7 Zeta potential of nanosuspensions

7.1 Background for nanocrystals

A prerequisite to achieve an enhancement of the oral bioavailability with drug nanocrystals is that the crystals are finely dispersed in the gut liquid and do not aggregate. In case they start aggregation, the bioavailability decreases with increasing aggregate formation. This is attributed to the fact that they loose special properties of nanoparticles such as their adhesive property to the mucosal wall. Therefore it is necessary to prepare nanosuspensions with a physical stability as high as possible, the aim of the present study. In this study electrostatic stabilisation is combined with steric stabilisation. Optimum stabilisers/stabiliser combinations were identified in a systematic screening based on zeta potential measurements.

7.2 Short introduction to the zeta potential

The particle charge is one of the factors determining the physical stability of emulsions and suspensions. The higher particles are equally charged, the higher is the electrostatic repulsion between the particles and the higher is the physical stability. Typically the particle charge is quantified as the so called zeta potential, which is measured e.g. via the electrophoretic mobility of the particles in an electrical field. Alternatively the particle charge can be quantified in surface charge per surface unit, determined by colloid titration. In this thesis the charge is characterised by the zeta potential.

The zeta potential theory is described in very detail in the literature (Müller 1996), here only a brief explanation is given. In general particles posses a surface charge, which occurs due to the dissociation of surface functional groups, the so called Nernst potential. Of coarse the degree of dissociation of the functional groups depends of the pH of the suspension, therefore the zeta potential is pH dependent. This is important for the nanosuspensions because they undergo a pH change from the acidic medium in the stomach, increasing to around pH 7 in the gastro intestinal tract (GIT). Therefore in the study the effect of the pH on the zeta potential of the cyclosporine was important to be determined.

In electrolyte containing media, ions from the dispersion medium adsorb onto the particle surface. For this model description a negative Nernst potential is assumed. In general the first absorbed monolayer of ions consists of negatively charged, fixed and dehydrated ions, the so called inner Helmholtz layer. The second monolayer absorbed consists of positively charged, fixed but hydrated ions, the so called outer Helmholtz layer. Both Helmholtz layers together are called the Stern layer. The not yet compensated negative charge of the surface is compensated by

freely diffusing counter ions in the so called “diffuse layer”. The border of the diffuse layer is defined where the particle surface charge is fully compensated (simplified model description).

Figure 7-1 upper shows the course of the potential from the surface to the different layers. The negative Nernst potential increases further in the inner Helmholtz layer due to the adsorption of negative ions, followed by a slight decrease in the outer Helmholtz layer. Increase and decrease are linear. In the diffuse layer decays exponentially towards zero due to the positively charged counter ions.

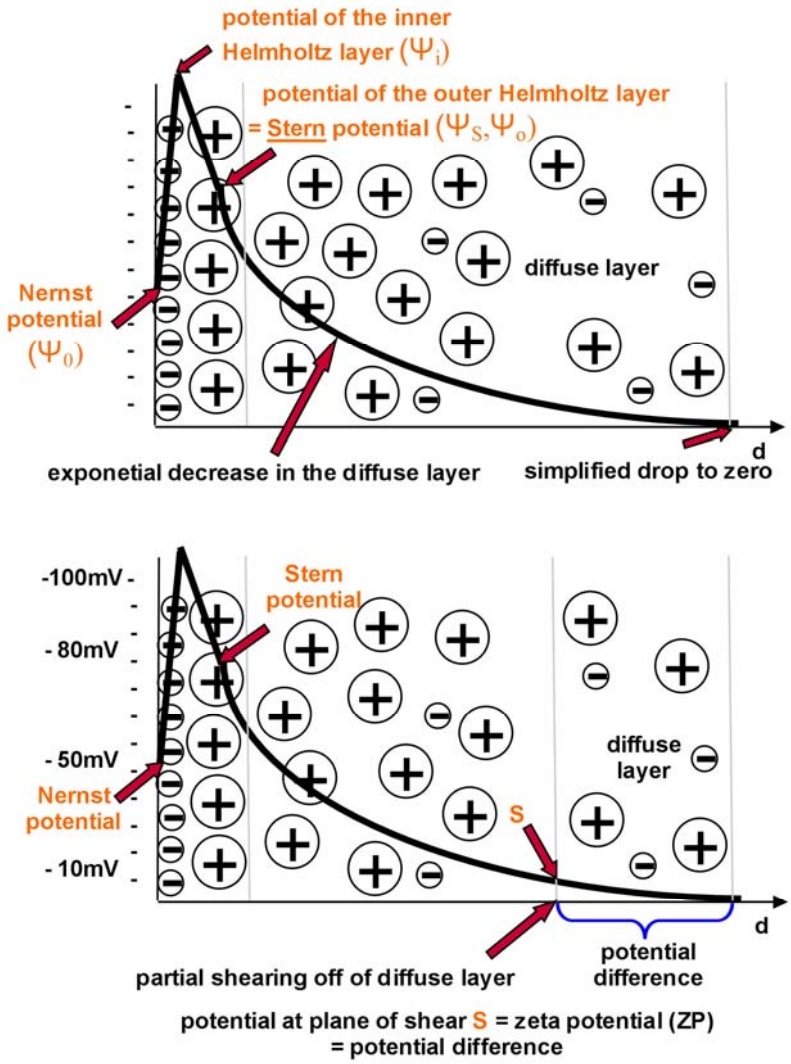

Figure 7-1: Stern model of the zeta potential theory showing the course of the potential in the different layers (upper) and the composition of the different layers (lower).

The zeta potential is determined by measuring the electrophoretic particle velocity in an electrical field. During the particle movement the diffuse layer is shorn off, hence the particle obtains a charge due to the loss of the counter ions in the diffuse layer this potential at the plane of shear is called the zeta potential.

With increasing electrolyte concentration, the surface charge will be compensated at a lower distance from the particle surface, which means the potential drops faster and the diffuse layer is thinner. Consequently, the measured zeta potential decreases with an increasing electrolyte concentration, whereas it decreases faster with increasing valency of the counter ions, that means increasing from sodium to e.g. calcium and aluminium. Consequently the stability of the suspensions is reduced. Therefore in this study the effect caused by salts with increasing valency of the positively charged ions was studied.

Charged surfactants like sodium dodecyl sulphate (SDS) adsorb with the negatively charged part of the molecule onto the particle surface and form the inner Helmholtz layer. Adsorption takes place according to the theory of adsorption isotherms. The surface coverage increases with an increasing SDS concentration until full surface coverage is obtained (plateau of the adsorption isotherm). Consequently the potential of the inner Helmholtz layer increases with the increasing surface coverage, leading subsequently to an increase of the zeta potential and an increase in the physical stability of the suspensions. Therefore in this study also the effect of an increasing concentration of stabiliser was investigated. At high stabiliser concentrations, well above of the plateau of the adsorption isotherm, electrolyte stabilisers can cause a decrease in the diffuse layer leading to a decreased zeta potential and a decreased physical stability. Therefore it is important to find the optimal concentration for a stabiliser.

The measurement itself is a particle electrophoresis, the particle velocity is determined via the doppler shift of the laser light scattered by the moving particles. The field strength applied was 20 V/cm. The electrophoretic mobility was converted to the zeta potential in mV using the Helmholtz-Smoluchowski equation. At standard measuring conditions (room temperature of 25 °C, water) this equation can be simplified to the multiplication of the measured electrophoretic mobility (µm/cm per V/cm) by a factor of 12.8, yielding the ZP in mV.

To study different aspects of the adsorption of ionic and non ionic stabilisers, different measuring media had to be applied. For the determination of the Stern potential measurements were performed in bidistilled water having its conductivity adjusted to 50 µS/cm. Using a standard conductivity adjusted with a 1:1 electrolyte avoids fluctuations in the zeta potential due to variations in the conductivity of distilled water which can range from approx. 1 up to 10 µS/cm, especially when the sample itself contains also electrolytes when adding it to the distilled water. For detailed explanations see (Müller 1996).

As a rule of thumb, suspensions with zeta potential above 30 mV (absolute value) are physically stable. Suspensions with a potential above 60mV show excellent stability. Suspensions below 20mV are of limited stability, below 5mV they undergo pronounced aggregation (Müller 1996).

7.3 Combined steric and electrostatic stabilisation

As outlined above the zeta potential and physical stability decrease at increasing electrolyte concentration. Electrolytes are present in the gastrointestinal tract and the contact of the nanocrystals with these electrolytes cannot be avoided. Electrostatic stabilisation is reduced in its efficiency in an electrolyte containing environment. To compensate for this it is ideal to use steric stabilisers, which are less impaired in their effect by electrolytes, ideally one combines electrostatic and steric stabilisation. The contribution of the electrostatic stabilisation results from the charged groups on the nanocrystal surface and/or on additionally adsorbed electrostatic stabilisers. The steric contribution results from added steric stabilisers such as Poloxamer or TPGS.

Of course it should be noted that adsorption of a steric stabiliser layer leads to a reduction of the measured zeta potential, which is however not an indication of a reduced electrostatic repulsion. The adsorption layer of the stabiliser shifts the plain of shear, at which the zeta potential is measured, to a larger distance from the particle surface (Figure 7-2). Consequently the measured zeta potential is lower. In such cases zeta potentials of about 20mV are still sufficient to fully stabilise the system in combination with steric stabilisation.

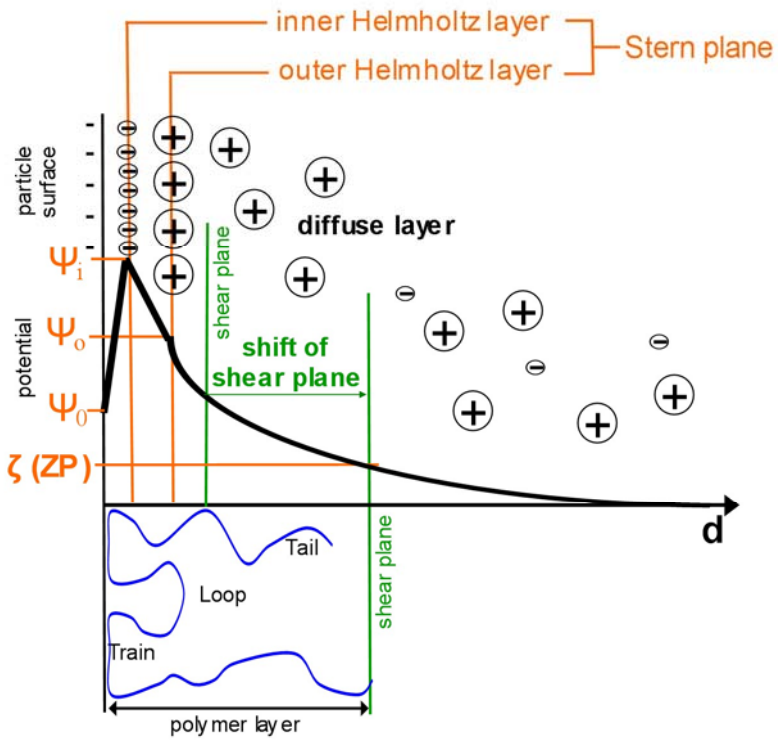

Figure 7-2: Adsorbed polymer layer shift the plane of shear further away from the particle surface as a function of the adsorbed layer thickness, protecting the covered part of the diffuse layer against being shorn off in the electrophoretic zeta potential measurement , thus leading to lower measured zeta potentials (despite an unchanged surface charge and remaining electrostatic stabilisation)

7.4 Considerations for selection of stabilisers

All stabilisers were carefully selected according to the following considerations:

SDS is an excellent electrostatic stabiliser with high affinity to absorb onto particle surfaces leading to high zeta potentials. It is a regulatory accepted stabiliser for oral dosage forms (e.g. tablets and capsules) and is therefore suitable to be used in nanosuspensions.

Dehyquart is a positively charged electrostatic stabiliser and is of interest to increase the adhesion of positively nanocrystals to the negatively charged GIT wall, thus further enhancing the oral bioavailability.

Sodium glycocholate was selected because it is an electrostatic stabiliser and bile salt, known to play an important in enhancing the bioavailability of orally administered Sandimmun. Therefore it might be beneficial to use it in combination with cyclosporine nanocrystals.

Poloxamer 188 is a well known efficient steric stabiliser leading to adsorption layers of up to 7nm in thickness (Müller 1991). It proved to be sufficient for an efficient steric stabilisation despite the fact that the theoretical minimum thickness for complete steric stabilisation is about 10nm. Poloxamer 188 is also regulatory accepted, even for intravenous administration, therefore also highly suitable to be used in nanosuspensions. In addition, Poloxamer 407 was used because it has a higher molecular weight and leads to even thicker adsorption layers of up to 12nm (Müller 1991). However, it is not yet accepted for i.v. use, but formulations for oral administration are in clinical phases.

Tween 80 was selected because it proved previously to be an efficient stabiliser for nanosuspensions (Jacobs 2000). It is also very well tolerated because it is accepted for i.v. injection.

Chitosan is a compound which combines the electrostatic stabilisation due to its positive charge, and the steric stabilisation because of its polymeric nature. In addition it is a well know mucoadhesive. That means theoretically it should be the ideal stabiliser because it combines electrostatic and steric stabilisation, together with bioavailability enhancement due to mucoadhesion.

The stabilisers mentioned above increased physical stabilisation, or combined it with bioavailability enhancement by mucoadhesion (chitosan). TPGS has a steric stabilisation effect by its PEG part in the molecule; in addition it can enhance the bioavailability due to p-glycoprotein inhibition. Therefore it was selected to complement the spectrum.

7.5 Influence of stabiliser concentration

To study the effect of different stabilisers, a cyclosporine nanosuspension was produced using 0.5% of Poloxamer 188 as stabiliser (this suspension is abbreviated in the figures below). To this stock suspension the stabilisers discussed in 7.4 were admixed. This approach allowed it to produce one stock cyclosporine nanosuspension for the complete study and saved expensive cyclosporine.

The alternative would have been to prepare a separate batch of 40.0g with each stabiliser.

In the discussion of the results it should be noted that zeta potentials are discussed as absolute values, not considering the sign of the zeta potential. That means a change from e.g. -20mV to -50mV is an increase in the zeta potential (and not a decrease when one would consider the sign). This is in agreement with the general literature to have the correlation that an increase in zeta potential leads to an increase in physical stability. For the increase in stability it does not matter if the zeta potential is positive or negative.

The two negatively charged stabilisers SDS and sodium cholate led to a zeta potential of about -30mV even at the low concentration at 0.01mmol. SDS proved most efficient with a zeta potential of about -70mV at 10mmol, from this being the most suitable stabiliser of the two.

The two positively charges stabilisers led – as expected – to a charge reversal. The zeta potential for both surfactants was in the range of about +20mV to +30mV. The magnitude of the zeta potential is exactly in the range as observed previously for such steric stabilisers (Müller 1991), the reduction being caused to the shift of the plane of shear. These nanocrystals should provide still a sufficient electrostatic stabilisation, in combination with a positive charge to adhere to the wall of the GIT.

The Poloxamer polymers show a decrease of the zeta potential with increasing molecular weight from Poloxamer 188 to 407. In addition there is a general tendency of a further decrease with increasing polymer concentration (especially for Poloxamer 407). The decrease in zeta potential confirms the formation of a sterically stabilising adsorbed polymer layer.

Tween 80 and TPGS show an even more pronounced reduction in zeta potential with increasing concentration compared to the two Poloxamers. This was unexpected because of the lower molecular weight of the two compounds. The only potential explanation is a more flat adsorption of the Poloxamers and a densely packed, thicker adsorbed layer of Tween 80 and TPGS - due to a difference in affinity to the surface. To sum up, from the zeta potential concentration profiles all investigated stabilisers can be judged as being suitable for the stabilisation of cyclosporine nanocrystals.

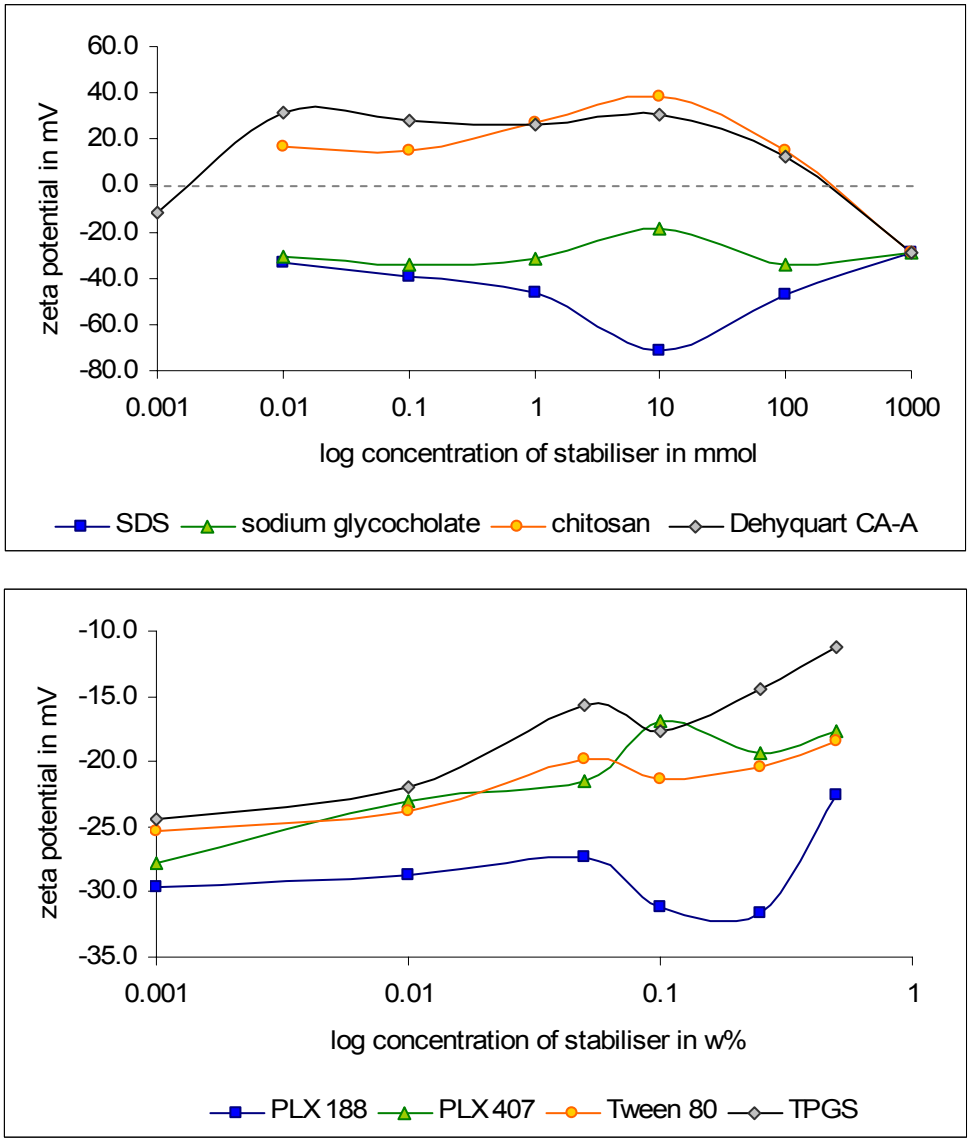

Figure 7-3: Dependence of the zeta potential on the concentration of stabilisers used.
upper: negatively charged surfactants; SDS and sodium glycocholate, positively charged surafactants; chitosan chloride and Dehyquart.
lower: steric stabilisers; Poloxamer 188 and 407, Tween 80 and p-glycoprotein inhibitor TPGS

7.6 Influence of pH

Figure 7-4 shows the zeta potential profil of the stock nanosuspension (NS; stabilised with 0.5% Poloxamer 188) and the suspensions after addition of another 0.5% Poloxamer 188 (NS+ PLX 188 0.5%) and the suspension after addition of another 0.5% Poloxamer 188 and 0.5% Poloxamer 407). With decreasing pH the electrolyte concentration increases, leading to a reduced zeta potential. This is even more pronounced because the measured zeta potential is reduced anyway in case of adsorbed polymers (shift of plane of shear). At low pH values 1 and 2 the zeta potential is practically zero (i.e. in stomach), that means the nanocrystals are only stabilised by the steric stabilisation effect. When reaching pH 2, the zeta potential for all Poloxamer mixtures is around -20mV, sufficient for a stable suspension in case of combined steric and electrostatic stabilisation. In general the zeta potentials are lower with increasing Poloxamer concentrations in the nanosuspension, being lowest for Poloxamer 188 and Poloxamer 407 combined stabilised nanosuspensions.

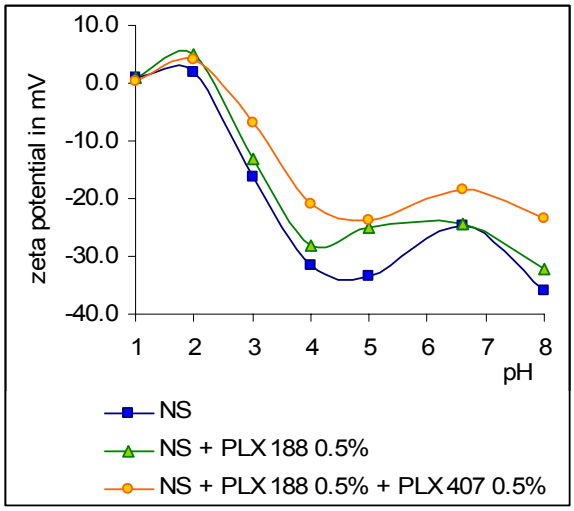

Figure 7-4: Zeta potential-pH profile of cyclosporine stock nanosuspension NS (stabilised with 0.5% PLX 188) and nanosuspensions after addition of additional PLX 188 0.5% and after addition of a mixture of PLX 188 0.5% + PLX 407 0.5%)

Figure 7-5 shows the nanosuspension after the addition of a mixture of PLX 188 0.5% + SDS 10^{-3}M, compared to the original stock suspension (NS) and the stock dispersion NS + PLX 188 0.5%. The concentration of 10^{-3}M SDS was chosen because in previous studies it provided the highest zeta potential as a function of SDS concentration. The figure clearly shows that SDS addition had practical no effect ad low pH values of 1 and 2, because the electrolyte concentration is very high, reducing the thickness of the diffuse layer and the zeta potential. Some limited effect was seen for SDS addition around pH 5-7 in low electrolyte conditions.

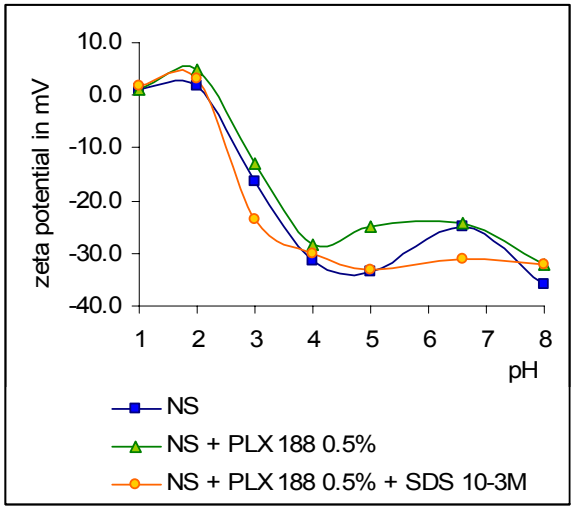

Figure 7-5: Zeta potential-pH profile of cyclosporine stock nanosuspension NS (stabilised with 0.5% PLX 188) and nanosuspensions after addition of additional PLX 188 0.5% and after addition of a mixture of PLX 188 0.5% + SDS 10^{-3}M

Highest zeta potential values were observed with the nanosuspension co-stabilised with SDS 10^{-3}M. Admixing of chitosan to the stock nanosuspension NS led to a charge reversal as already seen in Figure 7-3 (upper). The positive charge of chitosan of around +30mV was very little influenced by the pH in the pH range from 2-7, thus making it ideal as a stabiliser for the cyclosporine nanosuspensions. Due to the excellent properties of chitosan Dehyquart was not further investigated (Figure 7-6).

TPGS showed a pronounced pH dependency, leading to a zeta potential below approximately -10mV at pH 3 and a zeta potential of around 0 at pH 1 and 2 (Figure 7-6). Because TPGS has a lower molecular weight than the Poloxamers, instabilities in the stomach cannot be excluded.

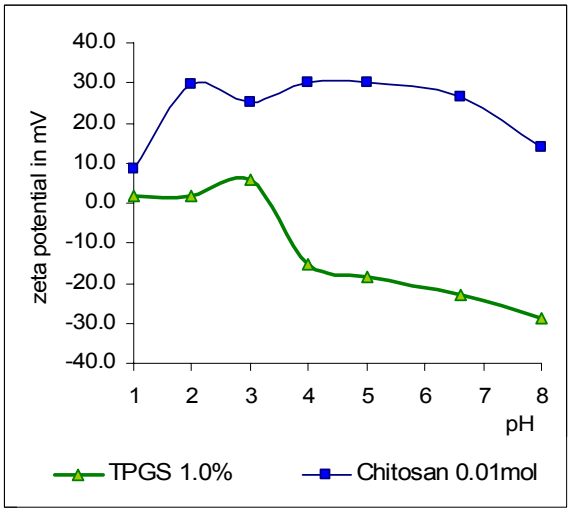

Figure 7-6: Zeta potential-pH profile of cyclosporine nanosuspensions after addition of additional chitosan 1.0% and after addition of a TPGS 1.0%

However - as discussed above – the packing density on the surface might be higher compared to more flatly adsorbed Poloxamer, potentially preventing destabilisation. Final judgement is only possible after an in vivo study. To avoid nanocrystal aggregation in the stomach, the tablets can be coated with an enteric coating releasing the nanocrystals after the passage of the stomach.

Exemplarily for the size effects one figure is presented (Figure 7-7). Of course the electrolyte concentration and the effect of the zeta potential affect the particle size. Highest zeta potential at simultaneously low electrolyte concentration led to preservation of the smallest particle size. Consequently at the pH of water (approximately pH 5) the size is lowest for the stock nanosuspension NS, even more pronounced for the better stabilised nanosuspensions with additional PLX 188 0.5% and the combined mixture of PLX 188 0.0% + SDS 10^{-3}M (Figure 7-7). Moving to lower or higher pH values with increased electrolyte concentration leads consequently to higher sizes. There is no steady increase in size with e.g. decreasing pH in Figure 7-7, values show rather fluctuation in size. At the first glance this seems to be contradictory, but might be explained by the fact that aggregation under reduced stability conditions is a statistical phenomenon and does not create necessarily a linear relationship at least the data clearly show a reduced stability as indicated by the size increase at lower pH values. Some smaller particle sizes measured especially at pH 1 and 8 can not be explained.

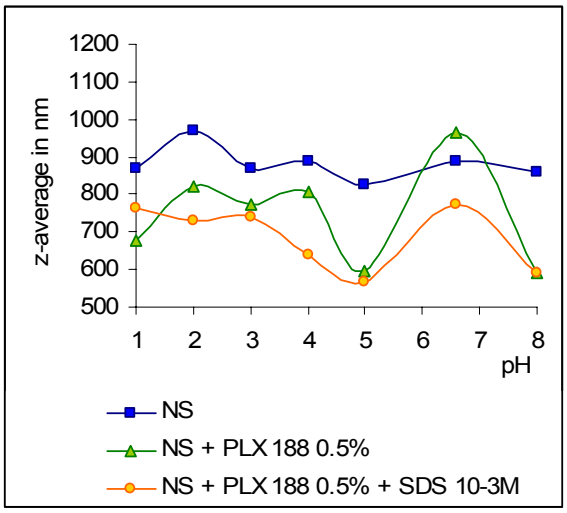

Figure 7-7: Particle size of nanosuspensions at different pH values adjusted with HCL and NaOH, respectively, determined by PCS approx. 15min after pH adjustment

7.7 Influence of electrolytes

In chapter 7.6 the effect of electrolytes in the form of 1:1 electrolytes (HCL, NaOH) could already be demonstrated when adjusting the different pH values. As the next part of this study a systematic investigation was performed adding 1:1, 2:1 and 3:1 electrolytes in increasing

concentrations to the nanosuspensions. Nanosuspensions and electrolyte solutions were mixed this way that they yielded increasing percentages as shown in Figure 7-8 and Figure 7-9. The electrolyte concentrations were chosen this way that the concentration was increased stepwise from 0 to 0.9% (physiological concentration in case of NaCl), in addition an approx. "double isotonic" concentration of 2% was tested.

Theoretically the 2:1 and the 3:1 electrolytes should show a much more pronounced decrease in zeta potential compared to 1:1 electrolytes. However the percentages selected were so high that the differences between the different types of electrolytes were minimised, a strong drop in zeta potential occurred with all salts already at 0.1% for all negatively charged nanosuspensions (below -5mV). A less pronounced drop in zeta potential to about -10mV was observed for the nanosuspensions additionally stabilised with SDS (Figure 7-8).

In contrast, the positively charged nanosuspensions, stabilised with Dehyquart, exactly showed the behaviour expected from the theory. The decrease in zeta potential was more pronounced when moving from NaCl to $MgCl_2$/ $CaCl_2$ and to $AlCl_3$. In the case of $AlCL_3$ the zeta potential dropped straight to zero at 0.1 %.

In Figure 7-9 the situation is shown in a clear and simplified way. It compares the zeta potentials of the different stabilisers investigated at an isotonic concentration of sodium chloride. All negatively charged nanosuspensions possess a zeta potential between approx. -1 and -5mV, the nanosuspension additionally stabilised with SDS shows a potential of -10mV. The Dehyquart nanosuspension has also a zeta potential of almost 10mV, of course +10mV because of the positive charge of the stabiliser.

To summarise, the zeta potential of all nanosuspensions investigated shows a strong decrease with an increasing electrolyte concentration, thus the steric contribution for stabilising the systems is very important. The best stability against electrolyte addition showed the nanosuspensions additionally stabilised with SDS and additionally stabilised with the positive stabiliser Dehyquart, both exhibiting zeta potentials of about 10mV. Nanosuspensions stabilised with chitosan showed some instabilities after one day of storage, therefore the use of chitosan stabilised nanosuspensions appears only sensible in form of dry product, e.g. nanocrystals in a tablet.

The conclusion from the study is therefore to combine a steric stabiliser such as Poloxamer with either SDS to obtain a negatively charged suspension or to combine it with a positive stabiliser such as Dehyquart to achieve additionally adhesion to the GIT wall. Regarding to the zeta potential both systems are of similar physical stability.

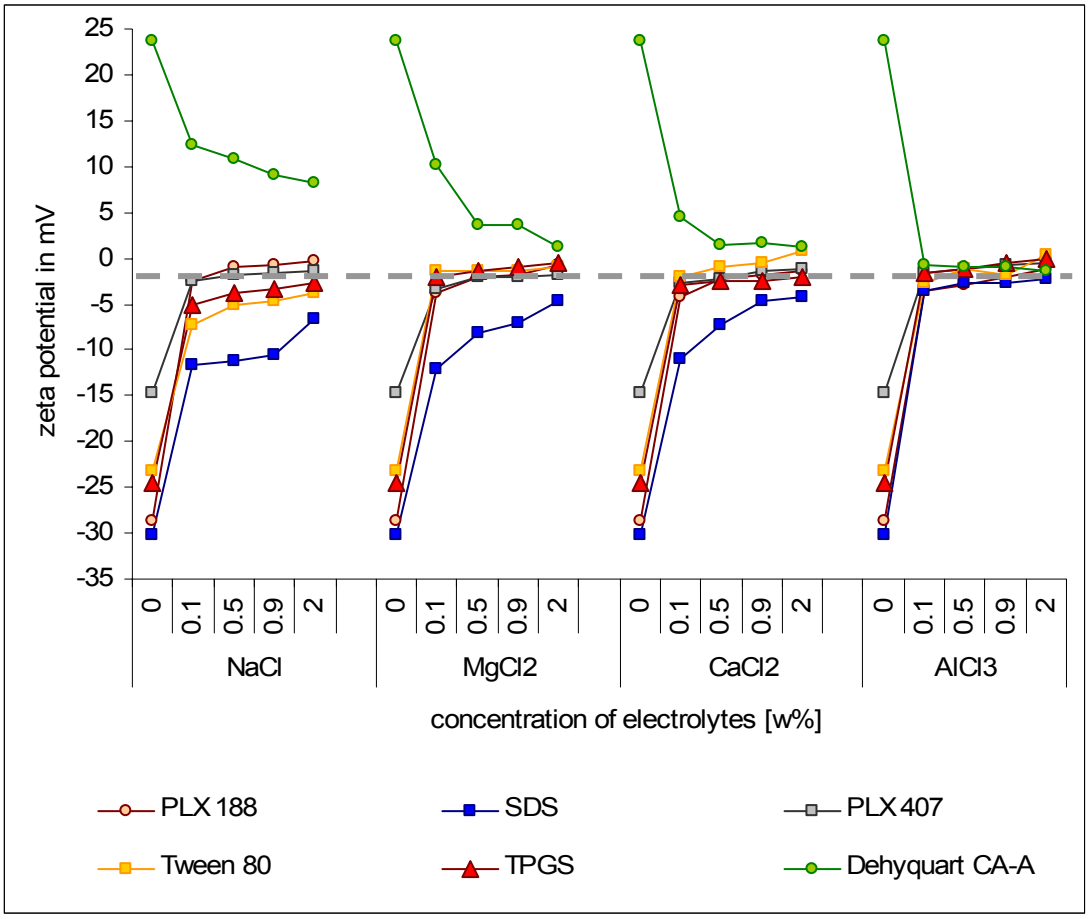

Figure 7-8: Zeta potentials of nanosuspensions stabilised with additional various stabilisers as a function of increasing concentration of 1:1 (NaCl), 2:1 ($MgCl_2$, $CaCl_2$) and 3:1 ($AlCl_3$) electrolytes.

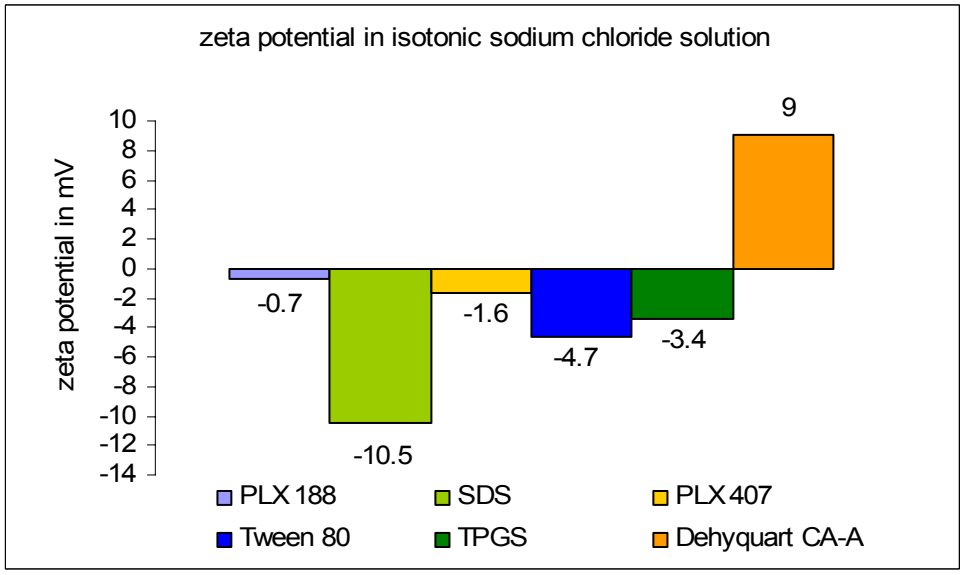

Figure 7-9: Zeta potentials of nanosuspensions stabilised with additional various stabilisers in isotonic sodium chloride solution