# 7. Summary

The purpose of this thesis was to develop a system in which the effect and interaction of nanoparticles in infected cellcultures could be measured. Mice macrophage cell line J774-A1 and GFP-transfected *Toxoplasma gondii* were used as a model system. In addition the particles were loaded with drugs to analyze whether the therapy of toxoplasmosis could be improved with drug-carrier-systems. With the use of macrophages which are capable of absorbing drug-loaded particles via phagocytosis it was anticipated that this would lead to an intracellular accumulation of the drug.

A few important physiochemical parameters of nanoparticles were analyzed for a more detailed characterization. The particles used consisted of a non biodegradable polystyrene-core and a polybutylcyanoacrylate-shell which contained the drug (Spiramycin or pentamidine). They were used as standard particles. After leading to promising results in the cell cultures other nanoparticles were synthesized as references. These included core-shell-particles with a polystyrene-core and a polymethylmethacrylate- or polystyrene-shell as well as pure polybutylcyanoacrylate-nanoparticle.

For the investigation in the cell cultures two different sizes of particles were produced to investigate the following statement: The bigger the particles, the better the absorption of the cells (Müller et al., 1997). The measurement of the particle size was done by photon correlation spectroscopy (PCS). The particle charge which was measured as zetapotential (ZP) has also an impact on the uptake of nanoparticles by the cells of the RES. It is shown that colloidal particles are phagocytized the more the higher the value of the zetapotential is (Müller, 1991). The high negative ZP indicated that the particles we used for the investigations were stable dispersions during storage and were appropriate for tests with macrophages. In addition to the effective particle charge density was measured, too. This was an additional parameter for the characterisation of the exact surface composition. These way relations between the effective charge and the chemical character were visible. Another parameter which was important for the uptake of particles is surface hydrophobicity. Hydrophobic nanoparticles are phagocytized in favour of hydrophilic ones. Hydrophobic particles interact with blood components over hydrophobic interdependencies which lead to a higher particle uptake by the macrophages. The loading of drugs had no significant impact on parameters investigated. While standard-particles had a fast biodegrading Polybutylcyanoacrylate (PBCA)-shell, reference core-shell-particles had a slowly degradable respectively not degradable shell. The enzymatic degradation of PBCA showed that the particles were degraded – at least altered in a

way that incorporated drug was released - within 200 minutes. This assured that the particles were degraded and the drug was set free within the 3 days of testing. The enzymatic degradation with porcine liver esterase was traced by measurement of absorption while degradation of particles by hydrolysis was measured via photon correlation spectroscopy PCS.
A prerequisite for the use of nanoparticles as drug-carrier-systems is a high rate of loading with drugs. With that the necessary particle dosis for an application can be reduced.
The relative rate of load of the particles was determined by the amount of drugs remaining in dispersion medium. It was shown that the rate of loading of the standard-particles with the PBCA-shell was higher than 95 percent. The rates of loading of the reference particles with PMMA- and PS-shells were between 65 and 80 percent.
Drug release via enzymatic degradation was carried out in a bimodal process but yielding 50 – 60 percent it was incomplete. A study about the loss of drugs during storage showed that standard particles with a PBCA-shell were stable for more than one year with a remaining drug concentration of more than 90 percent.

In the context of this thesis an *in-vitro* model was developed which made it possible to monitor and evaluate the infections of macrophages with *Toxoplasma gondii* and the treatment with nanoparticles.
An infected cell-culture system, in which the effect of nanoparticles with or without drugs was measured over a period of three days (that is equivalent to several cycles of parasite multiplication) is similar to an animal-model and represents a valuable addition to test-systems Uptake and fate of nanoparticles and parasites was measured with a fluorescence activated cell sorter (FACS). That made it possible to determine the number of macrophages and the percentage of infected or with nanoparticles loaded cells as well as the extracellular parasites. Measuring the green fluorescence of the *T. gondii* and the red fluorescence of nanoparticles the intensity of fluorescence of infected or particle loaded cell corresponds with the concentration of intracellular *Toxoplasma gondii* and nanoparticles respectively. Initially all experiments with FACS should be controlled by microscopy, to prove that the interpretation of the FACS-data is consistent with the images seen under the microscope. With the established model repeatable results could be attained.
During the experiments with the nanoparticles loaded with drugs it was shown that pentamidine had no influence on the system – in opposite, there was a better effect of particles without this substance.

The loading of nanoparticles with spiramycin which is used for therapy of pregnancy-toxoplasmosis was just a little more effective with smaller MC81cs-particles. The bigger nanoparticles were more effective without the drug.
The comparison between drug loaded nanoparticles showed that spiramycin is more effective than pentamidine a suspected result.
The efficiency of the particles without drugs is explained by stimulation of macrophages by internalised particles which could be detected by a strong increase of unspecific autofluorescence of cells after particle uptake. *Toxoplasma gondii* caused an inhibition of macrophages-activity after cell intrusion which could be neutralised by addition of nanoparticles.
An experiment which had to reveal whether nanoparticles could be used as a prophylaxis before a toxoplasmosis showed that this was not the case because the effect of particles on infected cells was the same as on prophylaxis. Prophylaxis and treatment of infected cells resulted both in a reduction of parasite multiplication. On the other side, infection did not alter the uptake of nanoparticles - infected and non-infected cells internalised the same amount of nanoparticles.
The comparison of standard-particles (polystyrene-core and polybutylcyanoacrylate-shell) with the reference-particles of a polystyrene-core and a polystyrene- or polymethylmethacrylate-shell showed that the inhibition of parasite multiplication depends on the amount of internalised particles. The reference-particles had a lower effect on toxoplasmosis although they were phagocytised more than the standard-particles. The same effect was observed comparing bigger and smaller nanoparticles. While the bigger MC80cs-particles were more internalised from the cells the smaller MC81cs-particles showed a better toxoplasmicide effect. According to this a stimulatory effect of nanoparticles on the cells was diminished/reduced by an uptake of to many particles.
An interesting fact is that the smaller nanoparticles were taken up faster on the first day while the bigger particles were taken up continuously over the three-day-period of testing. The cumulative amount of phagocytized bigger particles during the period of testing, resulted in a worse toxoplasmicide effect.
In later experiments the influences of nanoparticles coated with proteins on the system was investigated. It turned out that the saturation had no positive effect compared to the non saturated standard-particles.
Minor differences could be seen between drug-loaded and unloaded-particles while measuring the protein absorption pattern. They were sufficient to achieve a different uptake by the cells. A difference existed in the adsorption of the opsonin IgM for example.

With the presented model effect of nanoparticles on intracellular parasites could be measured and numerous investigations of effects and interactions of nanoparticles on infected cellcultures could be done. With that the system is capable to contribute to the characterisation/optimization of therapeutical interesting nanoparticles with regard to

- size
- charge
- and load (e.g. with drugs)

as well as an alternative to animal-models.