8 Summary

This thesis mainly shows two aspects of the phenomenon plasma protein adsorption. The first part of the presented data shows the change of plasma protein adsorption of two different model nanoparticles by varying the physicochemical parameters and keeping the chemical properties of the nanoparticle surface constant. The second part shows a close correlation between the plasma protein adsorption of drug consisting nanoparticles and an *in vivo* animal study.

The 2-D PAGE study of the styrene nanoparticles generated a lot of new information concerning protein adsorption onto different surfaces. The first time it was possible to calculate an exact amount of single protein molecules on the nanoparticle surface using calibration lines. A striking difference between the composition of the protein pattern between the real calculated values and the protein pattern calculated by MELANIE 3 could be shown. Especially the values for albumin show a high deviation. An adsorption value of about 10 %Vol calculated by MELANIE 3 is enough to be the dominating plasma protein on the particle surface. This shows once more the importance of albumin in its function as a dysopsonine. Fibrinogen shows a different behavior. Although its three chains show a high abundance on the pherograms its real proportion on the protein pattern was significantly lower than calculated with MELANIE 3.

The second type of styrene particles had chains on the surface that were positively charged. Although the particle surface was extremely hydrophilic a high protein adsorption could be observed. Due to the hydrophilia of the particle surface it was impossible that hydrophobic interaction between the proteins and the nanoparticles could be responsible for this phenomenon. In this special case it can be assumed that charge interaction is the reason for the protein adsorption onto the cationic surface. Since the most of the proteins have opsonizing properties an application as i.v.-carrier system appears to be not useful.

The second part of the thesis was the detection of the protein adsorption onto PBCA nanoparticles containing the drug Doxorubicin on the surface. An animal study which was attended by a 2-D PAGE study showed some new insights into plasma protein adsorption and a resulting brain targeting.

First of all it could be shown that the plasma protein patterns of PBCA particles that were incubated with Poloxamer 188 were basically identical with those which were incubated with Polysorbate 80. The animal study showed that the *in vivo* efficacy of both nanoparticle formulations were also the same. This fact is a proof for the participation of some plasma proteins in the transport of Doxorubicin over the BBB. Here it could be shown that not only Apolipoprotein E is a mediator for brain targeting because no traces of ApoE could be detected on the pherograms of the formulations. The conclusion was that one or more other plasma proteins are also possible to mediate a drug transport over the BBB. On both gels a significant high amount of ApoA-I and ApoJ could be detected. Both plasma proteins are ligands for certain receptors that are present on the surface of the endothelial cell wall of the BBB. ApoA-I has a high affinity to the so called scavanger receptor class B, type I (SR-BI) which mediates the uptake of the cholesteryl esters of ApoA-I into the cells. ApoJ is a ligand of Megalin, a receptor that mediates the transport of ApoJ into the brain by endocytic uptake. ApoJ would transport the drug into the brain in the same way like ApoE does. If ApoA-I is the main mediator for the transport of drugs into the brain the mechanism would be completely different. ApoA-I could act as an anchor for the drug-containing nanoparticle in front of a cell. It leads to longer holding time of the nanoparticle in front of the endothelial cell. Due to the degradation of the nanoparticle the concentration gradient between the blood stream and the inner cell rises. The drug can diffuse into the endothelial cell and the brain. Both ways are theoretically possible. But the mechanism via ApoA-I seems to be much more sparing for the endothelial cells. The data presented in the thesis do not disprove the meaning of ApoE for brain targeting but do enlarge the old ApoE-theory with two other plasma proteins. In the middle of the nineties the SR-BI was not known as a receptor on the BBB and could not be included into the theory. The theory of ApoA-I is also supported by the data of Gessner generated by analyzing the protein adsorption patterns of “lipid drug conjugates” (Gessner, Olbrich et al. 2001). Animal studies to

proof the concept of ApoA-I and ApoJ mediated targeting of the brain are in preparation.

Another important aspect of the study with the PBCA particles was that the protein patterns of the nanoparticles after incubation in plasma depends on the species. The adsorption patterns of the nanoparticles that were generated with human plasma were significantly different to those which were generated with rat plasma.

If the adsorption of ApoA-I is the key step for a transport of Doxorubicin into the brain it seems to be very likely that in humans a transport of Doxorubicin will not take place. On the pherograms of the human plasma the amount of adsorbed ApoA was too low. Here are big differences – at least in the adsorption kinetics. The aspect of species-depending protein patterns of nanoparticulate drug carrier systems must be considered by the performance and evaluation of future studies.