulted in an improved stabilization of these particles at physiological conditions compared to the homopolymers. The PEO-blocks affected the stability of the particles critically in copolymers made of PEO and glutamic acid. An effective stabilization of the maghemite particles required a PEO concentration in the range $1 - 2 \times 10^{-7}$ mol g^{-1} independent of the type of the copolymer (block or graft copolymer) and of the number of PEO chains in the polymer. It could be shown that even PEO chains with a very low number of glutamic acid units (2 or 6 units, respectively) stabilized iron oxide particles effectively. The glutamic acid units act obviously as "anchors" for the PEO chains to fix them at the surface of the particles. This could not be verified for aspartic acid, since only two polymers with aspartic acid were available.

The complexation between the water soluble drug diminazene and blockcopolymers out of PEO and a poly(L-glutamate) block was described in Chapter 5. In this study, we have found that complexes in the form of nanoparticles with a mean hydrodynamic radius of 16 nm and low polydispersity (P.I. = 0.1) were spontaneously formed by the complexation of poly(ethylene oxide)-*block*-poly(L-glutamate) (PEO-*b*-PLGlu) with diminazene. Only one of two possible binding sites of each diminazene molecule was involved in complexation. As determined by UV-VIS difference spectra measurements, the complex binding constant is in the order of $1\text{-}2 \times 10^{4}$ M^{-1}. Circular dichroism measurements showed that the highly water-soluble diminazene can induce and stabilize the α-helical secondary structure of a poly(L-glutamate) block. The helix-to-coil transition of the poly(L-glutamate) blocks is remarkably shifted from pH 5 to pH 12. This effective stabilization of the α-helix structure seems to be due to the formation of a protective coating of diminazene and a shell of PEO. One may speculate that diminazene can also stabilize α-helical rich protein structures against pH-induced denaturation.

Superparamagnetic iron oxide particles (SPIO) of maghemite were prepared in aqueous solution and subsequently stabilized with polymers in two layer-by-layer deposition steps as reported in chapter 6. The first layer around the maghemite core was formed by poly(ethylene imine) (PEI), and the second one by poly(ethylene oxide)-*block*-poly(glutamic acid) (PEO-PGA). The hydrodynamic diameter D_H of the particles increased stepwise from 25 nm (parent) via 35 nm (PEI) to 46 nm (PEI plus PEO-PGA) due to stabilization. This was accompanied by a switching of their zetapotentials from moderately positive (+28 mV) to highly positive (+50 mV) and finally slightly negative (-3 mV). By

contrast, the polydispersity indexes (PDI) of the particles remained constant (PDI = 0.15). Mössbauer spectroscopy revealed that the iron oxide, which formed the core of the particles, was only present as Fe(III) in the form of superparamagnetic maghemite nanocrystals. The combination of Mössbauer spectroscopy and X-ray diffraction revealed that the size of the maghemite domains was 12 nm and that only maghemite was present. The coated maghemite nanoparticles were tested to be stable in water and in physiological salt solution for longer than 6 months. In contrast to novel methods for magnetic nanoparticle production, where organic solvents are necessary, the procedure proposed here can dispense with organic solvents. Magnetic resonance imaging (MRI) experiments on living rats indicated that the nanoparticles are useful as a MRI contrast agent. The preliminary MRI experimental results showed that the particles cause a strong MRI contrast and indicated that they possess biocompatibility.

Summarizing the results of this thesis shows that it is possible to synthesis nanoparticles out of maghemite and biocompatible block copolymers with different zetapotentials. These particles are stable under physiological conditions and gave a strong contrast in MRI experiments on rats. With such particles it is possible to investigate the behavior of particles of different zetapotentials and same size and outer shell *in vivo* or *in vitro*.

The ability of the tested blockcopolymers to build complexes with a drug and to stabilize nanoparticles under physiological conditions makes it possible to develop drug loaded contrast agents that combine diagnostic and therapeutic aspects.

The work described in this thesis gives an insight into the broad field of possible applications of peptide functionalized blockcopolymers in diagnostic and therapeutic applications.