7 Summary

This thesis investigated the adsorption of plasma proteins on various colloidal drug carriers by the mean of two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). The main focus was on solid lipid nanoparticles (SLN), as they showed a very good tolerability *in vivo* and do not have any problems with respect to large scale production. Moreover, fat emulsions, gelatin nanoparticles and core-shell latex nanoparticles (MC81cs) were investigated.

For the transfer of 2-D PAGE analysis to SLN, gel filtration was established as reliable method to separate all types of SLN from excess plasma. Beside this, the optimal parameters of sample preparation of cetyl palmitate-SLN via centrifugation were determined (three washing steps using 20 mM phosphate buffer pH 7.4). Incubation of SLN in plasmas of different donors, different stabilisation of the plasmas (sodium citrate vs. sodium EDTA) and freezing of the plasmas over four months at –70°C had no influence on the resulting protein adsorption patterns. Incubation of selected SLN formulations in serum revealed no complement activation. Furthermore, it was shown that linear gradient IPG-strips are a good alternative to distinguish between adsorbed amounts of apoC-III, apoC-II and apoA-II.

Thereafter, SLN were surface-modified with a range of interesting surfactants to assess the influence on the resulting protein adsorption patterns. Priority objective was enrichment of apoE on the surface of SLN, to investigate an *in vivo* well-tolerable carrier, which is able to transport drugs across the blood brain barrier (BBB). An important result was that apoE and apoA-VI were preferentially adsorbed, when the SLN were stabilised with block-copolymers having a low number of polyethylene oxide units. Especially poloxamer 235, as an inhibitor of P-glycoprotein (P-gp), is a promising surfactant to be used to deliver drugs to the brain, because it showed the highest apoE/apoC-II ratio. Moreover, it was shown that the amount of adsorbed apoE and apoA-VI on SLN decreased with increasing HLB values of the used polysorbate surfactants. Beside poloxamer 235 and polysorbate 80, poloxamer 184, lecithin and TPGS (another P-gp inhibitor) seemed to be the most promising surface-modifiers to optimise plasma protein adsorption patterns on SLN.

In the next step, it was shown that the matrix lipids play an important role for the resulting patterns. However, in case of similar surface hydrophobicities of the bulk

media (e. g. Cetyl palmitate, Compritol or Witepsol E85, respectively), adsorption patterns were very similar.
Furthermore, the adsorption kinetics of plasma proteins on SLN were determined. Employing diluted human plasma, a transient adsorption of fibrinogen was observed on the surface of SLN, which in plasma of higher concentrations was displaced by apolipoproteins. This phenomenon is called “Vroman-effect” and was previously determined on other solid surfaces. However, over a period of minutes and hours, no relevant changes in the composition of the adsorbed proteins on SLN were detected, which was in agreement with former investigated fat emulsions but in contrast to polymer nanoparticles. As there was no competitive displacement of apolipoproteins, the stable patterns may be better exploited for drug targeting than particles with a pattern being very dependent on contact time with plasma. Moreover, the adsorption patterns on physically long-term stable SLN dependent on their age were determined. No considerable changes were found, supporting the same organ distribution independent on storage time (“physiological stability”). These results provide an important basis for the development of SLN for intravenous drug targeting to the BBB.

In the next part of this thesis, the adsorption patterns of fat emulsions as delivery system for intravenous anaesthesia of xenon were determined. Modification of Abbolipid 10% with polysorbate 80 did not lead to the expected higher amounts of apoE. Nevertheless, this formulation showed the best clinical effect. It has to be taken into account, that correlation of plasma protein adsorption data with the observed clinical effects was difficult, because Xenon is able to pass the BBB on his own. Beside this, the clinical results with i. v. injected Xenon-emulsions were quite promising to solve the known economical problems, which occur when using Xenon as an inhalational anaesthetic.

Moreover, plasma protein adsorption patterns on gelatin nanoparticles (gelatin-NP) were determined for the first time. The results showed that not all adsorption patterns of drug delivery systems are dominated by apolipoproteins. The highest percentage of apolipoproteins on gelatin-NP was 3%. In order to bind DNA by electrostatic interactions onto the surface of the gelatin-NP, the quaternary amine cholamine was covalently coupled to the particles. The adsorption patterns of these positively

charged gelatin-NP were slightly modified. However, subsequent binding of an oligonucleotide to the surface of these particles had no effect on the adsorption patterns. Additionally, protein adsorption patterns after incubation in rat plasma were determined. The same conclusions could be drawn when looking on the resulting patterns.

In the final part of this thesis, protein adsorption on core-shell latex nanoparticles (drug loaded and free of drug, respectively) were investigated. The resulting patterns seemed to be similar and once more the apolipoproteins were the dominant proteins (>90%). Moreover, adsorption patterns after incubation in fetal bovine serum (FBS) were determined, as this medium was used for *in vitro* cell-studies. As a result of different protein composition in the media, protein patterns looked different in comparison to human plasma protein patterns. However, among each other they seemed to be similar again. Surprisingly, *in vitro* cell-studies with macrophages (infected by *Toxoplasma gondii*) showed that nanoparticles free of drug (MC81cs) had a therapeutic effect. This effect was even (slightly) stronger than that of nanoparticles loaded with Pentamidine or Spiramycine. It is possible that the effect of MC81cs in counteracting or neutralising the suppression of defence of host cell by the parasite is more effective than the sole or additional effect of the drug (which has a low efficacy anyway). Moreover, percentages of adsorbed opsonins on MC81cs were (slightly) higher in comparison to drug loaded particles, which could be a reason for more efficient phagocytosis, showing the importance of (even minor) variations in plasma protein adsorption patterns.

Investigations of different, intravenously injectable colloidal drug carriers showed that apolipoproteins have a great affinity to SLN, because these particles also have a lipid core like lipoproteins. Surface modifications of SLN using surfactants like poloxamer 235 and polysorbate 80 resulted in accumulation of apoE and the absence of typical opsonins on the surface. The adsorption patterns of SLN were stable, neither they depended on contact time with plasma nor on storage time of the dispersions. In conclusion, SLN are suitable carriers to be biofunctionalised with apoE in the blood and may have a high potential to deliver drugs to the brain.