6 Cyclosporine nanosuspensions

6.1 Production and characterisation by application of the optimised analytics and comparison with the conventional analytics

In the past many work was done to show the applicability and the efficiency to produce stabile nanaocrystals by applying high pressure homogenisation. The review of publications offers a broad range of compounds which were transferred into nanocrystals by high pressure homogenisation (Müller 1995; Muller and Peters 1998; Müller 1999; Grau et al. 2000; Jacobs 2000; Kayser 2001; Krause and Muller 2001; Krause and Muller 2001; Muller et al. 2001; Jacobs and Muller 2002; Kayser 2002; Akkar and Muller 2003; Kayser et al. 2003; Akkar 2004; Fichera 2004; Möschwitzer and Müller 2004; Mueller and Keck 2004). However in the previous chapters it was shown how sensitive and changeable all results are, which were obtained from laser diffractometry. The aim of this work was to investigate the capability and the meaning of the results obtained by the application of the newly established guide lines from the previous chapters by a comparison of the results which would have been obtained by the application of the "older" standard. On purpose a sensitive drug, namely cyclosporine, was chosen. Cyclosporine is special because of its unique properties and for the need of a more safe and reliable dosage form on the market. It is inversingly proportional soluble to the temperature. It shows liquid crystal behaviour (O'Leary et al. 1986; O'Donohue et al. 1995; Augustijns et al. 2000; Lechuga-Ballesteros et al. 2003). However it is still the most common used drug after organ transplantation. Problems with the formulations are poor bioavailability, as well as undesired side effects such as nephrotoxicity or an increase in blood pressure which can lead to organ drop out or death (Runge 1998; Penkler 2003). Also it is a substrate for cytochrome (cyp) 450 3A4 and a substrate of p-glycoprotein; also it inhibits p-glycoprotein in a dose dependent manner. Up to today there is no optimal dosage form for application on the market. The most common ready to use drug is Sandimmun Optoral®, which is a microemulsion pre-concentrate. Problems here are less a variable bioavailability but the high plasma peak shortly after administration, which can cause nephrotoxicity. Though this work not only investigates the advantages of an optimised analytic, but it also investigates a new formulation approach for cyclosporine. In respect to its limited bioavailability due to p-glycoprotein, as a novel approach only stabilisers which are known to modulate the function of p-glycoprotein were chosen to formulate the nanosuspensions. Those stabilisers are Tween 80, SDS, PLX 188, PLX 407, polyvinylpyrrolidone (PVP) 25k and PVP 40k and especially TPGS, known to be a potent inhibitor of p-glycoprotein. Other stabilisers were chosen

because of its property to enable mucoadhesivness. Stabilisers used because of this reason were chitosan and Dehyquart. Special focus was put on the investigation of the long term stability and its particle size characterisation. The measurement of the zeta potential was performed in order to investigate the stability and to investigate the potential of aggregation due to changes in pH, which occur during the passage of the gastrointestinal tract (GIT).

6.1.1 Production of cyclosporine nanosuspensions

The production of a nanosuspension is the transformation of a macrosized powder into a mostly aqueous nanosized suspension. Therefore the first step is the wetting of the dry powder with a solution of the stabiliser of the suspension. Nanosuspensions can be prepared with concenentrations of up to 40% (w/w). High pressure homogenisation is a high energy process and stresses the coumpound. In order to minimise the stress for the drug, as well as to minimise the friction of the homogenisation material (e.g. valve, valve seat) some pre-homogenisation steps are performed. First the macro suspension is homogenised with an Ultra Turrax (UT), aggregates are destroyed and very large particles are diminuted. Normally the homogenisation via Ultra Turrax is performed for about 60s without repetition. In previous work it was shown, that it is more effective but if the procedure is repeated three times and the time of one homogenistaion step is only 30s instead of 60s. The procedure avoids an increase of the temperature and reduces the appearance of foam, which is caused by the addition of the stabiliser. For an optimal energy input, the rotor-stator of the Ultra Turrax needs to be placed near to the bottom of the beaker. The speed of the Ultra Turrax can reach up to 10,000 rpm. It needs to be increased slowly to avoid splashing and extraordinary foaming of the suspension (Keck 2003). Prior to the homogenisation of 1500bar, several cycles are processed at lower pressures (normally 3x100bar, 3x500bar, 3x1000bar). High pressure homogenisation with solid compounds normally requires the application of about 20, sometimes 40 cycles at 1500bar. After this the maximal dispersity of the suspension is reached. However in some cases the final dispersity is already reached at an earlier stage. Further homogenisation of those suspensions can lead to an increase in particle size and a destabilisation of the suspension. Therefore the progress of the homogenisation needs to be monitored as a function of cycles. The monitoring is performed by laser diffractometry and photon correlation spectroscopy. In this study also light microscopy was applied. The homogeniser used for small scale production was the APV 40. The quantity of one batch is 40 ml. The APV 40 is a discontinuous homogeniser, which means that the homogenised product remains in the product container. For the next homogenisation cycle it needs to be poured back into the bulk container by hand. The procedure therefore is the transfer of the suspension from the product

container into a beaker, followed by the transfer from the beaker to the bulk container. A direct transfer from product to bulk container is not possible as the product container is too large and the opening of the bulk container is too small. This procedure requires some skills and experience. A loss of volume occurs due to the transfer, there is always a loss of suspension between each cycle. The APV homogeniser can homogenise volumes as little as 20ml. If the volume of the suspension becomes smaller, the volume must be increased, to ensure a correct homogenisation. This is typically done by diluting the suspension with the stabiliser solution. The increase in volume also increases the dilution medium of the particles, which may lead to a change of the system. Up to now no investigation was done on this dilution step.

The aim of the study was to investigate the optimal parameters for the production of cyclosporine nanosuspensions. Parameters such as changes due to dilution, influence of production temperature, concentration of cyclosporine and type of stabilisers were investigated. All analytics were performed using the guide lines, which were established ahead. For comparison the old methods for measuring LD were applied as well, to show possible differences of the results obtained.

6.1.1.1 Influence on production temperature

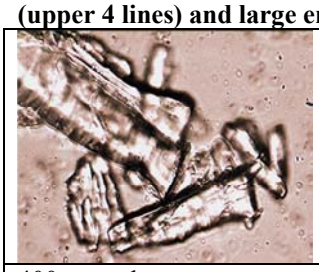
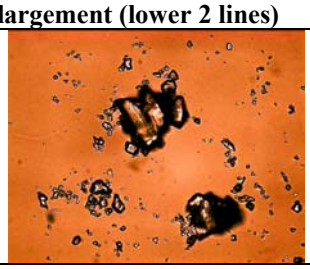
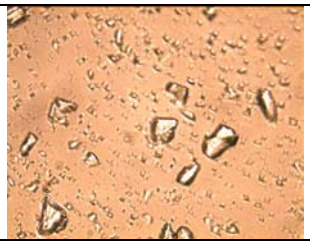
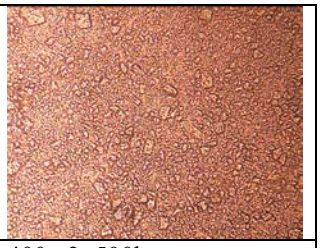
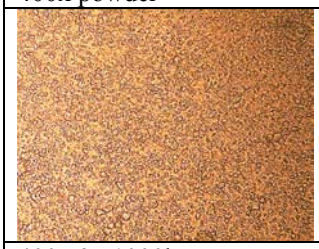
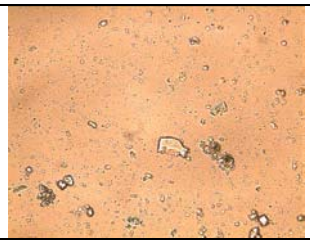
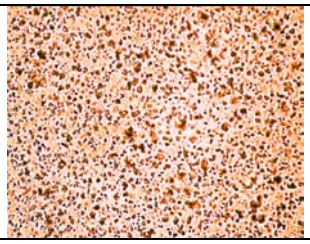
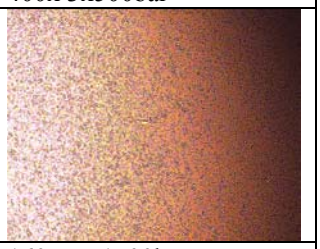
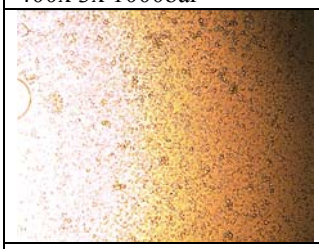
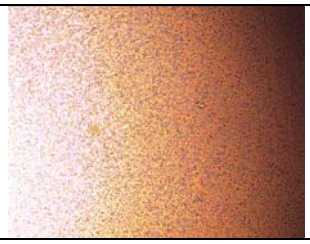
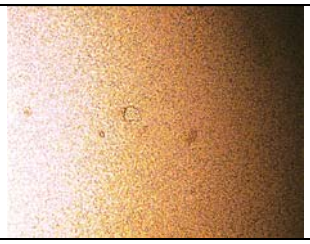
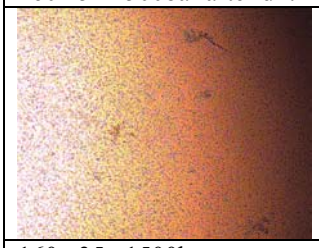
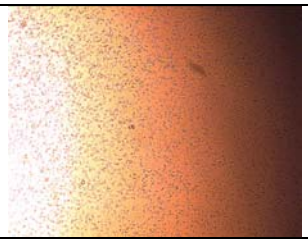
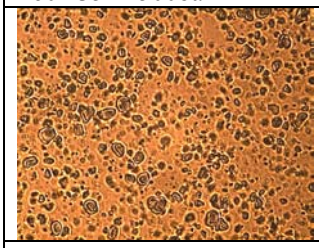
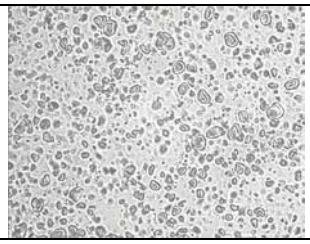
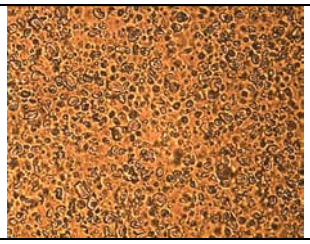
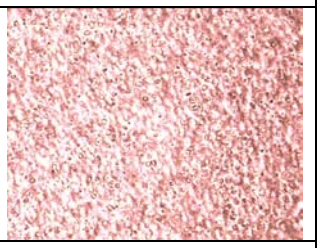
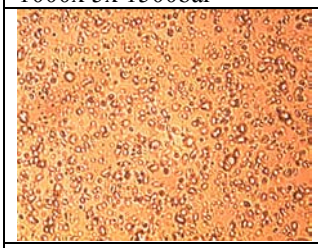
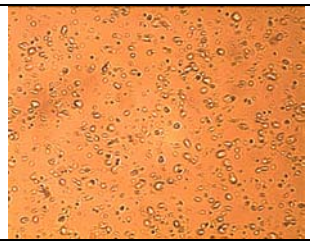
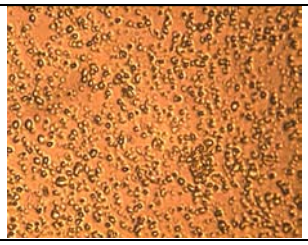
A macrosuspension containing 4% (w/w) of cyclosporine and 0.5% of Poloxamer 188 as stabiliser was produced. The suspension was pre-homogenised using an Ultra Turrax (3x30s, maximal 10,000rpm). Then the suspension was homogenised at 100bar, 500bar and 1000bar (3 cycles each), followed by 40 cycles at 1500bar. Particle size characterisation was performed after homogenisation with the Ultra Turrax and after three cycles of 100bar, 500bar and 1000bar. The progress of diminution at 1500bar was observed at cycle 1, 5, 10, 20, 30, 35, and 40. Additionally the particle size has been investigated after the dilution of the suspension which was performed after cycle 5 at 1500bar.

The temperature of the nanosuspension during the production was varied in order to investigate the influences on the quality and the stability of the resulting nanosuspensions. For that productions were conducted without temperature control (= previous method) and at temperatures below 50°C and below 30°C.

6.1.1.1.1 Conventional method – with no temperature controlling

Table 6-1 shows the microscopic images obtained from the production without temperature control. A smaller magnification (160fold or 400fold) was chosen as it enables a better observation of changes within the system.

Tabelle 6-1: Microscope images from the production of cyclosporine nanosuspension small magnification (upper 4 lines) and large enlargement (lower 2 lines)

			
400x powder	400x 3x UT	400x 3x 100bar	400x 3x500bar
			
400x 3x 1000bar	400x 1x 1500bar	400x 3x 1500bar	160x 5x 1500bar
			
160x 5x 1500bar after dil.	160x 10x 1500bar	160x 20x 1500bar	160x 30x 1500bar
			
160x 35x 1500bar	160x 40x 1500bar		
			
1000x 5x 1500bar	1000x 5x 1500bar after dil.	1000x 10x 1500bar	1000x 20x 1500bar
			
1000x 30x 1500bar	1000x 35x 1500bar	1000x 40x 1500bar	

Good diminution was reached from dispersion by Ultra Turrax and low pressures 100-1000bar. After one cycle of 1500bar large aggregates and crystals appeared. Further homogenisation destroyed the aggregates and the particle sizes decreased again. The dilution of the suspension after cycle No.5 at 1500 bar lead to a new appearance of aggregates. The observed aggregates could be destroyed during the next 5 cycles. After 20 cycles of homogenisation new large crystals and some aggregates were observed. With the following cycles no de-aggregation could be reached, also the amount of large crystals increased. In the last two lines images with 1000fold magnification are shown, proving that too large magnification cannot observe marginal changes of the system. Here only a large bulk of small particles is visible, which would lead to the non finding of the changes in the system during the homogenisation.

Laser diffractometry analysis

The samples were analysed using PIDS technology (3runs each), then the measurement was repeated by excluding PIDS. The data were analysed by using Fraunhofer optical mode (conventional) method and Mie modus, with the optical parameters 1.49 and 0.03. In Figure 6-1 the obtained results are shown.

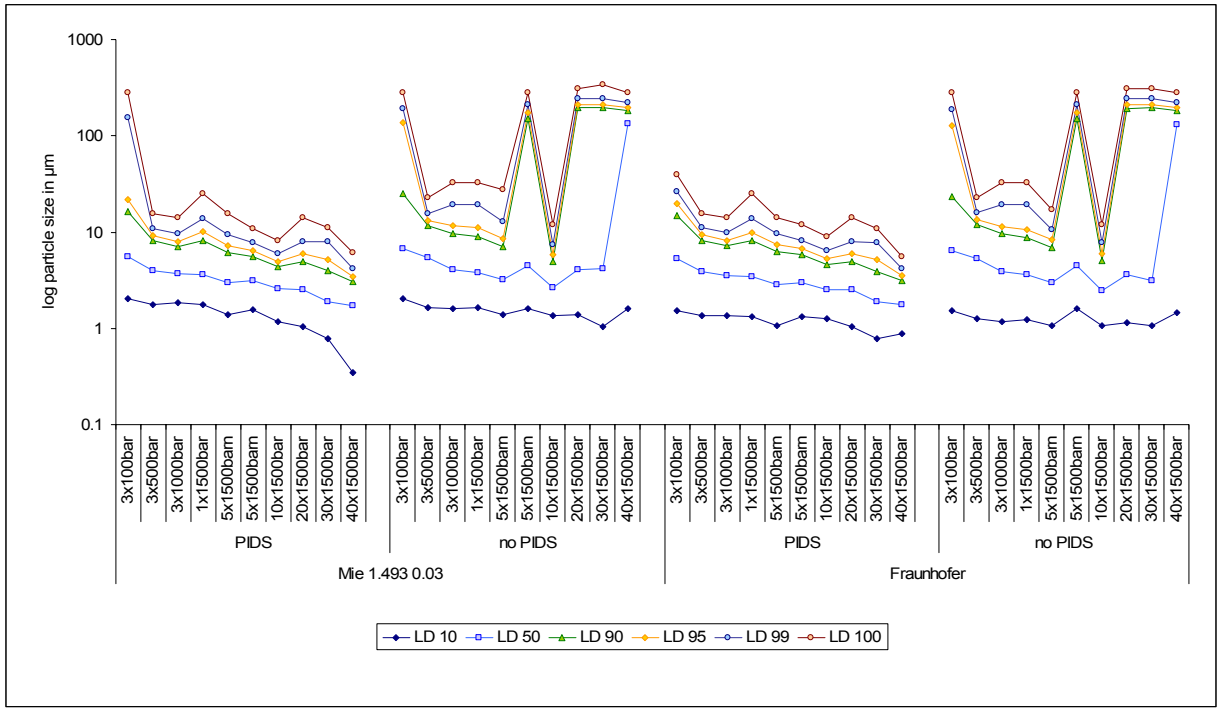


Figure 6-1: Results of LD data for different optical modules obtained from measurements with and without PIDS

As visible the results of this analysis vary tremendous. Analysis with included PIDS technology gives the idea that the decrease in particle size progresses with every cycle of homogenisation. However it could still detect the increase of particle size due to the

dissolution step at cycle 5, even though only to a much smaller extent, as analysis without PIDS did. The differences between analysis in Mie mode and analysis in Fraunhofer mode are minimal and neclectable. When PIDS was excluded from the measurement, all observations from microscopy analysis were confirmed.

It clearly shows the increase of particle size at the cycles of 1000bar (only LD 99 and 100), the change of the system after dilution (after cycle 5 at 1500bar) and the further increase in particle size from cycle 20 to 40. Again it could be shown, that LD analysis with PIDS is not the appropriate method for the detection of larger particles, which are of interest in this study. Therefore only the measurements where PIDS was not included into the measurement were taken for the interpretation of the results of the study.

Figure 6-2 shows the temperature profile of the nanosuspension during the production with the corresponding LD data (measurements without PIDS analysed with Mie mode).

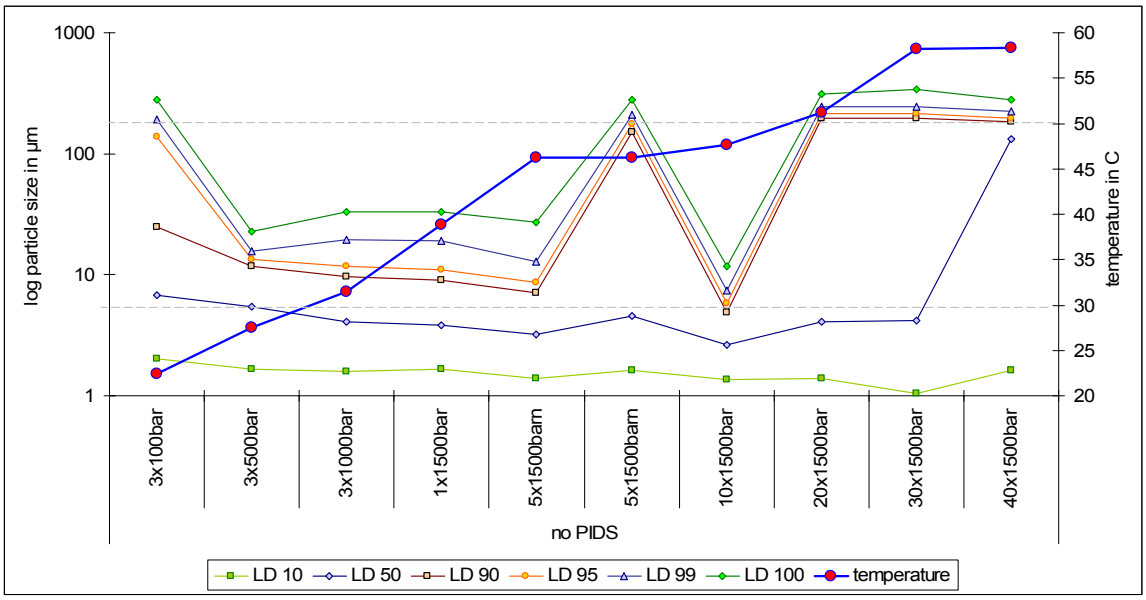


Figure 6-2: Temperature profile of the nanosuspension during production, compared with the changes in particle size (measurements without PIDS)

The data show, that the particle size not only decreases during the production process. Also increases in particle size indicating changes of the system, i.e. agglomeration, could be observed. The first change of the system correlates with the first rise of the temperature well above 30°C. The second change in the system observed occurred at cycle 20 at 1500bar, when the temperature exceeded values above 50°C (see Figure 6-2). Only because of the improved analytics, it could be seen, that the production of a cyclosporine nanosuspension is sensitive to elevated temperatures. Two temperatures seem to be important. First an increase over 30°C led to the appearance of the large crystals, which could be de-aggregated again, when the homogenisation process was continued. An increase of temperature over 50 degrees led to an

irreversible destruction of the suspension. From the literature it is known, that cyclosporine is inversingly soluble to the temperature. Therefore the appearance of crystals is thought to be a re-crystallisation process due to the increase of the temperature and the decrease in saturation solubility thereof. The destruction at temperatures above 50 degrees might be the result of a transformation as cyclosporine shows temperature dependent liquid crystal behaviour.

6.1.1.1.2 Production with temperature control

From the first study it was seen that elevation of the temperature over 30°C leads to aggregation and the appearance of larger crystals. When the suspension reached temperatures above 50°C the system agglomerated irreversible. To prove the observation from the first productions the production was repeated but the temperature was controlled. In the first repetition the temperature should not exceed 50°C. The result was expected to show only changes in the system, when temperature exceeds more than 30°C, further homogenisation should only decrease the particle size, further agglomeration or destruction was not expected to occur. In the second repetition the temperature should not exceed temperatures above 30°C; here no aggregation or re-crystallisation during the homogenisation is expected.

6.1.1.1.2.1 High pressure homogenisation below 50 °C

The temperature increases during homogenisation. For the avoidance of too high temperatures it is necessary to cool the suspension after each cycle of homogenisation. The aim of this production was to avoid temperatures above 50°C. However the critical temperature, where re-crystallisation occurred was 30°C in the first production. Therefore a decrease of the suspension below 30°C after the critical temperature was exceeded first should be avoided to exclude side effects. Optimally the temperature of the suspension after each cycle should be in the range from 45 – 48°C and higher than 30°C after cooling. From the temperature profile of the first production it was seen that the temperature rose quickly, when the pressure was increased (Figure 6-3). Therefore it was expected that the temperature after cooling and prior to homogenisation should be as low as possible. In conclusion 31°C was chosen as the temperature of the suspension prior to homogenisation. During the homogenisation this temperature was increased (up to 35°C) in order to find the maximal temperature, which would not lead to temperatures above 50°C after the homogenisation cycle (Figure 6-4). Temperatures of 31-34°C lead to temperatures well below 50°C, whereas temperatures around 35°C lead to temperatures, close to the critical temperature of 50°C. Therefore the suspension should be cooled to at least 33°C after each cycle, if the temperature after homogenisation should not exceed 50°C. Table 6-2 shows the images obtained from the production below 50°C.

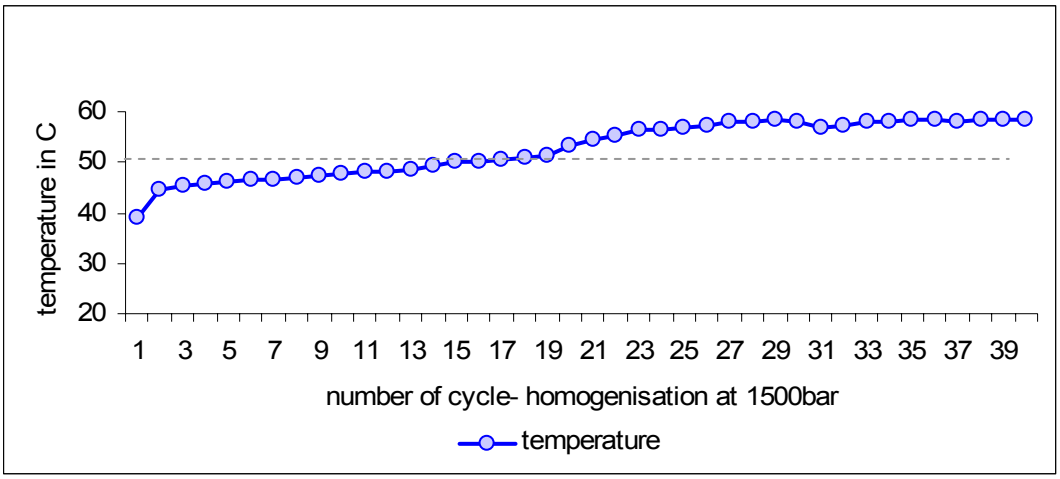


Figure 6-3: Temperature profile of suspensions homogenised without temperature control

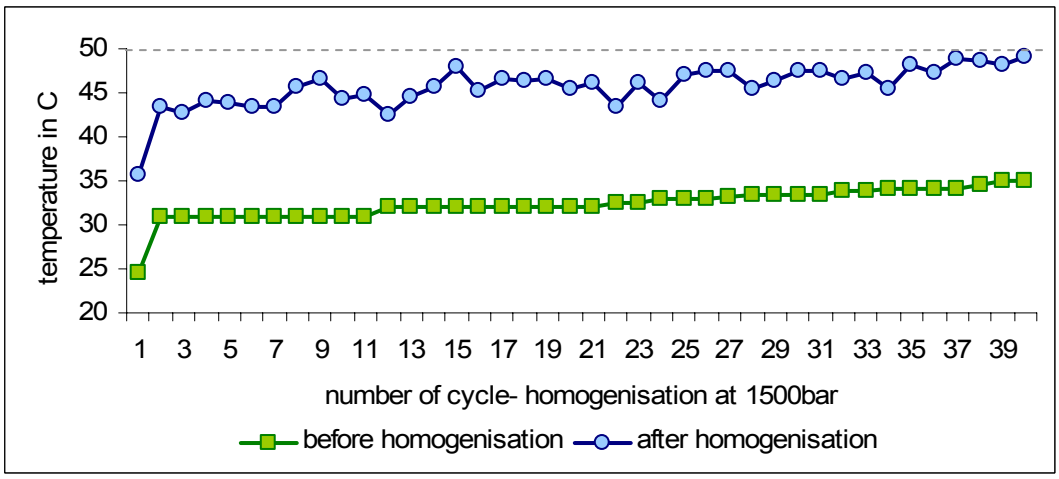
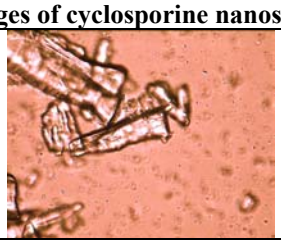
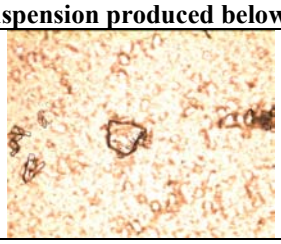
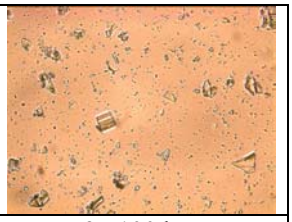
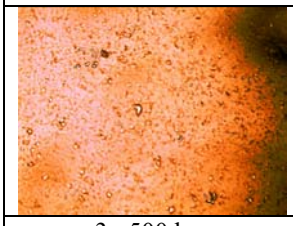
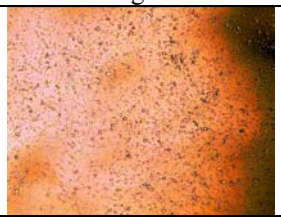
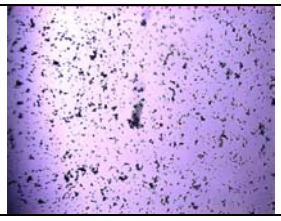
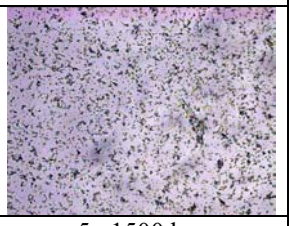
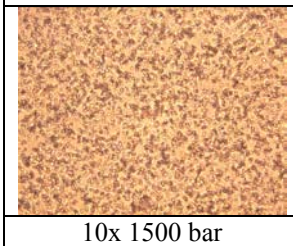
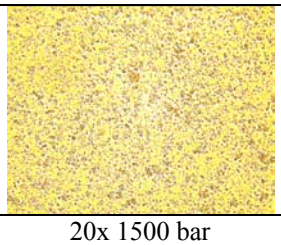


Figure 6-4: Temperature profile of suspensions homogenized below 50°C

Table 6-2: Microscopic images of cyclosporine nanosuspension produced below 50°C

Production of cyclosporine nanosuspension at temperatures below 50 °C			
			
	Original	3x Ultra Turrax	3x 100 bar
			
3x 500 bar	3x 1000 bar	1x 1500 bar	5x 1500 bar
			
10x 1500 bar	20x 1500 bar	30x 1500 bar	40x 1500 bar

The results obtained from microscopy show a good progress in diminution before the first homogenisation cycle at 1500bar was performed. After the first cycle at 1500bar the temperature exceeded 30°C, simultaneously aggregation of the system was observed. Further homogenisation at 1500bar could minimise the amount of aggregates (5 cycles at 1500bar). After 10 cycles at 1500bar no aggregates were observed anymore. The nanosuspension could be further diminuted successfully. No aggregation was observed in the final product. Also the results of laser diffractometry show the increase of particle size after the first cycle at 1500bar. After this the particle size decreases statically (Figure 6-5). The results fully confirm the results obtained by light microscopy.

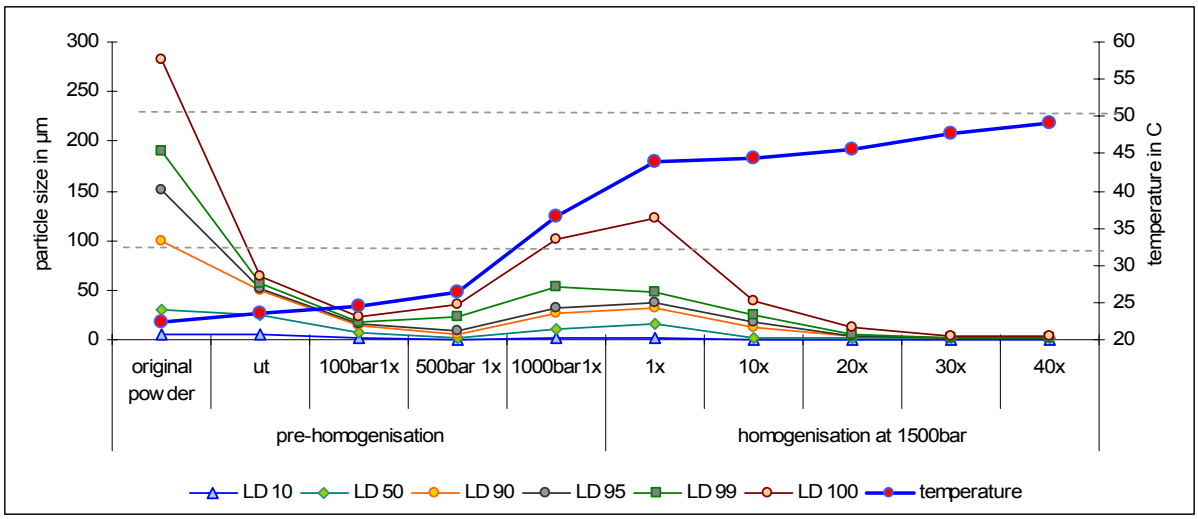


Figure 6-5: Changes in particle size during the production (LD data measured without PIDS)

6.1.1.1.2.2 High pressure homogenisation below 30 °C

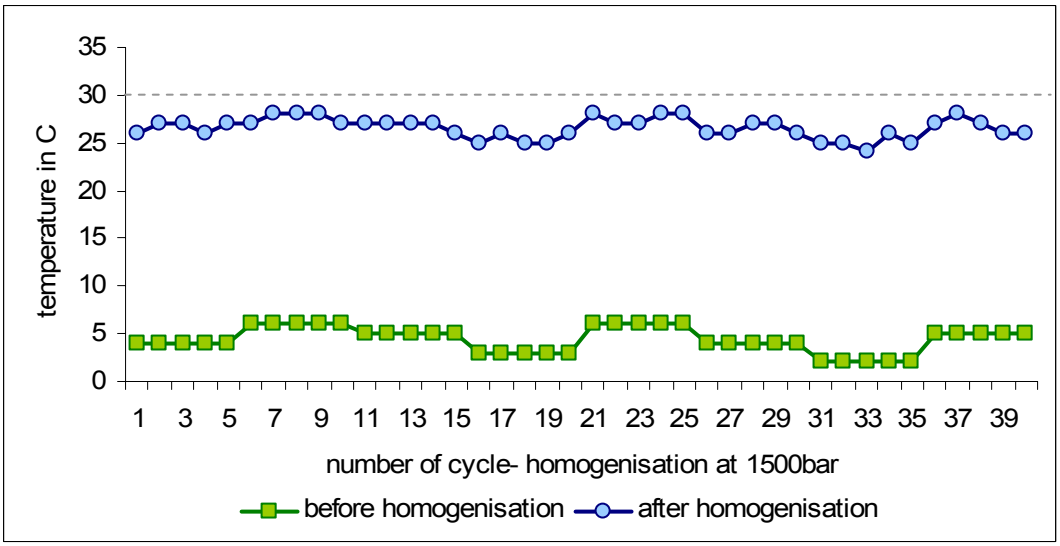


Figure 6-6: Temperature profile of suspensions homogenised below 30°C

The aim of this production was to keep the temperature of the suspension below 30°C. Due to the expected sharp increase in temperature the suspension was cooled down to 2°C. Between the homogenisation this temperature was slightly increased in order to investigate the maximal temperature were temperatures below 30°C after homogenisation at 1500bar are ensured (Figure 6-6). Microscopic analysis shows neither aggregation nor re-crystallisation during the whole production (Table 6-3). This clearly proves that the production of cyclosporine nanosuspensions is performed best at temperatures below 30°C. The laser diffractometry confirmed the results obtained by light microscopy, as no larger particles occurred within the production process (Figure 6-7).

Table 6-3: Microscopic images of cyclosporine nanosuspension produced below 30°C

Production of cyclosporine nanosuspension at temperatures below 30 °C	Original	3x Ultra Turrax	3x 100 bar
3x 500 bar	3x 1000 bar	1x 1500 bar	5x 1500 bar
10x 1500 bar	20x 1500 bar	30x 1500 bar	40x 1500 bar

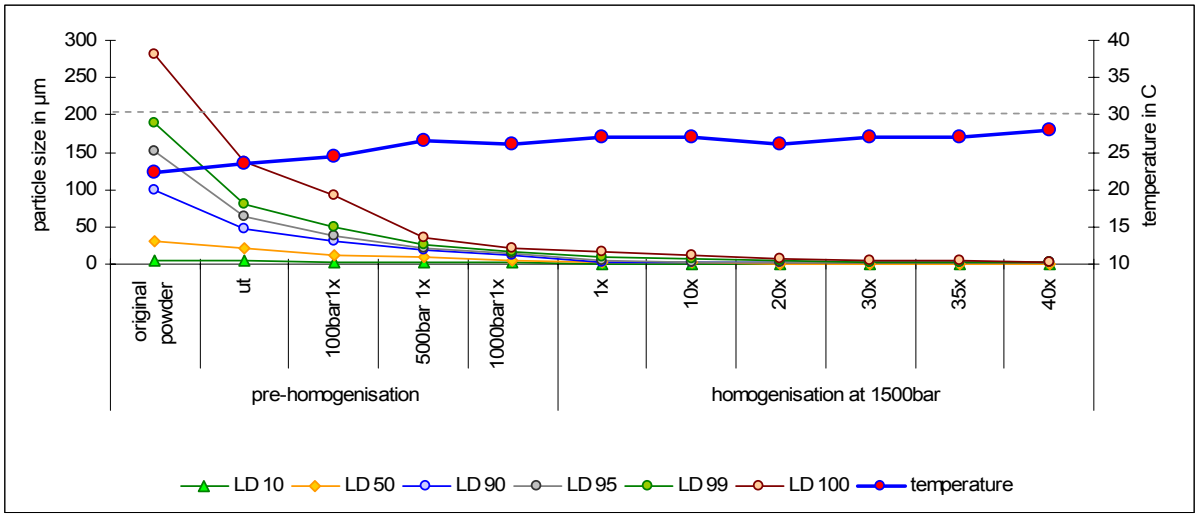


Figure 6-7: Changes in particle size during the production (LD data measured without PIDS)

6.1.1.1.3 Comparison of nanosuspensions produced below 30°C and below 50°C

The previous study showed that cyclosporine nanosuspensions are sensitive to elevated temperatures. Temperatures above 50°C destroy the dispersion by the formation of large aggregates. When the temperature is kept below 30°C no aggregation or re-crystallisation effect can be observed. Any increase over 30°C leads to re-crystallisation effects or/and aggregation of the suspension, but is reversible when the homogenisation is continued. When comparing the particle size of the final products (40 cycles at 1500 bar production below 30°C and below 50°C) the particle size is almost the same. There is a slight tendency that nanosuspensions produced at temperatures below 50°C are slightly finer and smaller in size than suspensions which were produced at temperatures below 30°C (Figure 6-8).

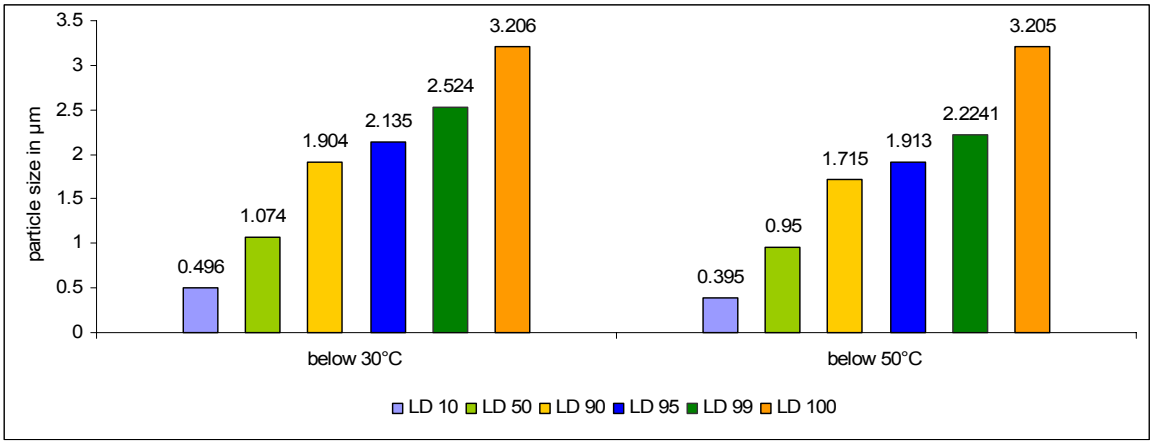


Figure 6-8: Comparison of particle size of cyclosporine nanosuspensions stabilised with poloxamer 188, immediately after production

The production of nanosuspensions with temperature control is much more time consuming than high pressure homogenisation without temperature control. For example; 1 production (APV 40, pre-cycles and 40x 1500bar) with no temperature control takes about 2-3h, depending on the experience of the producer. A production where the temperature is kept below 50°C takes between 3-4h. If the suspension needs to be kept below 30°C and the suspension needs to be cooled to 4°C after each cycle, the time of production takes at least 7h, but some times more than 12h. The production within 7h requires at least two persons, active and controlled cooling (e.g. fast shaking of the suspension in ice-salt mixture) and no break in the production process. The production with only one person and normal ice and size characterisation easily exceeds production times over 12h. In conclusion this way of production is very unproductive and inconvenient. Therefore it would be less problematic to produce cyclosporine nanosuspensions by keeping the temperature only below 50°C. This seems possible as the comparison of the particle sizes after the productions below 30°C and below 50°C were analysed to be the same. However this would be only possible if the particle

size of the different suspensions is stable in a similar way during a long-term of storage. The next aim of this study therefore was to investigate if the nanosuspensions produced in the described two different ways, also show the same changes and the same particle sizes over a period of time.

The nanosuspensions were divided into two parts. Sample 1 was stored at room temperature and sample 2 was stored at cold temperatures (refrigerator 4°C). The samples were analysed by light microscopy and laser diffractometry after 24h (d1) and after 7 and 14 days of storage.

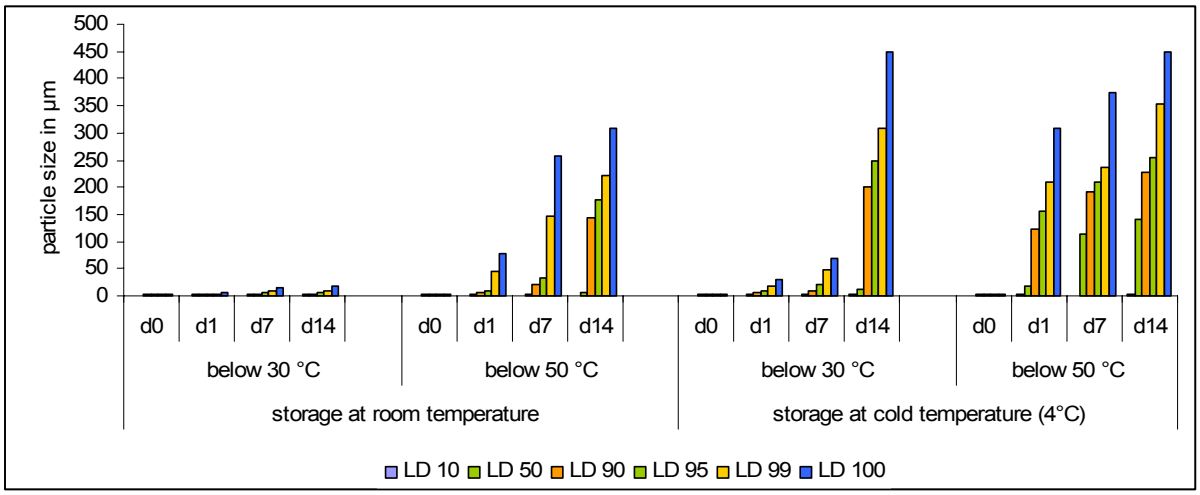


Figure 6-9: Comparison of nanosuspensions produced below 30°C and below 50°C (LD data without PIDS)

The data obtained clearly show that nanosuspensions produced below 50°C but above 30°C behave differently than those produced below 30°C. Nanosuspensions which exceeded the first critical point at 30°C are not stable. Those nanoparticles aggregate after a short period of time, i.e. less than 24h. Storage at room temperature seems to be better than storage at cold temperatures. Therefore it is not possible to produce nanosuspensions in a more convenient way (i.e. production below 50°C but above 30°C). The production needs to be performed at temperatures below 30°C.

6.1.1.2 Summary of study – set up of parameters for the production and characterisation of cyclosporine nanosuspensions

The production of cyclosporine nanosuspensions by high pressure homogenisation is sensitive to temperatures. Only production temperatures below 30°C will ensure a stabile final product. Therefore the suspension needs to be cooled down to 3-4°C after each homogenisation cycle. A further decrease in temperature did not lead to lower temperatures after the homogenisation cycle. For the cooling of the suspension it is advisable to use an ice sodium chloride mixture, were the temperature is much lower due to freezing point depression. This shortens the time of production.

Dilution of the sample due to a loss of suspension during homogenisation cannot be avoided in most of the productions. However it changes the system. Dilution may lead to a change in particle size due to dilution of particles and/or aggregation. Further homogenisation is required to reach a fine and disperse product again. Therefore the dilution step, if required, should be performed at an early stage of the production.

Prior homogenisation pre-cycles with an Ultra Turrax (3x 30s) and at low pressures at 100bar 500bar and 1000bar (each 3 times) are required. Homogenisation at 1500bar should be repeated 40 times.

The characterisation by laser diffractometry is meaningless when PIDS technology is applied, as no larger particles are detected in this mode. Only the exclusion of PIDS leads to the findings, which were described in this study. Microscopy analysis gives no useful result when only large magnifications (e.g. 630x or 1000x) are used. An overview of the suspension is important for the detection of large crystals or aggregates. Therefore small magnifications (e.g. 160x or 400x) should be used for the characterisation of those suspensions.

6.1.2 Stability of cyclosporine nanosuspensions

Aqueous nanosuspensions are thermodynamically instable systems. The addition of stabilisers is required, to prevent aggregation and re-crystallisation of the nanocrystals produced. Usually electrostatic or steric stabilisers are used. The efficiency of the electrostatic stabiliser can directly be assessed by the measurement of the zeta potential. However, the investigation of the long-term stability is also required to get information about the changes of the system over time, as the stability is not only influenced by the zeta potential. Other influences e.g. temperature of storage or particle growth due to Ostwald ripening or an inefficient steric stabiliser can only be investigated by long time observation (particle size characterisation) of the systems. The aim of this study was to identify the most appropriate stabiliser of cyclosporine and the optimal storage conditions. For that a so called “stabiliser screening” was performed.

6.1.2.1 Stabiliser screening

The conventional method for the finding of an optimal stabiliser is called “stabiliser screening”. It was performed as follows: A single nanosuspension is produced typically using high pressure homogenisation with 40 applied cycles of 1500bar; the stabiliser used for it is Poloxamer 188, typically in a concentration of 0.5w/w%. After the production process the obtained nanosuspension is divided in equal parts of 2ml. Each sample obtained is diluted by a stabiliser solution, from which the stability is monitored. The method is time saving,

because from only one production with a yield of at least 30ml of nanosuspension more than 15 different nanosuspensions with different stabilisers can be prepared and investigated.

A cyclosporine nanosuspension was produced and the product obtained was divided in 12 equal parts. Each part was diluted with an equal amount of a doubled concentrated stabiliser solution, which also contained 0.5%ww PLX 188. The procedure leads to nanosuspensions with a total concentration of 0.5ww% PLX 188 and the required concentration of the co-stabiliser. The stabilisers used were chosen because of their special properties regarding the enhancement of the bioavailability. Cyclosporine is a substrate of p-glycoprotein. Poloxamers, SDS, PVP, Tween 80 and TPGS are known to inhibit the function of p-glycoprotein, whereas TPGS is the most effective one. Chitosan and Dehyquart were chosen because of their positive charge, known to increase muco-adhesiveness and bioavailability therefore.

The prepared systems of the study are listed in Table 6-4.

Table 6-4: Systems prepared for the stabiliser screening

concentration of cyclosporine	concentration of Poloxamer 188	co-stabiliser	concentration of costabiliser
1% (w/w)	0.5% (w/w)	Poloxamer 188 (PLX 188)	1.0% (w/w)
1% (w/w)	0.5% (w/w)	Poloxamer 407 (PLX 407)	0.5%(w/w)
1% (w/w)	0.5% (w/w)	sodium dodecyl sulfate 10^{-3}M (SDS)	10^{-3}M(w/w)
1% (w/w)	0.5% (w/w)	sodium dodecyl sulfate 10^{-2}M (SDS)	10^{-2}M
1% (w/w)	0.5% (w/w)	sodium glycocholate	10^{-3}M
1% (w/w)	0.5% (w/w)	ploy vinyl pyrolidone 25k (PVP 25)	1.0%(w/w)
1% (w/w)	0.5% (w/w)	ploy vinyl pyrolidone 40k (PVP 40)	1.0%(w/w)
1% (w/w)	0.5% (w/w)	chitosan	1.0%(w/w)
1% (w/w)	0.5% (w/w)	Dehyquart CA-A	1.0%(w/w)
1% (w/w)	0.5% (w/w)	Tween 80	1.0%(w/w)
1% (w/w)	0.5% (w/w)	TPGS	1.0%(w/w)

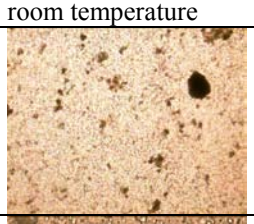
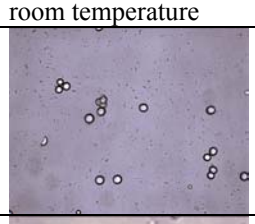
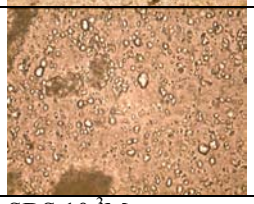
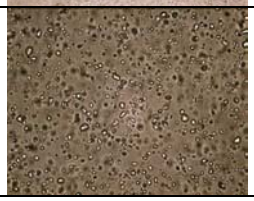
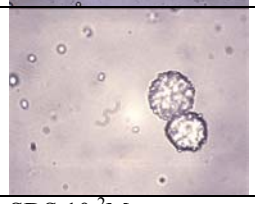
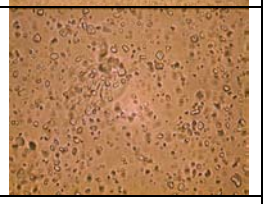
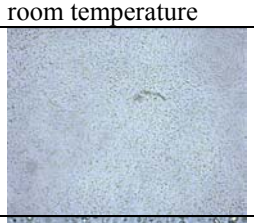
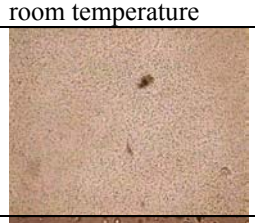
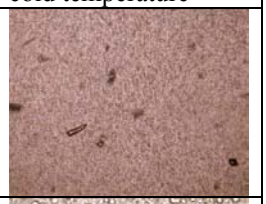
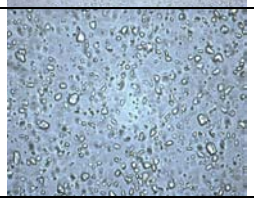
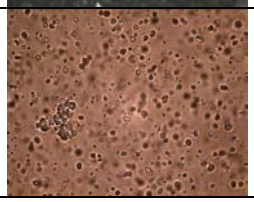
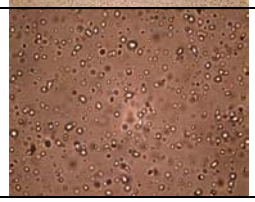
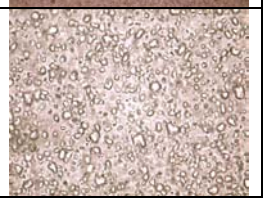
A cyclosporine macrosuspension was homogenised using an APV LAB 40. 40 cycles were applied. Several pre-cycles of 100, 250, 500 and 1000bar (three times each) were performed. The temperature was controlled before and after each homogenisation cycle. The suspension was cooled down to 4˚C before it was further homogenised, to prevent a heating during homogenisation above 30˚C, as this would lead to the destruction of the suspension due to the decrease in its saturation solubility and re-crystallisation of drug. The stability was observed using light microscopy, PCS and Laser diffractometry. Laser diffractometry was performed by using the newly established user guidelines.

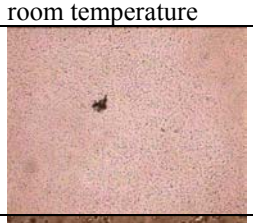
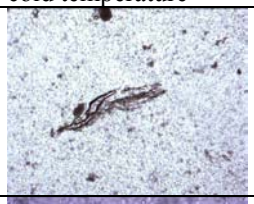
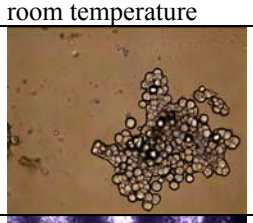
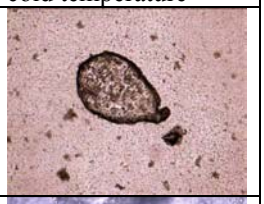
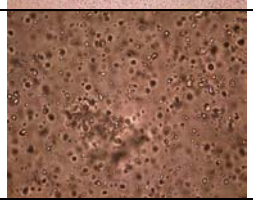
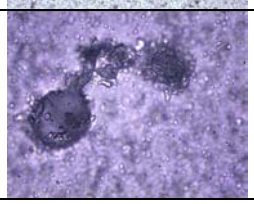
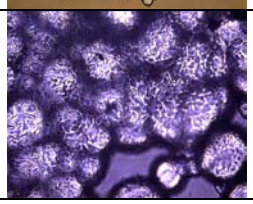
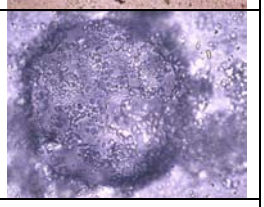
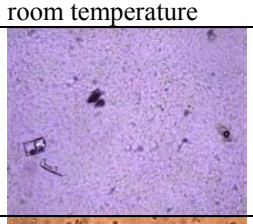
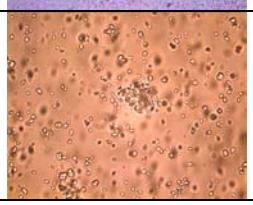
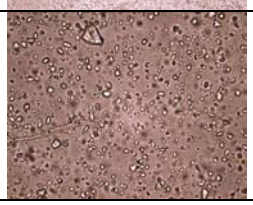
PIDS was not included in the measurement, as interest was set in the finding of larger particles. The optical model used was 1.49 for the real part and 0.03/0.3 for the imaginary

part. Saturation was performed in situ, to avoid re-crystallisation due to heating of the measuring medium. PCS was performed using the Zetasizer 4. Cyclosporine has a very low scattering intensity when it is analysed in saturated medium and it dissolves when pure water is used. Therefore it was analysed in a mixture of 50% (w/w) of glycerol and water. The measurement is possible as cyclosporine is not soluble in glycerol. Light microscopy was performed in parallel for each measurement of LD/PCS analysis to confirm the data obtained from these methods. Polarised light was also applied to enhance the visibility of large crystals in microscopy.

The images obtained from microscopy at day 28 (d28) of storage are shown in Table 6-5. The main observation gained, was the fact that storage at cold temperatures destabilised the systems in comparison to the storage at room temperature, only PLX 407 was an exception. All vials stored in the refrigerator showed crystal growth, visible as crystalline rim at the wall of the vials (not PLX 407).

Table 6-5: Microscopic images from the stabiliser screening at day 28 after production (RT= room temperature, CT= cold temperature, fridge at 4°C)

magnification	PLX 188		PLX 407	
	room temperature	cold temperature	room temperature	cold temperature
160x				
1000x				
	SDS 10^{-3}M		SDS 10^{-2}M	
	room temperature	cold temperature	room temperature	cold temperature
160x				
1000x				

magnification	sodium glycocholate room temperature	 cold temperature	chitosan room temperature	 cold temperature
160x				
1000x				
	Dehyquart			
	room temperature		cold temperature	
160x				
1000x				
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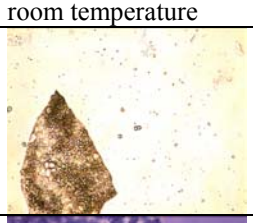
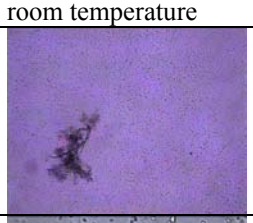
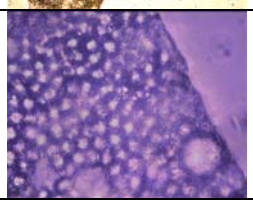
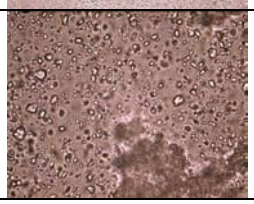
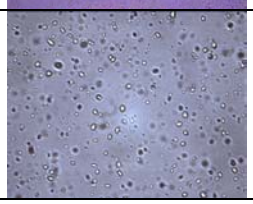
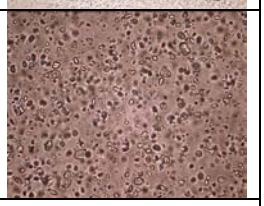
	room temperature	cold temperature	room temperature	cold temperature
160x				
1000x				
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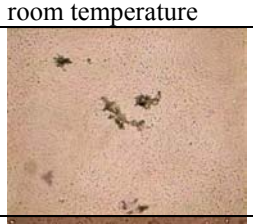
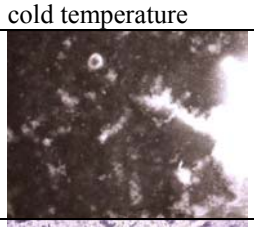
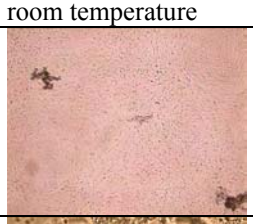
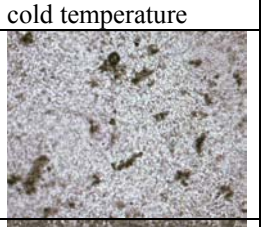
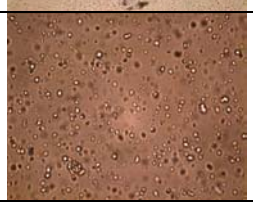
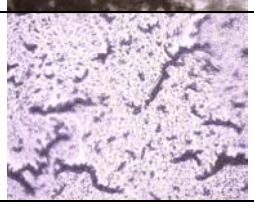
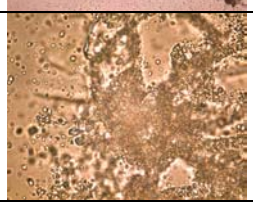
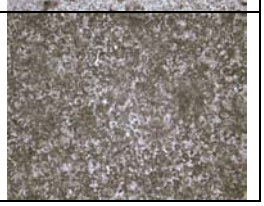
	room temperature	cold temperature	room temperature	cold temperature
160x				
1000x				

The effect could be avoided when the vials where stored at room temperature. Also the cold stored suspensions were more opaque and white when compared to the vials stored at room temperature. Suspensions stabilised by PLX 188, PVP, chitosan and sodium glycocholate were totally destroyed after the storage of 28 days in the refrigerator. Suspensions stabilised with TPGS, Dehyquart, PLX 407 and SDS seemed to be macroscopally stable, no crystals and sediment were detectable. In contrast to this the suspensions stored at room temperature, here the suspension stabilised with PLX 407 was destroyed after the storage time. Suspensions stabilised with Tween 80 and chitosan changed in the way, that the crystals disappeared and oil droplet like forms developed.

The data obtained from LD measurements are shown below. Figure 6-10 is the large scale diagram for a general overview, where especially the large particles found for Tween 80 and chitosan suspensions stored at room temperature can be seen. Figure 6-11 only shows particles size up to 1000µm for more detailed information; in contrast to the first diagram here the distinct nanosuspensions stored at room temperature or cold temperatures are directly compared between each other.

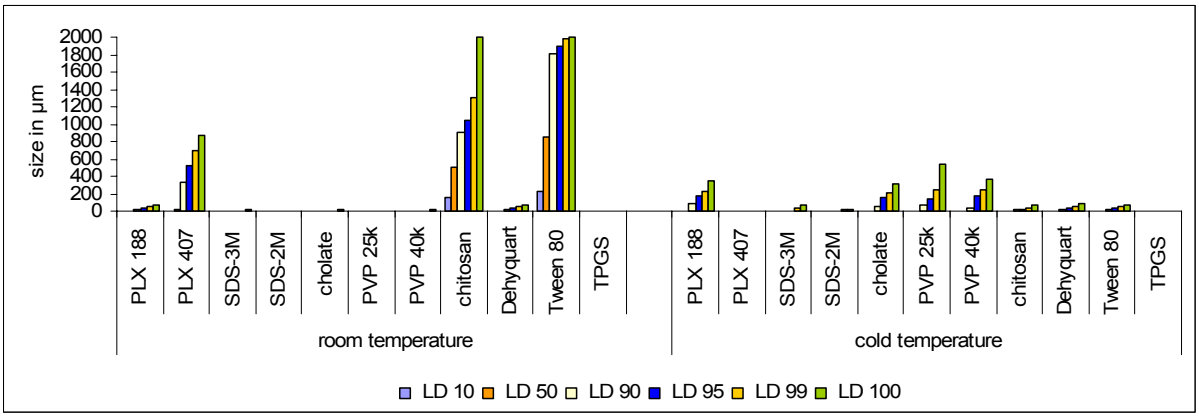


Figure 6-10: LD data from stabiliser screening at day 28 - large scale in y-axis

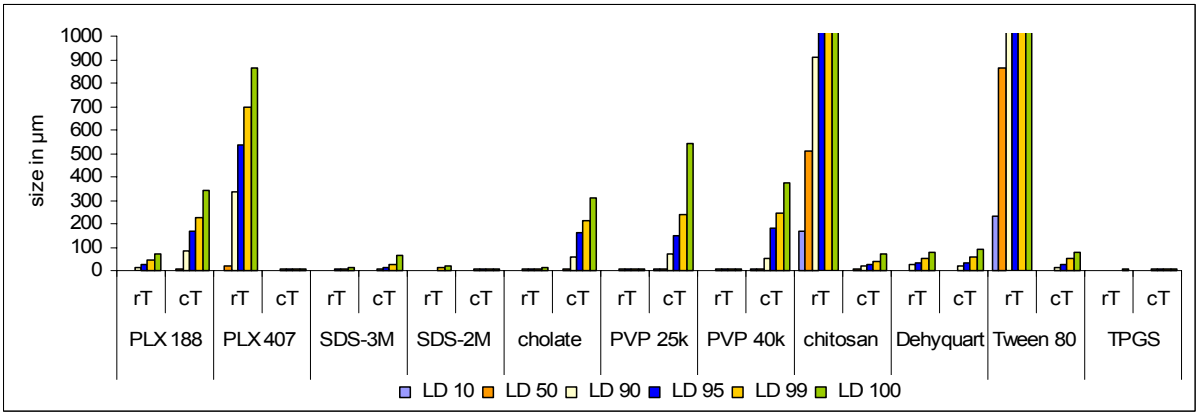


Figure 6-11: LD data from stabiliser screening at day28 – medium scale in y-axis (rT= room temperature, cT= cold temperature)

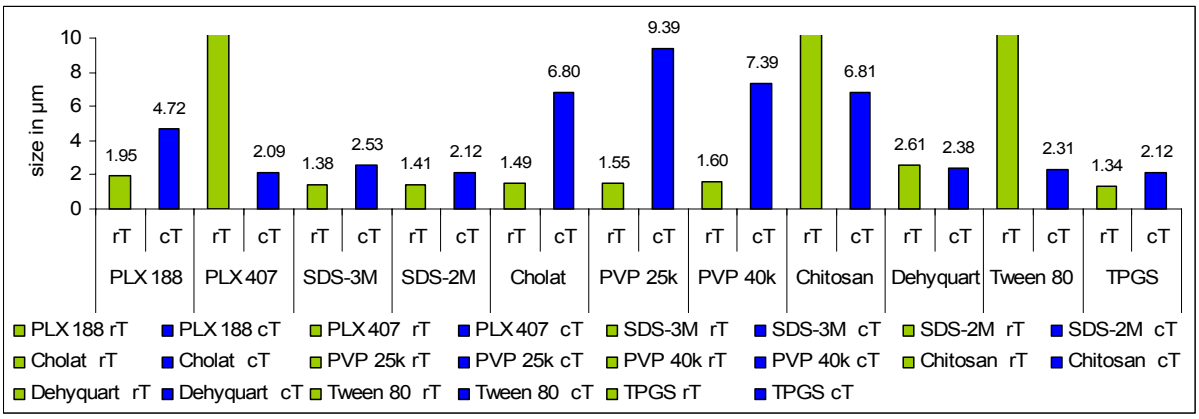


Figure 6-12: LD 50 of suspensions from stabiliser screening at day 28 (rT= room temperature, cT= cold temperature)

From the diagram it is clearly visible that storage at room temperature is better than storage at cold temperatures. In Figure 6-12 the LD 50 values are viewed for a direct comparison of the different systems. From this the suspensions stabilised with SDS 10^{-3}M or TPGS were best, as no larger crystals were detected and the LD 50 was sufficiently small. However from microscopy also these systems were found to contain larger particles. Table 6-6 list the complete LD diameters.

Table 6-6: List of LD data from the stabiliser screening at day 28

		LD 10	**LD 50**	LD 90	LD 95	LD 99	LD 100
storage at room temperature	PLX 188	0.75	**1.946**	84.93	169.2	225.1	339.9
	PLX 407	3.078	**20.96**	336	535.1	695.9	863.9
	SDS $^{-3}$M	0.706	**1.378**	2.601	4.529	9.057	12.99
	SDS $^{-2}$M	0.713	**1.405**	2.469	2.843	9.806	17.18
	sodium glycocholate	0.661	**1.491**	3.525	4.852	7.12	10.78
	PVP 25k	0.628	**1.548**	4.156	4.906	5.872	7.422
	PVP 40k	0.663	**1.597**	4.1	5.051	6.431	8.944
	chitosan	168.9	**506.6**	908.6	1040	1353	2000
	Dehyquart	0.909	**2.606**	22.59	34.34	51.88	76.43
	Tween 80	235.1	**861.9**	1808	1904	1981	2000
	TPGS	0.705	**1.34**	2.248	2.525	3.007	4.241
		LD 10	**LD 50**	LD 90	LD 95	LD 99	LD 100
storage at cold temperature	PLX 188	2.37	**4.718**	13.73	26.51	44.56	69.62
	PLX 407	1.148	**2.091**	3.472	3.881	4.625	6.761
	SDS $^{-3}$M	1.064	**2.528**	5.834	9.721	28.57	63.42
	SDS $^{-2}$M	1.006	**2.121**	3.733	4.251	5.279	8.148
	sodium glycocholate	1.322	**6.801**	57.71	161.1	211.9	309.6
	PVP 25k	3.709	**9.394**	70.46	145.4	241.1	541.9
	PVP 40k	3.333	**7.388**	53.01	182.3	247.7	373.1
	chitosan	2.644	**6.808**	19.17	24.66	41.36	69.62
	Dehyquart	1.169	**2.375**	19.8	31.45	57.6	92.1
	Tween 80	1.045	**2.309**	10.75	26.88	48.9	76.43
	TPGS	1.147	**2.123**	3.537	3.986	4.763	6.761

The data from the particle size measurements generally confirmed the information which were obtained by light microscopy. Only the few larger crystals in the SDS 10^{-3}M and TPGS systems were not detected.

Also the PCS data which are shown in Figure 6-13 and listed in Table 6-7 confirmed the observations of microscopy on laser diffractometry.

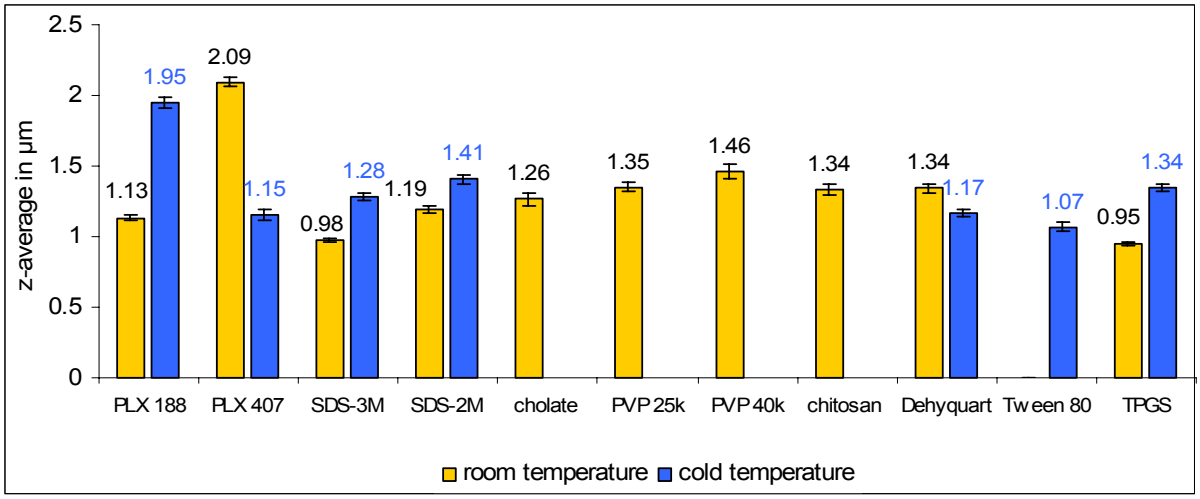


Figure 6-13: PCS data from stabiliser screening at day 28

Table 6-7: PCS data from stabiliser screening at day 28

	z-Average	
	room temperature	cold temperature
PLX 188	1.132	1.946
PLX 407	2.091	1.152
SDS^{-3}M	0.978	1.278
SDS^{-2}M	1.1882	1.405
sodium glycocholate	1.264	x
PVP 25k	1.352	x
PVP 40k	1.4626	x
chitosan	1.335	x
Dehyquart	1.34	1.1661
Tween 80	x	1.0705
TPGS	0.945	1.342

x – not analysed, as particle diameter was above the measuring range of the instrument

6.1.2.1.1 Conclusion

The stability of the produced suspensions is dependent on the temperature of storage. Suspensions stored at room temperature are more stable. This observation is logical, as the saturation solubility increases with a decrease in temperature. From this aspect it is clear that a nanosuspension will partly dissolve when it is cooled down. The saturation velocity is dependent on the particle size, where small particles dissolve faster than larger ones. Nanosuspensions produced by high pressure homogenisation are naturally polydisperse;

hence also here smaller particles dissolve faster, whereas larger ones survive. Over time the larger particles grow, because the saturation solubility is size dependent and the concentration of dissolved cyclosporine is now above its critical concentration. The phenomenon is known as Ostwald ripening. Clearly is can be avoided or at least slowed down, when the temperature is kept at elevated temperatures in case of cyclosporine. All over no suspension produced was stable over the period of 28 days. In the nanosuspensions stabilised with chitosan and Tween 80 the drug crystals disappeared and aggregates of droplets appeared. PVP 25 and PVP 40 could not stabilise the suspension at all, here large aggregates were obtained, leading to heavy and non re-disperseable caking. SDS seems to be a good stabiliser; here the problem was crystal growth. SDS strongly increases the saturation solubility of cyclosporine. When the original suspension was diluted with the SDS solution small particles were dissolved and larger remained, giving the start for pronounced Ostwald ripening. Poloxamer 407 was more stable at cold temperatures. The nanosuspensions stored at room temperature showed large crystals. Also it contained many air bubbles. The observation here was that the air bubbles seem to be an adhesion target for cyclosporine, crystals liked to adhere to the water-air interface. Dehyquart also showed crystal growth. From PCS or LD data, suspensions stabilised by TPGS and SDS and stored at room temperature seemed to be stable. However from microscopy it was obtained that also here agglomerates (in the case of TPGS suspension) and re-crystallisation occurred over the time of storage. The study therefore gave no stable cyclosporine nanosuspension.

6.1.2.2 Stabiliser screening without subsequent dilution

In the previous study cyclosporine nanosuspensions were produced via the conventional method. However no stabile nanosuspension could be obtained from this study. There was some evidence that dilution of nanosuspensions causes changes in the systems, which are not advantageous. Hence the suspension gets destabilised due to dilution. Dilution can cause aggregation, which was observed during the dilution process within the homogenisation process itself. Moreover, dilution changes the particle size of the suspension. This observation was done during the work of optimisation of measurements by laser diffractometry and is described in this chapter. From these facts it is concluded that nanosuspensions produced with the conventional method will not have the same behaviour over a longer time of storage, than suspensions which were produced without subsequent dilution i.e. produced directly in the stabiliser mixture to be investigated. Therefore the aim of this study was the production and characterisation of suspensions, were no subsequent dilution was undertaken.

Suspensions which are produced via the conventional method contain two kinds of stabiliser. The first stabiliser, usually Poloxamer 188, is added to the suspension prior to the homogenisation process and the second is added after the production. Poloxamer 188 is used to stabilise the suspension during the homogenisation. The second stabiliser is the one to be investigated, in respect of its efficiency to prevent the suspension from aggregation and particle growth. Suspensions produced in this study differ from these. They only contain one stabiliser - the one to be investigated - which is added to the suspension prior to homogenisation. In contrast to the conventional method, this method requires the production of a nanosuspension for each stabiliser of question. Therefore this kind of stabiliser screening will be more time consuming and more expensive, as more drug is needed and as much more suspensions need to be produced. Thus, the second aim of this study is the comparison of suspensions obtained from the production via the conventional method and those which are obtained from this alternative way of stabiliser screening. It will give evidence if results obtained will be the same or, can be related to each other or might be very different.

In order to investigate differences in particle size and shelf life of nanosuspensions with and without dilution, also nanosuspensions with subsequent dilution but no second stabiliser are required. Those can be obtained by dividing the nanosuspension produced into parts, though the main part is kept as the suspension without any dilution, whereas the other parts can be diluted and represent nanosuspensions with subsequent dilution for further characterisation. It was thought, that also the properties of the dilution medium used may play a role in respect to changes and stability of the suspension. Therefore not only water was used as dilution medium, but also glycerol. Glycerol is a viscose water miscible liquid. The addition of glycerol to an aqueous system increases the viscosity of the system, which decreases the mobility of particles and reduces the sedimentation velocity of them. Additionally glycerol does not change the zeta potential of a system. From this aspect glycerol should stabilise the system when added to it. Solubility studies of cyclosporine gave the result, that cyclosporine does not dissolve in glycerol, whereas the solubility in water was found to be 18.2 mg/l at 25°C in case of coarse cyclosporine powder and 24.0 mg/l at 25°C when a cyclosporine nanosuspension was used (c.f. 4.7.2.1.) Hence, the addition of glycerol should not destabilise the system, whereas the addition of water causes partially dissolution of the system, as the dissolution volume is increased. In those cases further homogenisation upon dilution is required to obtain a physically stable suspension. It was found that a minimum of 20 further homogenisation cycles is required to obtain a physically stable nanosuspension. From this, it does save not any work to enlarge the nanosuspension`s volume by water dilution. Instead of

diluting a nanosuspension and further homogenising it, a new nanosuspension can be prepared directly. However, even though no basic changes were expected upon the dilution of the nanosuspension with glycerol, the glycerol nanosuspensions were further homogenised to check weather unexpected effects would occur. The application of 10 homogenisation cycles proved to be efficient. The design of the study was constipated as follows:

A cyclosporine nanosuspension (concentration 4% (w/w)) was produced via high pressure homogenisation (pre-cycles and 40 cycles at 1500bar, dilution 1:1 after 5 cycles (i.e. concentration was now 2%), temperature below 30 °C, as described above). After 40 cycles at 1500 bar the suspension obtained was divided into 4 parts. One part was kept as original, non diluted suspension (concentration 2% (w/w)), the second part was diluted with aqueous stabiliser solution (1:1 (w/w) with a resulting concentration of 1% (w/w)), and the third part was diluted with glycerol (1:1 (w/w), resulting concentration 1% (w/w)). The third part obtained was divided into two parts again, from which the second was further homogenised (10 cycles at 1500bar, temperature was kept below 30°C). Though, four different nanosuspensions were obtained for further characterisation.

1. original suspension - non diluted - concentration of cyclosporine 2% (w/w)
2. suspension diluted with stabiliser 1:1 - concentration of cyclosporine 1% (w/w)
3. suspension diluted with glycerol 1:1- concentration of cyclosporine 1% (w/w)
4. suspension diluted with glycerol 1:1 and further homogenised - concentration of cyclosporine 1% (w/w)

As seen before, cyclosporine nanosuspensions are sensitive to the temperature of storage. Therefore each of the four suspensions was divided into two parts, where one part of the suspensions was stored at room temperature and the other part was stored at cold temperatures (4°C). Finally one production gave a total of eight nanosuspensions (Table 6-9), which were characterised by using light microscopy and laser diffractometry at the day of production (d0), at d1, d7, d14, d28 and after 3 months of storage.

Table 6-9: Overview of the eight different suspensions obtained for each stabiliser investigated

stored at room temperature	stored at cold temperature (4°C)
1. original suspension - non diluted	2. original suspension - non diluted
3. suspension diluted with stabiliser 1:1	4. suspension diluted with stabiliser 1:1
5. suspension diluted with glycerol 1:1	6. suspension diluted with glycerol 1:1
7. suspension diluted with glycerol 1:1 and further homogenised	8. suspension diluted with glycerol 1:1 and further homogenised

The complete procedure was performed for each stabiliser to be investigated. The stabilisers used correspond to those used in the previous study where the conventional stabiliser screening was performed. They are listed again in Table 6-10.

Table 6-10: Stabilisers investigated

stabiliser	concentration used (w/w)
Poloxamer 188 (PLX 188)	1.0%
Poloxamer 407 (PLX 407)	1.0%
sodium dodecyl sulphate (SDS)	10^{-3}M
sodium dodecyl sulphate (SDS)	10^{-2}M
sodium glycocholate	10^{-3}M
poly vinyl pyrolidone 25.000 (PVP 25k)	1.0%
poly vinyl pyrolidone 40.000 (PVP 40k)	1.0%
chitosan	1.0%
Dehyquart A-CA	1.0%
Tween 80	1.0%
α-Tocopheryl – PEG – succinate (TPGS)	1.0%

6.1.2.2.1 Unexpected results during production

The production of cyclosporine nanosuspensions was not possible with all of the stabilisers of interest. When homogenising the suspension agglomerated and no further homogenisation was possible. This occurred when the following stabilisers were used:

- sodium dodecyl sulphate (SDS)
- sodium glycocholate
- polyvinyl pyrolidone 25.000 (PVP 25k)
- polyvinyl pyrolidone 40.000 (PVP 40k)
- chitosan
- Dehyquart A-CA

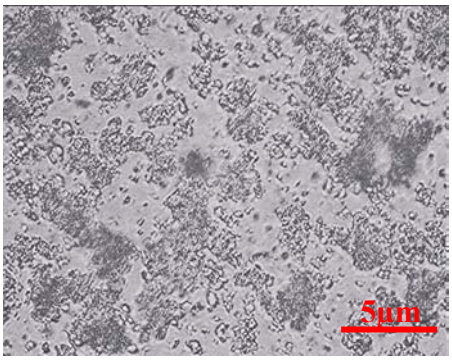


Figure 6-15: Agglomerated cyclosporine suspension (1000 fold)

All productions with these stabilisers have been repeated by varying the concentration of stabiliser (from 10^{-5}M-10^{-1}M for SDS and sodium glycocholate and from 0.25% - 2.0% (w/w) for all other stabilisers) and the concentration of cyclosporine (from 0.5% - 10%). Beside SDS none of the suspensions could be transferred into a nanosuspension. Agglomeration occurred within the first cycles of every homogenisation. In case of SDS a reduction of the cyclosporine concentration from 4% (w/w) down to 2% (w/w) and a concentration of SDS of 10^{-3}M led to acceptable results. Higher concentrations of SDS and/or cyclosporine also led to aggregation of the suspension. The observation done here, the higher the concentration of SDS and the higher the concentration of cyclosporine, the earlier does the nanosuspension agglomerate. The findings led to the exclusion of these stabilisers for further investigations, only SDS 10^{-3}M with a concentration of cyclosporine of 2% (w/w) was used for further investigations. Even though the results seen here were unexpected, 5 from 11 possible stabilisers could be excluded for further investigations within a short period of time, making this way of stabiliser screening more effective than the conventional method.

The remaining stabilisers for further investigations were Poloxamer 188, Poloxamer 407, Tween 80, TPGS and SDS (10^{-3}M) with half of the concentration of cyclosporine in comparison to the other suspensions. All suspensions were produced as described and all parameters of production were kept constant (e.g. APV LAB 40, homogeniser no.1 and production tower no.1, cooling with ice sodium chloride mixture)

6.1.2.2.2 Comparison of suspension at day of production

The tables listed below show the images of the suspensions which were obtained directly after production. Each suspension was observed at a small magnification (160x) to obtain an overview of the suspension (upper). The second image shows the suspension at a 1000fold magnification, for a more detailed impression of the suspension (lower). For a direct comparison images have been arranged that way, that all suspensions containing the same stabiliser are listed in one table. The first column of images corresponds to the non-diluted original suspension. The second column corresponds to the sample diluted with aqueous stabiliser solution. The third column represents the suspension which was diluted with glycerol, whereas the fourth column shows the pictures obtained from the suspension which was diluted with glycerol and further homogenised. For a better overview of all suspensions obtained from this study, all tables are arranged together on the next two pages. Tables are ordered after the stabiliser used, where Poloxamer 188 is always the first suspension described, followed by Poloxamer 407, Tween 80, TPGS and SDS. This order is kept for the whole chapter to avoid confusion.

Poloxamer 188 gave a fine disperse suspension; however some agglomerates can be detected in the original suspension. Those agglomerates remained when the suspension was diluted either with water or glycerol. Further homogenisation of the suspension diluted with glycerol reduced the size of the agglomerates, but could not totally remove them.

The 1000x fold magnifications of all the suspensions show remarkable differences within the suspensions obtained. The total amount of particles in the non diluted suspension is much higher than in the other suspensions, which was expected, as the concentration of cyclosporine is doubled, when compared to the diluted suspensions. However, the concentration of cyclosporine is equal in the other suspensions, but also here the amount of particles seems to be different. In contrast to the suspensions diluted with glycerol (3rd column) less participles, but larger in size can be found in the suspension, which was diluted with stabiliser solution (2nd column). Here there was the first evidence that dilution of a suspension prior homogenisation changes the system remarkable. The effect observed for Poloxamer 188 is also observed for all other suspensions produced here. All suspensions diluted with stabiliser solution (2nd column) show fewer particles when compared to those which were diluted with glycerol. Not all of the suspensions diluted with glycerol seem to be smaller in size than those which were diluted with water. However, this observation might be related to different saturation solubilities of cyclosporine in the different stabiliser solutions used. Dissolution phenomena have been described in 4.7.2. High pressure homogenisation is a top to down production process, leading naturally to polydisperse systems. Therefore nanosuspensions, produced via high pressure homogenisation, consist of smaller and larger nanoparticles. The dissolution velocity and the saturation solubility are inversingly proportional to the particle size. Therefore, if a nanosuspension is diluted, particles will dissolve, where smaller particles dissolve faster than larger particles do. The dissolution velocity also depends on the saturation solubility of the drug within the dissolution medium. The higher the saturation solubility in a medium, the faster is the dissolution process when concentration and dissolution volume are kept constant. Dilution of a suspension can therefore lead to total dissolution of particles or to partial dissolution. If total dissolution occurs in the case of small particles, larger particles remain in the suspension, leading to an increase in particle size. If only partial dissolution occurs, the particle size decreases. From this it is expected to see differences within the different suspensions produced. Larger but less particles are expected in the diluted suspensions stabilised with SDS and Tween, as here the saturation solubility is much higher than in the other stabilisers used (Fichtinger 2004).

Table 6-11: Comparison of suspensions stabilised with Poloxamer 188 1% at day of production

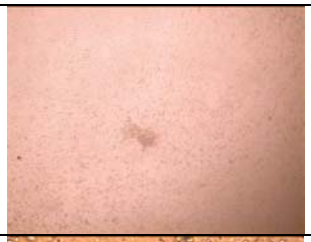
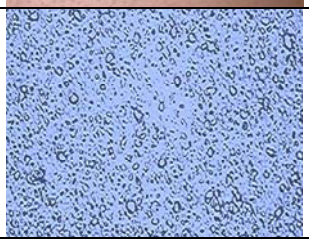
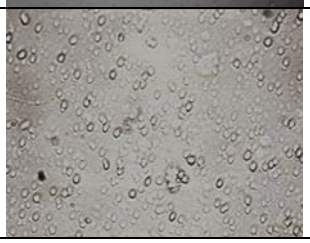
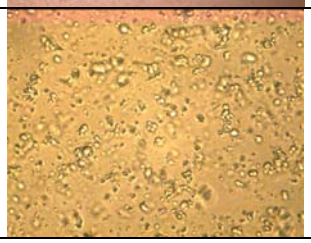
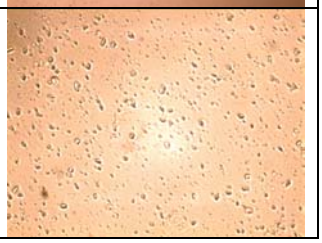
	original suspension non diluted	suspension diluted with stabiliser solution 1:1	suspension diluted with glycerol 1:1	suspension diluted with glycerol 1:1 and further homogenised
160x				
1000x				

Table 6-12: Comparison of suspensions stabilised with Poloxamer 407 1% at day of production

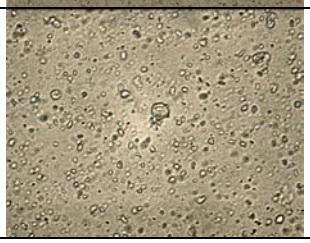
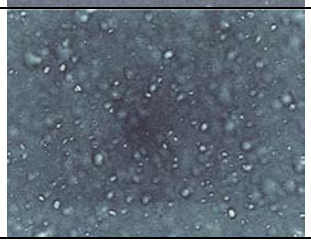
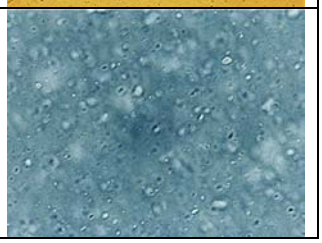
	original suspension non diluted	suspension diluted with stabiliser solution 1:1	suspension diluted with glycerol 1:1	suspension diluted with glycerol 1:1 and further homogenised
160x				
1000x				

Table 6-13: Comparison of suspensions stabilised with Tween 80 1% at day of production

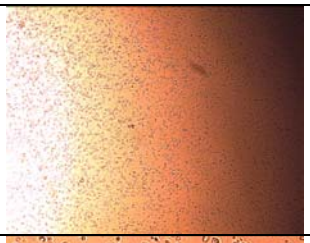
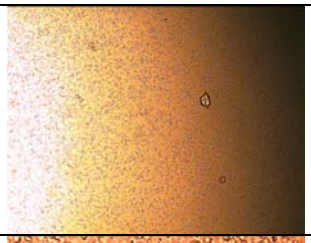
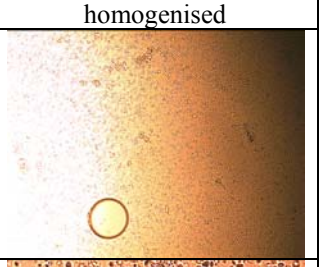
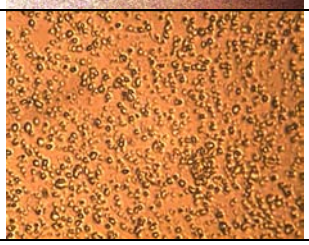
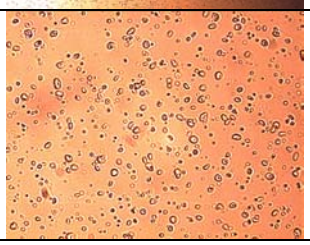
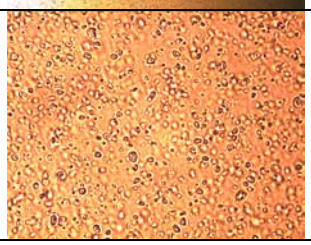
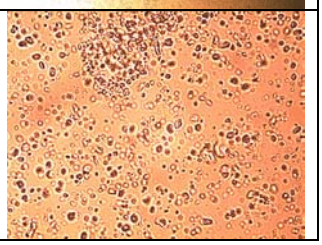
	original suspension non diluted	suspension diluted with stabiliser solution 1:1	suspension diluted with glycerol 1:1	suspension diluted with glycerol 1:1 and further homogenised
160x				
1000x				

Table 6-14: Comparison of suspensions stabilised with TPGS 1% at day of production

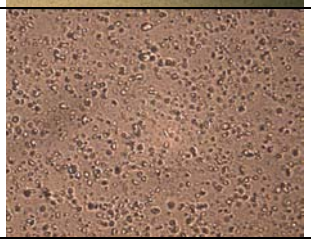
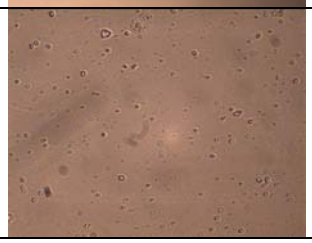
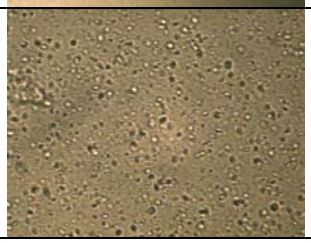
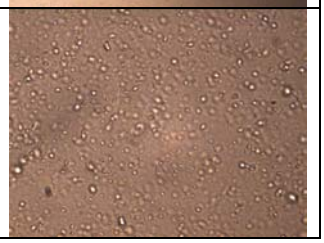
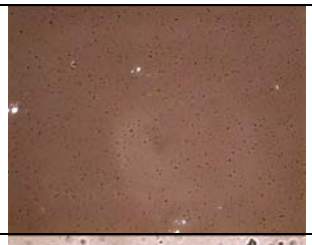
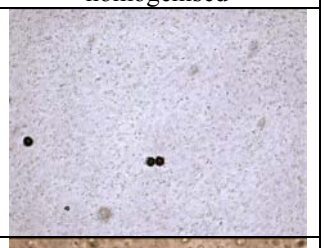
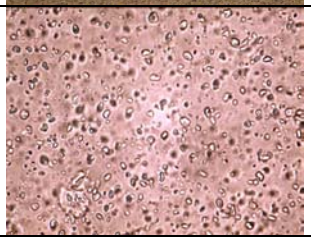
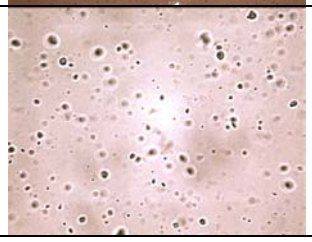
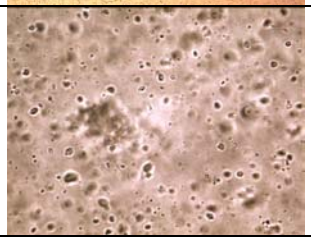
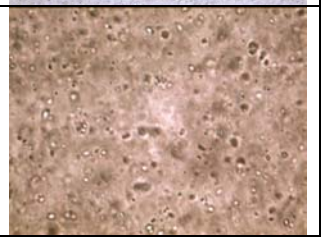
	original suspension non diluted	suspension diluted with stabiliser solution 1:1	suspension diluted with glycerol 1:1	suspension diluted with glycerol 1:1 and further homogenised
160x				
1000x				

Table 6-15: Comparison of suspensions stabilised with SDS 10^{-3}M at day of production

	original suspension non diluted	suspension diluted with stabiliser solution 1:1	suspension diluted with glycerol 1:1	suspension diluted with glycerol 1:1 and further homogenised
160x				
1000x				

The results obtained from microscopy nicely show the results expected. Furthermore it was observed that some suspensions which were diluted with glycerol contain air bubbles (black dots in the pictures). This was observed for PLX 407, Tween 80 and for SDS in the further homogenised suspensions. However, all suspensions stabilised with Tween 80 contain agglomerates, indicating that Tween 80 is probably not efficient to stabilise the cyclosporine nanosuspension. The nanosuspensions stabilised with TPGS and SDS seem to be finer than those stabilised with PLX 407, PLX 188 and Tween 80.

Results of Laser diffractometry

The complete study gave a large bulk of data, which were condensed into diagrams. The diagrams are listed here, whereas the complete set of data is listed in the appendix.

Figure 6-16 gives an overview of particle sizes obtained from all suspensions produced at the day of its production (d0). The blue columns correspond to the original non diluted suspensions, the columns coloured magenta show the particle sizes obtained from the suspension diluted with water. Yellowish columns show the size of the suspensions which were diluted with glycerol. The green columns correspond to the suspensions diluted with glycerol and further homogenisation. Within laser diffractometry analysis only some suspensions diluted with glycerol show larger particles larger 5µm.

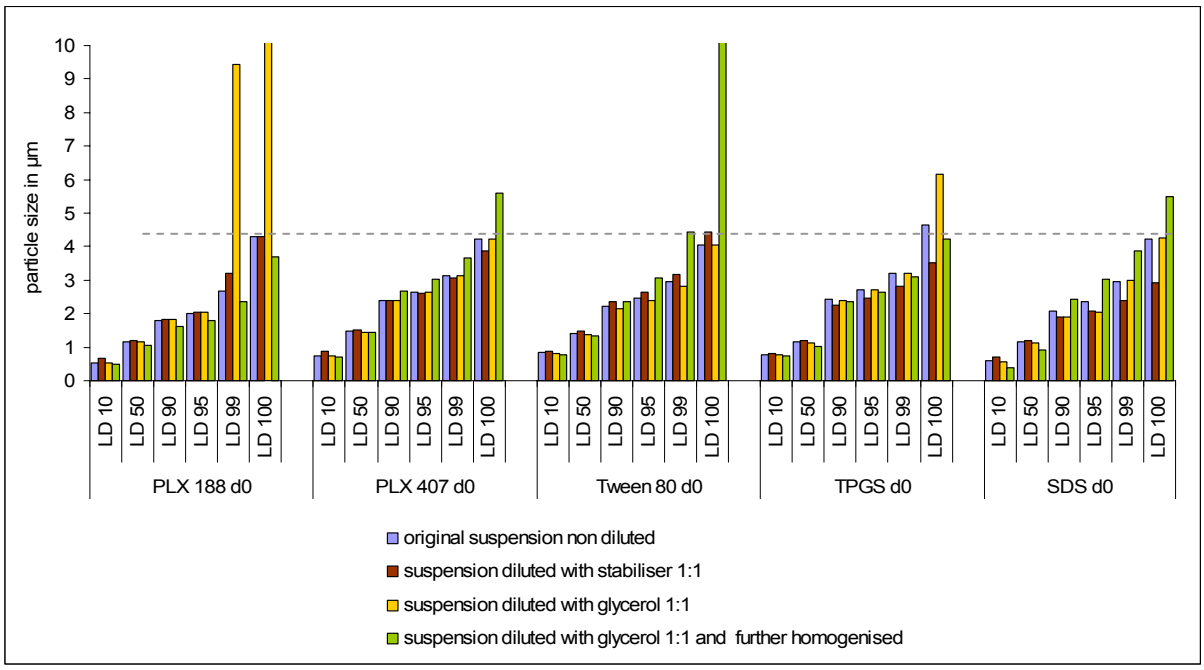


Figure 6-16: LD data of all cyclosporine nanosuspensions at day of production

However from microscopy it was seen that the suspension stabilised with Poloxamer 188 and Tween 80 contained agglomerates in every suspension produced. Therefore it is concluded, that agglomerates observed in microscopy are only loosely agglomerated and could be destroyed by the stirrer of the laser diffractometer.

The large air bubbles within the Tween 80 stabilised suspension were also detected by LD. Larger particles were also found for the suspensions stabilised with SDS and Poloxamer 407, which is interpreted as air bubbles seen in microscopy. Also the suspension stabilised with TPGS and diluted with glycerol shows some larger particles, which is interpreted as agglomerates, also seen in microscopy.

The second observation from microscopy was the change of the amount of particles and the change in particle size due to dilution. In Figure 6-17 only the LD 10 and LD 50 of the single

suspensions are compared. The results nicely confirm the observations obtained from microscopy. Dilution of suspensions with water led to an increase of particle size, as smaller particles dissolved completely and larger particles remained (magenta columns). Dilution with glycerol did not remarkably change the size of the particle, only a trend of a slight decrease in particle size could be observed. SDS and Tween 80 increase the solubility of cyclosporine. Therefore the effects should be more remarkable within those suspensions. Indeed, changes are more intense in the suspension stabilised with SDS, which confirms the theoretical expectation. The effect is also seen with Tween 80, but not as intense as within the suspensions stabilised with SDS. The reason for this might be the content of agglomerates, which can change the dissolution behaviour and the particle size distribution analysed.

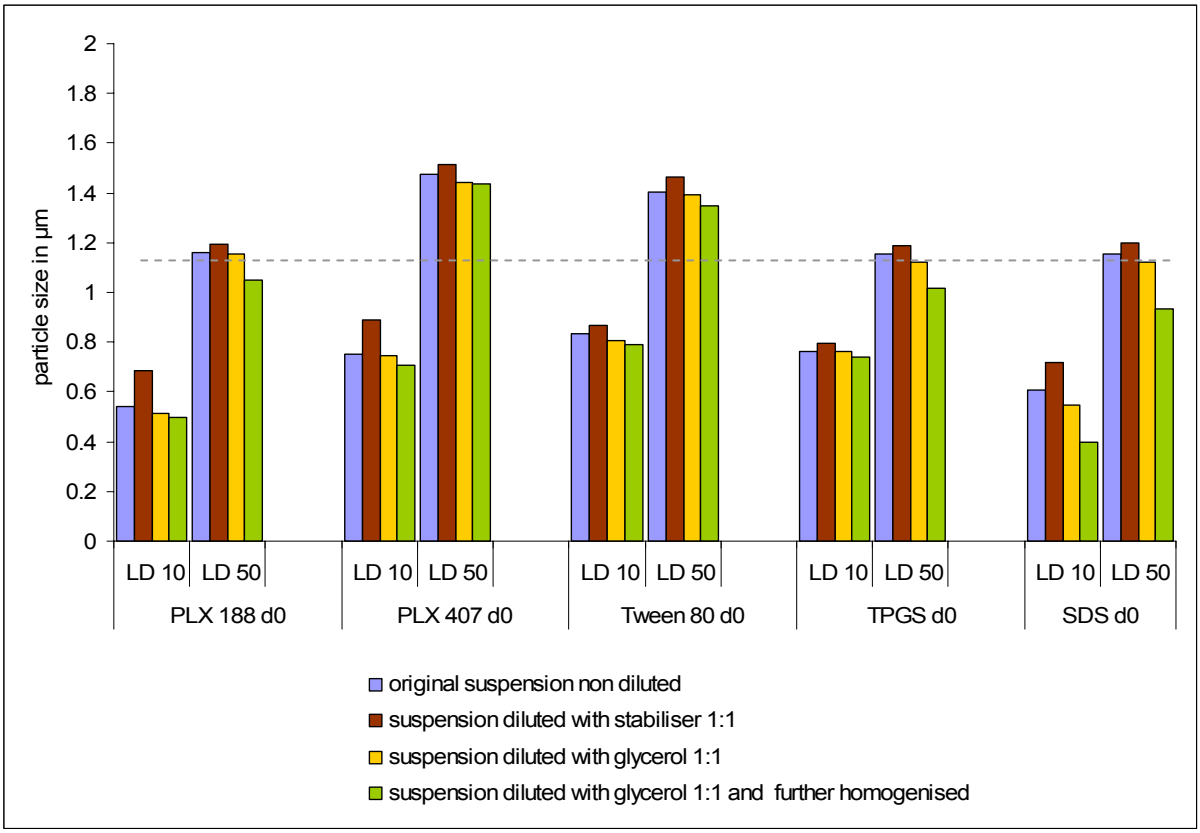


Figure 6-17: LD 10 and LD 50 of all cyclosporine nanosuspensions at day of production

In conclusion the analysis of the suspensions produced by the application of the optimised measurement standards (combined analysis of light microscopy and laser diffractometry where no PIDS is included in the measurement) gave a sound information about differences in size and the consistency of the suspensions. Larger particles, detected by laser diffractometry, could be proven to be agglomerates or air bubbles because of the additional information from microscopy. Also the disappearance of particles due to dilution with water can only be seen from microscopic analysis.

Results obtained by application of the older standard:

The older standard does not necessarily contain microscopic analysis. Only measurements of laser diffractometry and photon correlation spectroscopy are performed. The measurements of laser diffractometry do always include PIDS analysis. The analysis mode used was Fraunhofer in case of nanosuspensions (Grau 2000; Akkar 2004; Buttle 2004; Templin 2004; Krause 2006). Lately Mie mode was used with a standard refractive index of 1,456 or 1,46 and an imaginary value of 0.01 (Bushrab 2006; Hernandez-Trejo 2006; Möschwitzer 2006). Figure 6-18 shows the LD data obtained from all suspensions at the day of its production measured with included PIDS technology and Fraunhofer analysis mode.

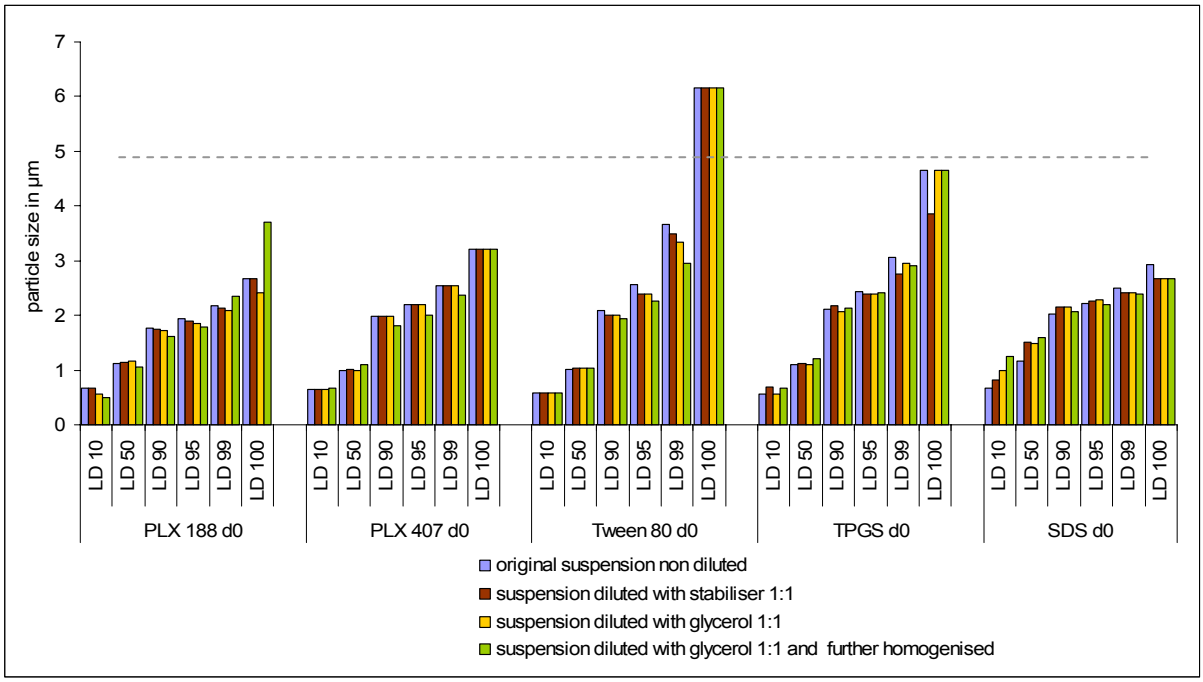


Figure 6-18: LD data of all cyclosporine nanosuspension from measurements with included PIDS and Fraunhofer analysis

The dotted line is a visual border of 5µm. Only suspensions stabilised with Tween 80 exceed this border. In contrast to the optimised method no remarkable differences between the individual suspensions can be obtained. Agglomerates, seen in microscopy, cannot be detected in any suspension. The interpretation would likely be that the suspension stabilised with Tween 80 contains some larger particles, whereas all other suspensions are nicely homogenised. The non finding of larger particles is contributed to the inclusion of PIDS technology, which overestimates small particles and fails to detect larger particles beside a small sized bulk population (Keck and Müller 2005). For more detailed information the LD 10 and LD 50 of all suspensions are compared (see Figure 6-19). The results obtained from this method of analysis gives larger values for the suspension stabilised with SDS, followed by TPGS and Poloxamer 188, the smallest suspensions here are the suspensions stabilised

with Tween 80 and Poloxamer 407. The result therefore is just the opposite of the result obtained from the optimised measurement. The reason for this controversy is the dissolution of smaller particles in the measuring medium. Therefore larger particles remain and the particle size measured is larger. Hence, the smaller the particles within a sample, the larger is the particle size analysed (Keck and Müller 2005). However, dissolution phenomena can be monitored and avoided by using saturated dissolution media and by monitoring the (PIDS) obscuration values during the measurements.

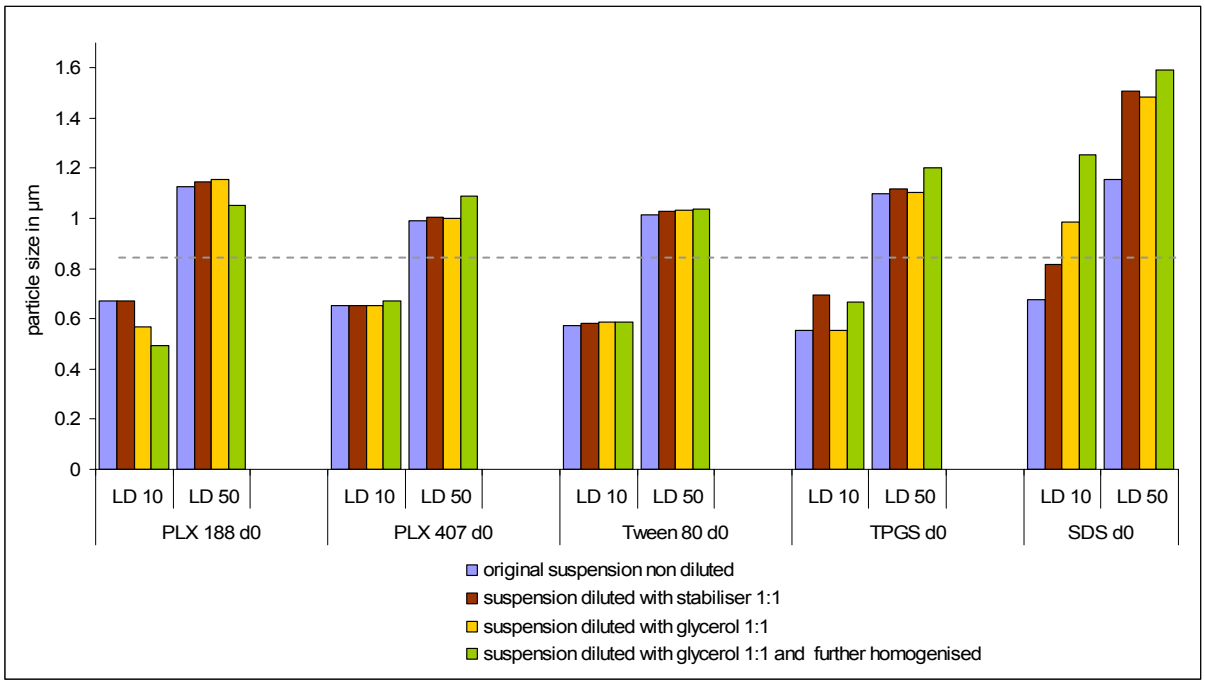


Figure 6-19: LD10 and LD 50 of all cyclosporine nanosuspension from measurements with included PIDS and Fraunhofer analysis, LD was performed in water, not in saturated medium

Any decrease of the (PIDS) obscuration during the measurement indicates dissolution of particles. In some of the cases also an increase in PIDS obscuration can be observed, which indicates de-aggregation of particles.

In case of TPGS and SDS some increase in LD 50 due to dilution can be observed. In conclusion, results obtained here give neither a correct result nor any sound information for interpretation of the systems obtained.

6.1.2.2.3 Comparison of suspensions at d0, d1 and d7

The tables (Table 6-16 – 6-26) contain the microscopic images of the suspensions obtained on their day of production (d0), 24 hours after production (d1) and after one week of storage (d7). The order of the suspensions analysed is kept as before. Suspensions stabilised with Poloxamer 188 are first, followed by Poloxamer 407, Tween 80, TPGS and SDS. The microscope analysis gave two tables for each suspension. The first table shows the images obtained from the suspensions stored at room temperature, whereas the second table shows the images obtained from the suspensions stored at cold temperatures.

Table 6-16: Suspensions stabilised with Poloxamer 188 at d0, d1 and d7 stored at room temperature

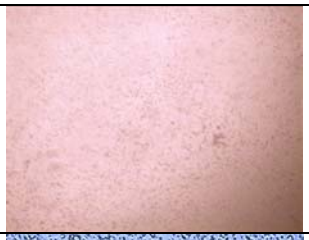
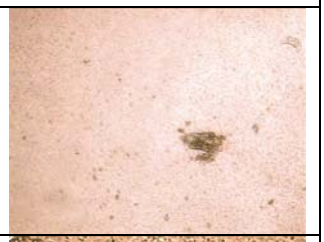
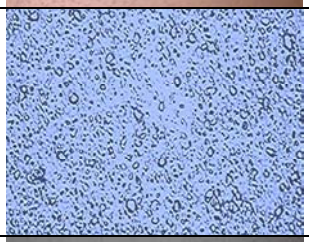
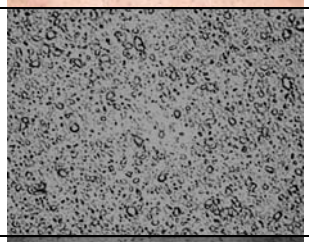
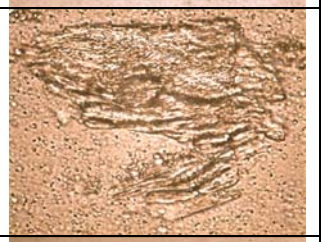
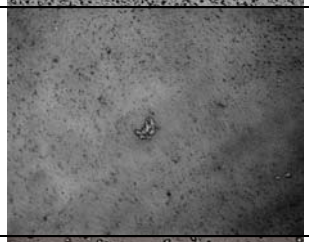
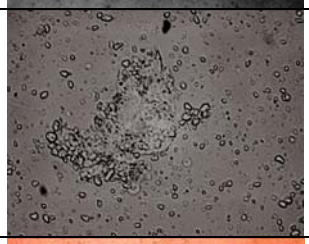
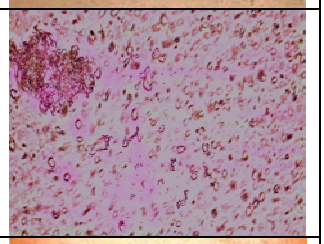
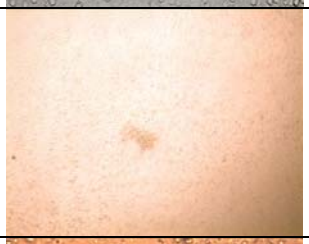
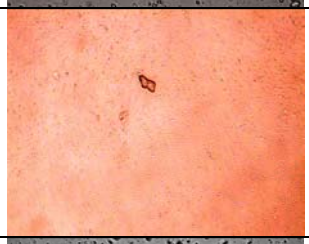
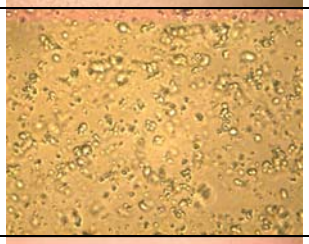
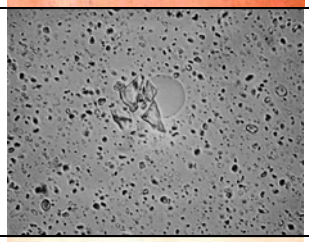
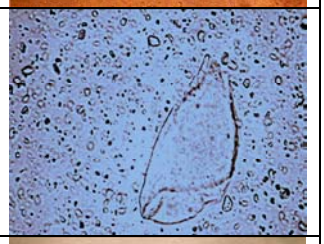
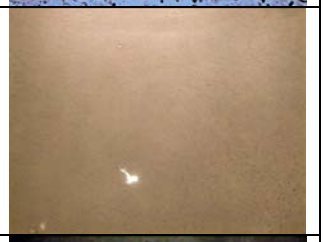
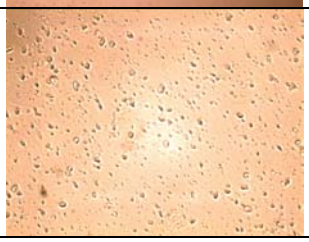
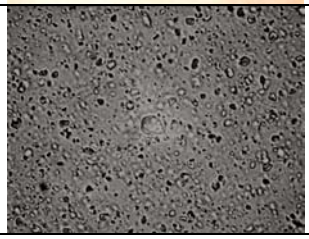
		d0	d1	d7
original suspension non diluted	160x			
	1000x			
suspension diluted with stabiliser solution 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1 and further homogenised	160x			
	1000x			

Table 6-17: Suspensions stabilised with Poloxamer 188 at d0, d1 and d7 stored at cold temperature (4°C)

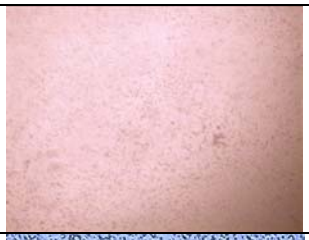
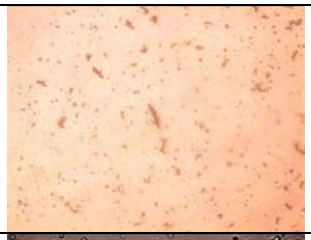
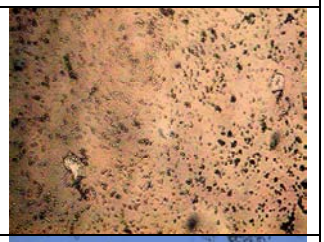
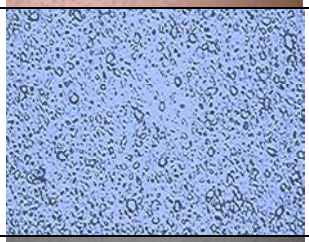
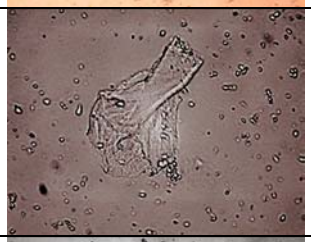
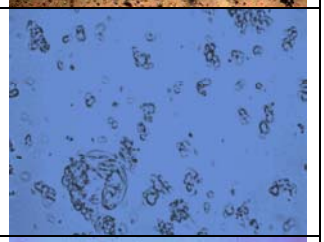
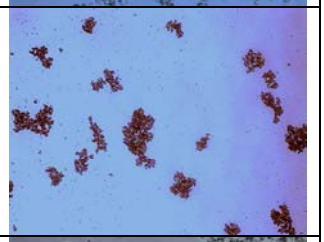
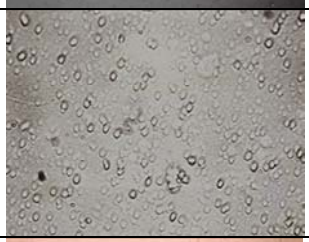
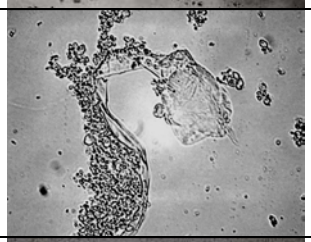
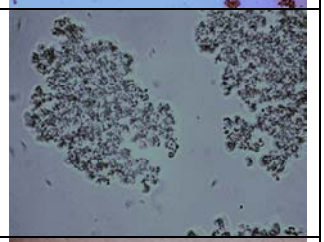
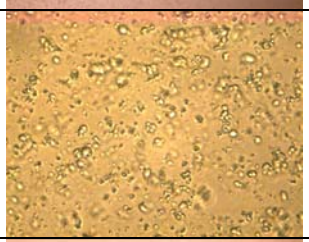
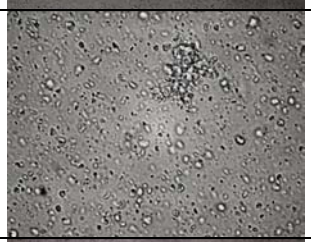
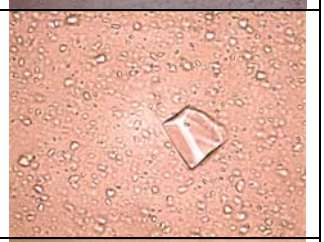
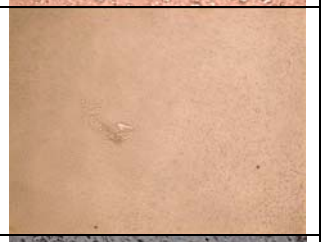
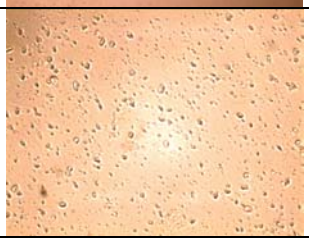
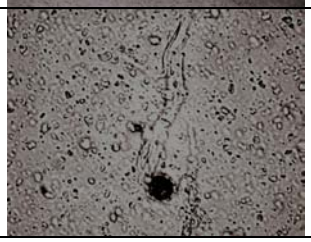
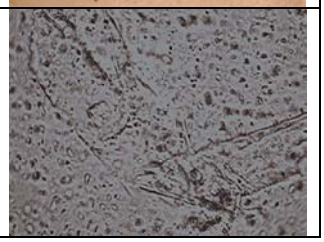
		d0	d1	d7
original suspension non diluted	160x			
	1000x			
suspension diluted with stabiliser solution 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1 and further homogenised	160x			
	1000x			

Table 6-18: Suspensions stabilised with Poloxamer 407 at d0, d1 and d7 stored at room temperature

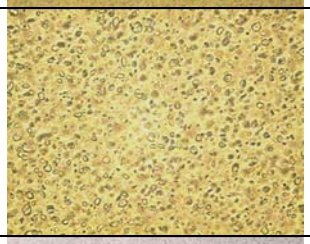
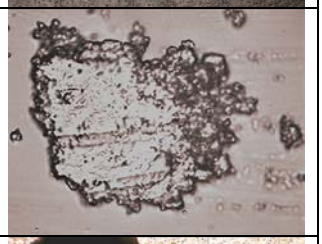
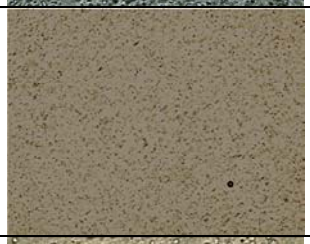
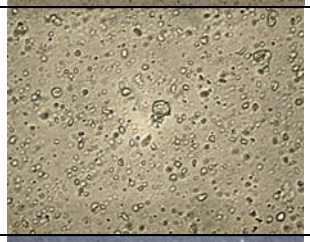
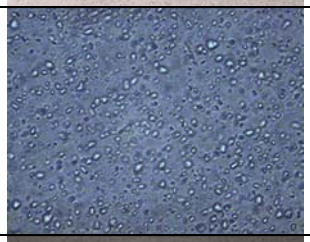
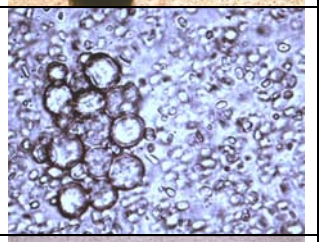
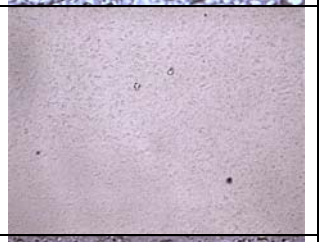
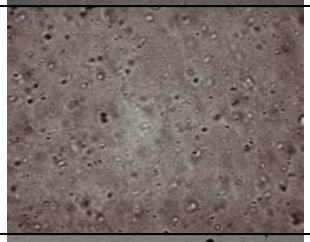
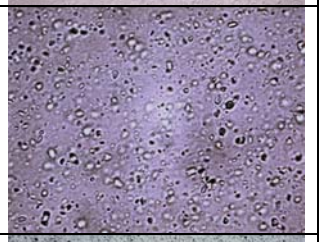
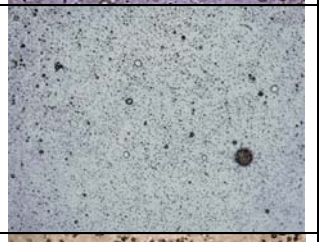
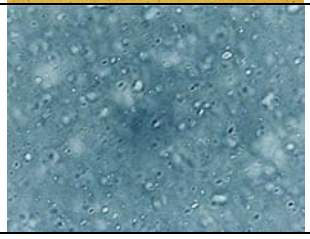
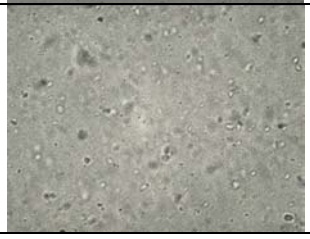
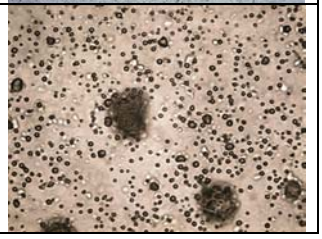
		d0	d1	d7
original suspension non diluted	160x			
	1000x			
suspension diluted with stabiliser solution 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1 and further homogenised	160x			
	1000x			

Table 6-19: Suspensions stabilised with Poloxamer 407 at d0, d1 and d7 stored at cold temperature (4°C)

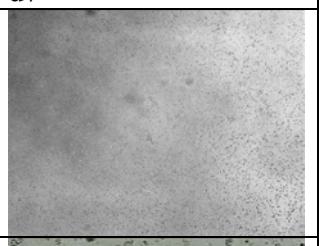
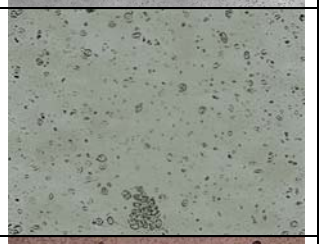
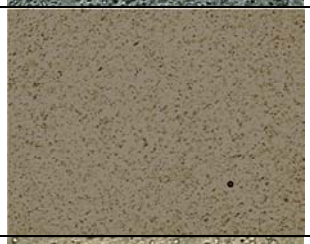
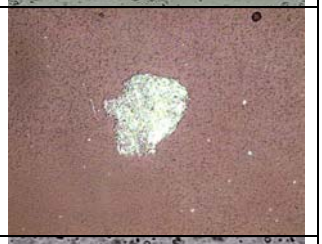
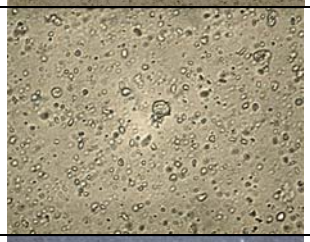
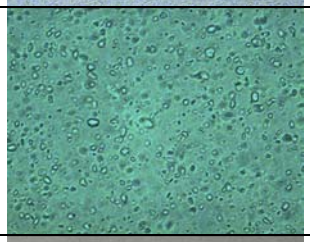
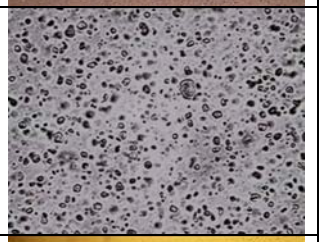
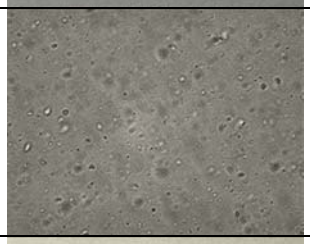
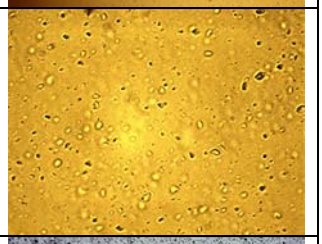
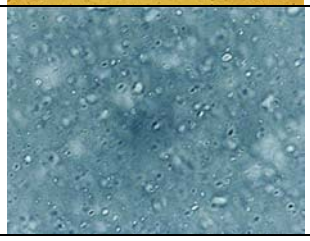
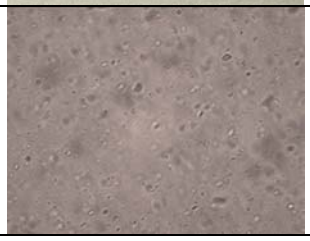
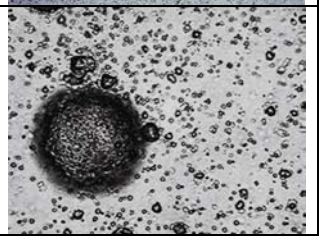
		d0	d1	d7
original suspension non diluted	160x			
	1000x			
suspension diluted with stabiliser solution 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1 and further homogenised	160x			
	1000x			

Table 6-20: Suspensions stabilised with Tween 80 at d0, d1 and d7 stored at room temperature

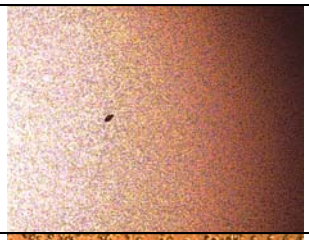
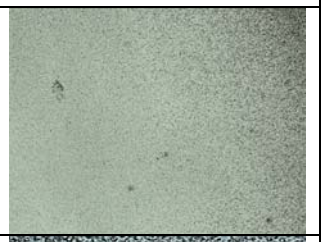
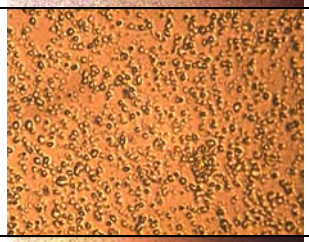
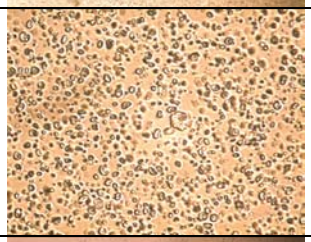
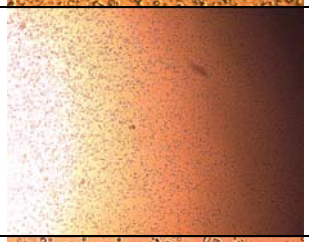
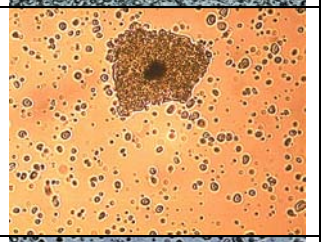
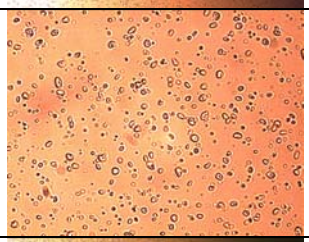
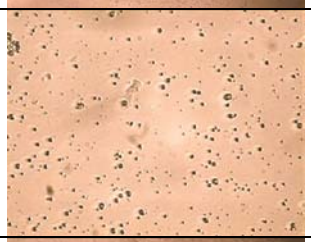
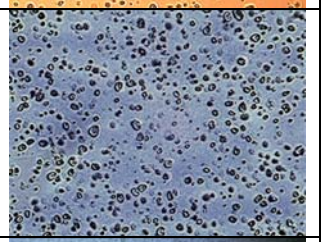
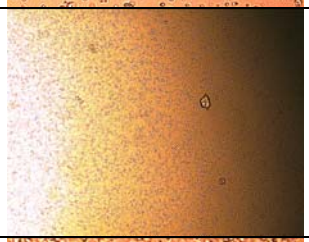
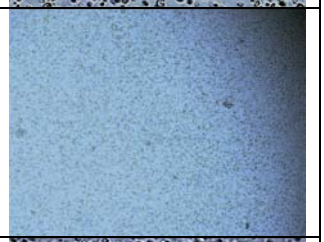
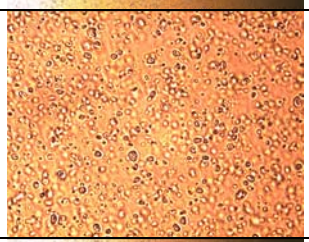
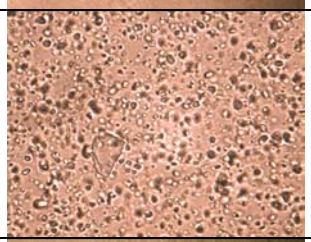
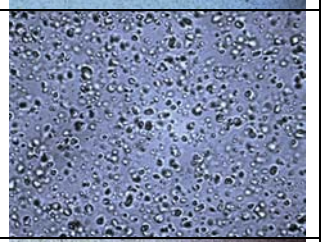
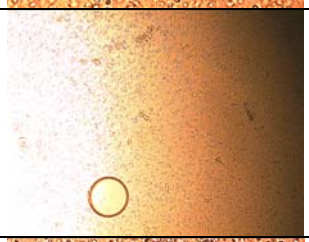
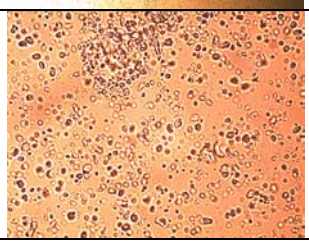
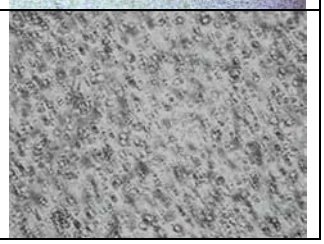
		d0	d1	d7
original suspension non diluted	160x			
	1000x			
suspension diluted with stabiliser solution 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1 and further homogenised	160x			
	1000x			

Table 6-21: Suspensions stabilised with Tween 80 at d0, d1 and d7 stored at cold temperature (4°C)

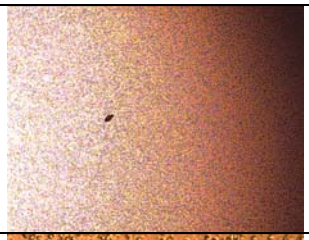
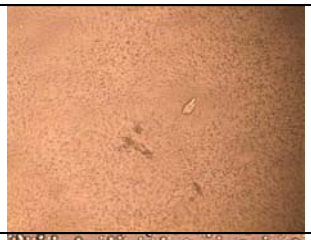
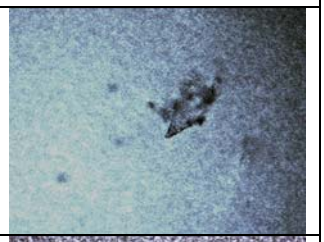
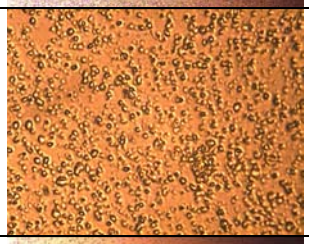
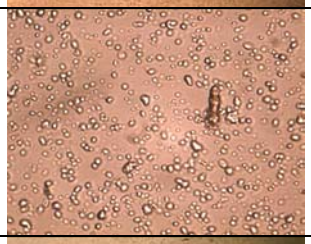
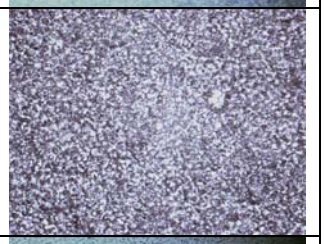
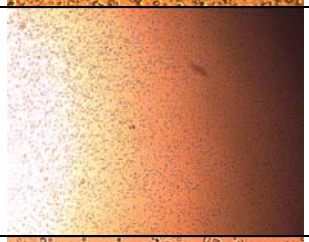
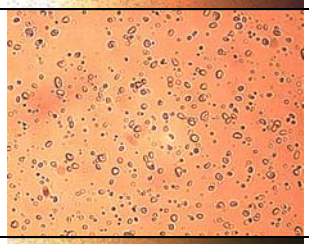
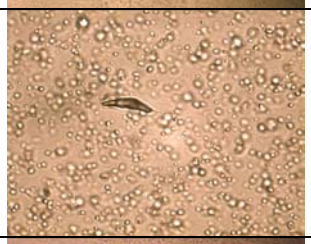
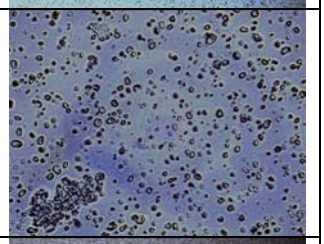
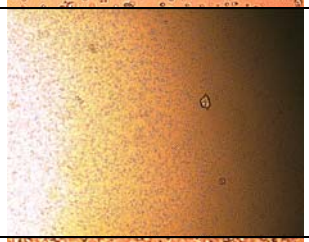
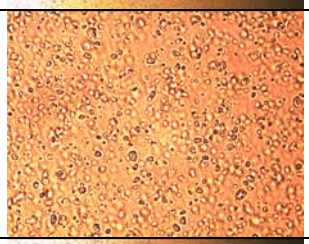
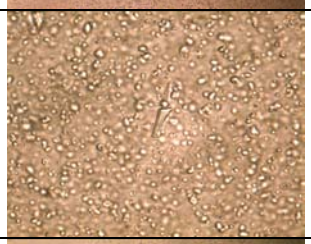
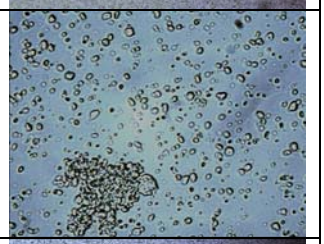
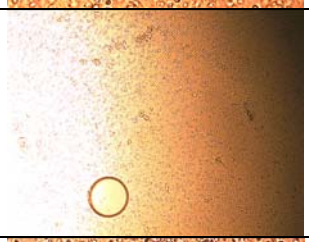
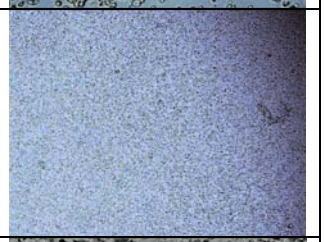
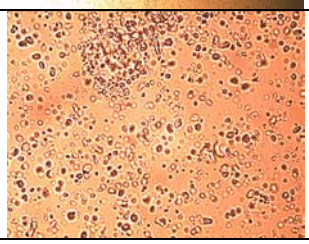
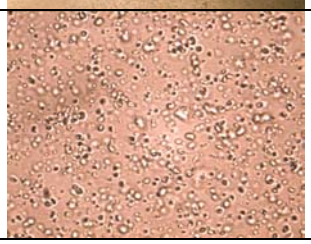
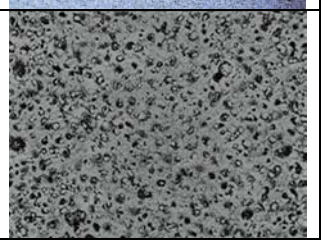
		d0	d1	d7
original suspension non diluted	160x			
	1000x			
suspension diluted with stabiliser solution 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1 and further homogenised	160x			
	1000x			

Table 6-22: Suspensions stabilised with TPGS at d0, d1 and d7 stored at room temperature

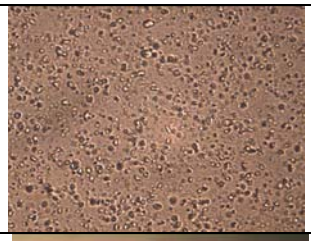
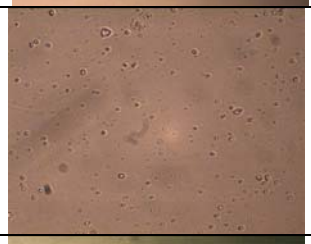
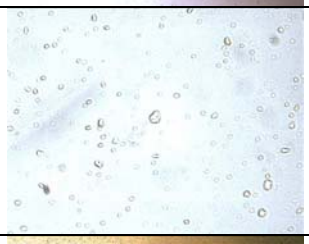
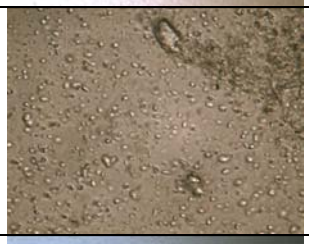
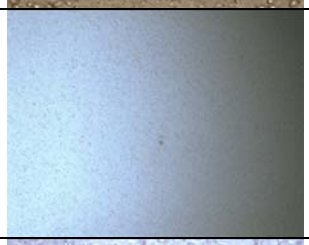
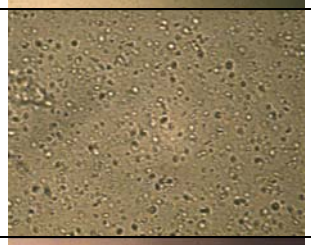
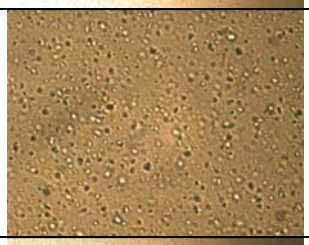
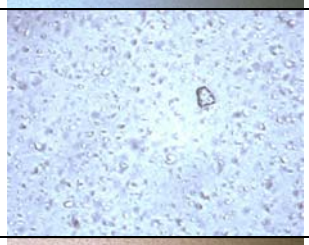
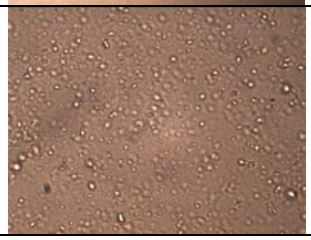
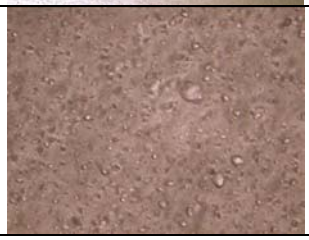
		d0	d1	d7
original suspension non diluted	160x			
	1000x			
suspension diluted with stabiliser solution 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1 and further homogenised	160x			
	1000x			

Table 6-23: Suspensions stabilised with TPGS at d0, d1 and d7 stored at cold temperature (4°C)

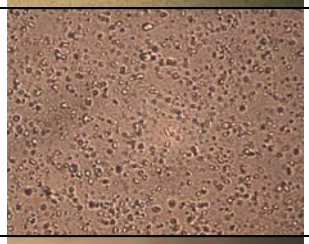
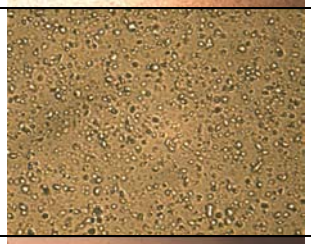
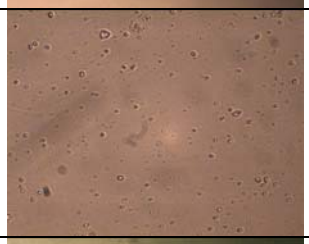
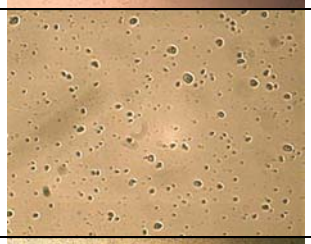
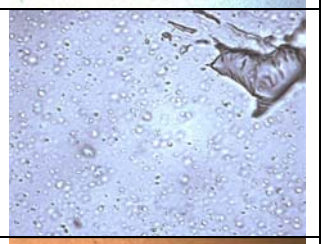
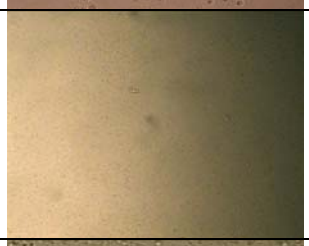
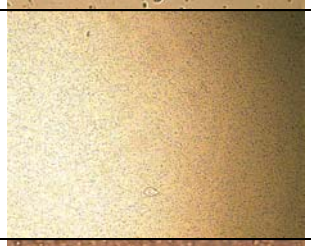
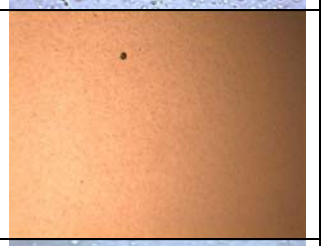
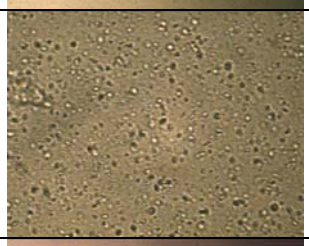
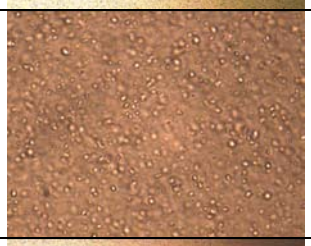
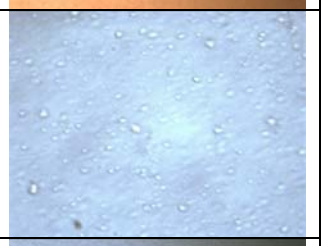
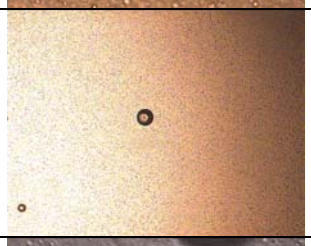
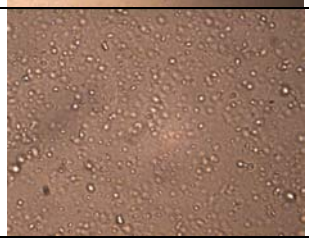
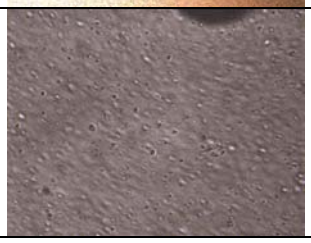
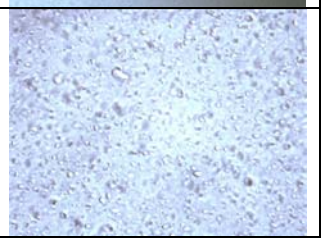
		d0	d1	d7
original suspension non diluted	160x			
	1000x			
suspension diluted with stabiliser solution 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1 and further homogenised	160x			
	1000x			

Table 6-24: Suspensions stabilised with SDS at d0, d1 and d7 stored at room temperature

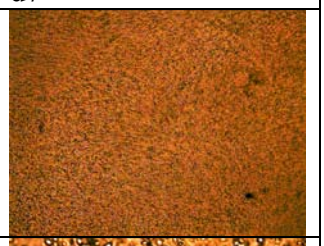
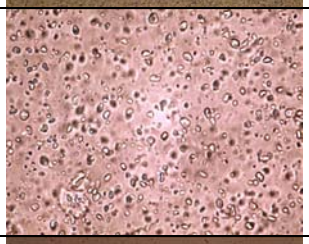
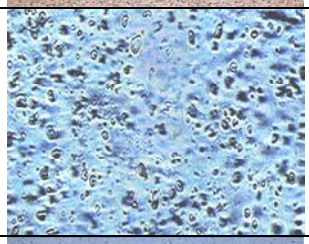
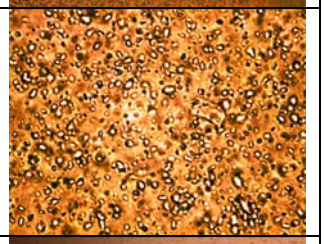
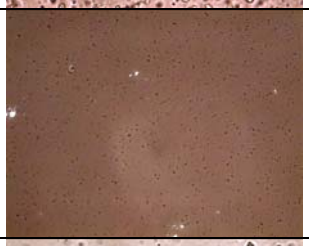
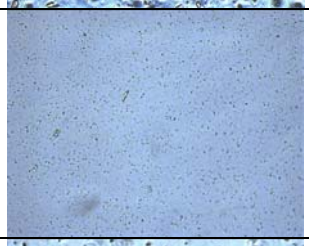
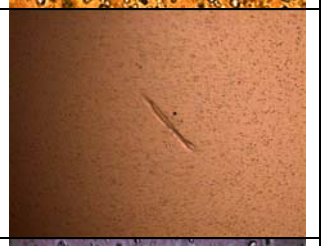
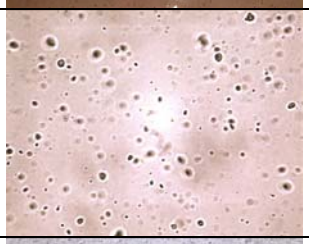
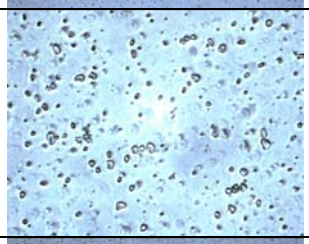
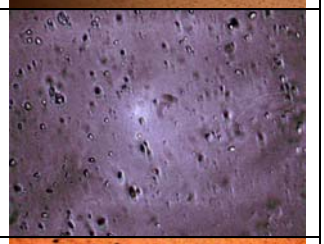
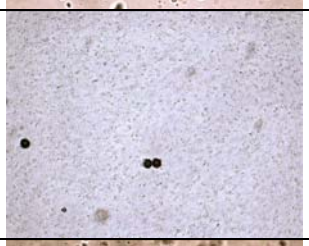
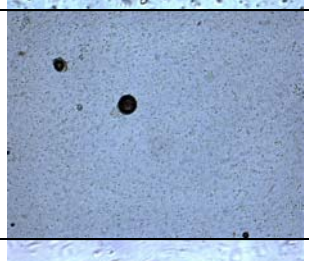
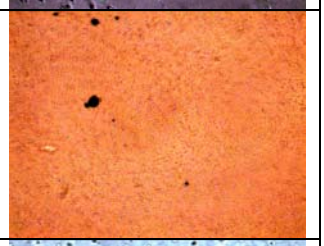
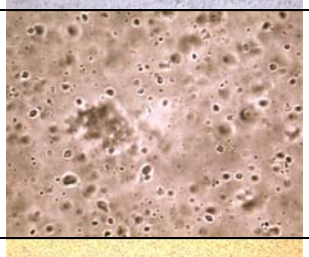
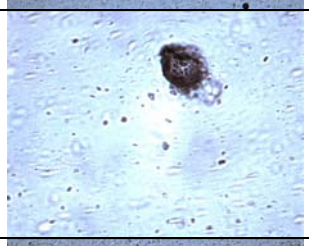
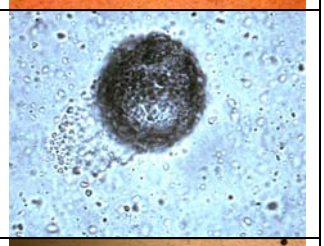
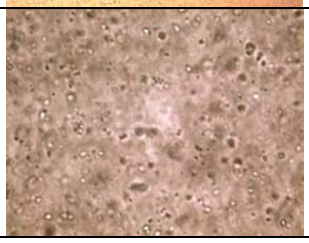
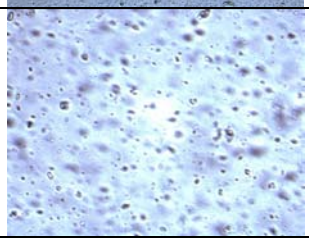
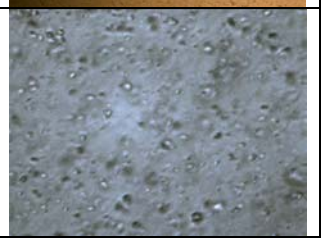
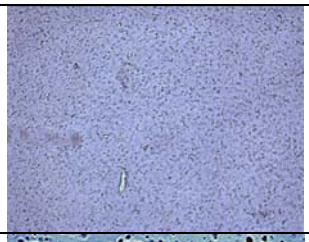
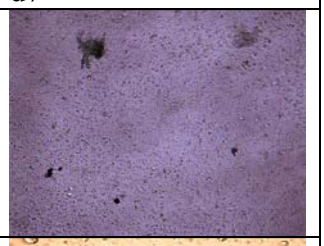
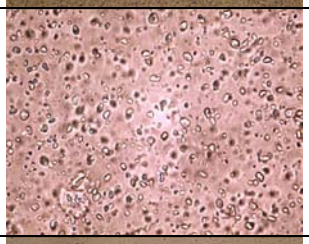
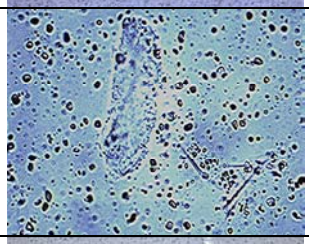
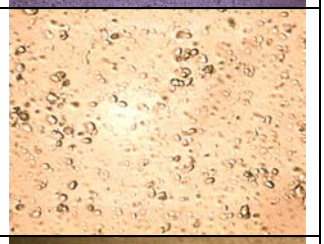
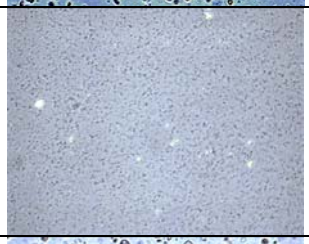
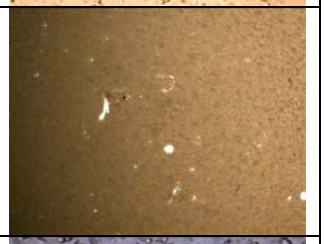
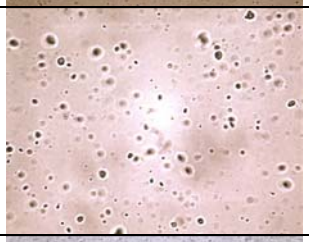
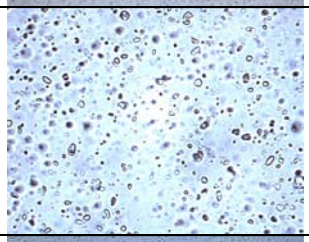
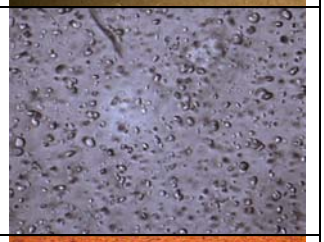
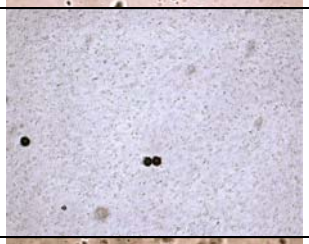
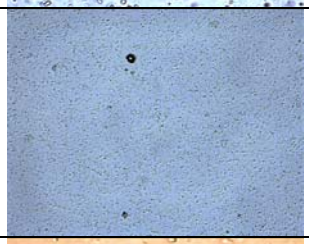
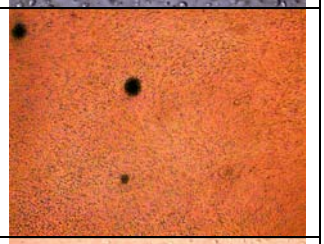
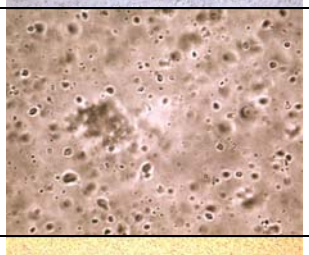
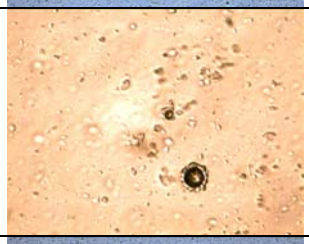
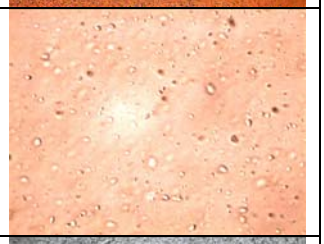
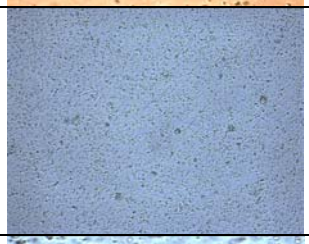
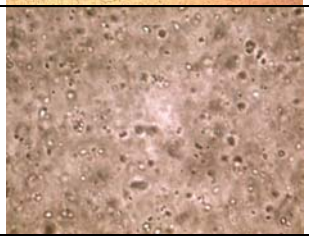
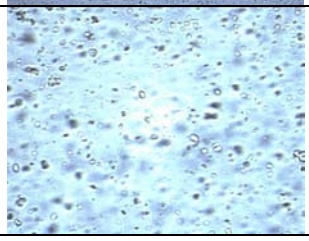
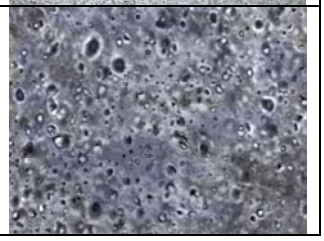
		d0	d1	d7
original suspension non diluted	160x			
	1000x			
suspension diluted with stabiliser solution 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1 and further homogenised	160x			
	1000x			

Table 6-25: Suspensions stabilised with SDS at d0, d1 and d7 stored at cold temperature (4°C)

		d0	d1	d7
original suspension non diluted	160x			
	1000x			
suspension diluted with stabiliser solution 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1	160x			
	1000x			
suspension diluted with glycerol 1:1 and further homogenised	160x			
	1000x			

The aim of the study was to investigate if nanosuspensions, which were diluted after the homogenisation process behave the same as those which were not diluted during storage.
Cyclosporine partially dissolves in water and aqueous stabiliser solutions. Though, when stabiliser solution was used as dissolution medium, the cyclosporine nanocrystals dissolved to a certain amount, which led to an increase in particle size already visible at the day of production. Storage of the suspensions for only 24 hours led to further dissolution and a loss of particles therefore in some cases (e.g. suspensions diluted with Poloxamer 407 and TPGS stored at room temperature). However in some other cases, especially in case of diluted suspensions stabilised with SDS and stored at room temperature, the particle size was decreased after 24 hours of storage. This might be due to a slight further dissolution of nanoparticles, which does not lead to a loss of particles but only to a decrease in particle size.
In conclusion it was shown, that dilution of suspensions with aqueous stabiliser solution led to changes in the suspensions. After only one day of storage clear differences between non diluted and diluted suspensions can be seen, indicating already at this stage of the study, that the conventional method of stabiliser screening, where suspensions undergo subsequent dilution after its homogenisation process, is not an appropriate way to investigate the efficiency of the stabiliser used.
Dissolution effects also occurred within the suspensions stored at cold temperatures. Here the effect also occurred with the original non diluted suspensions. The explanation for those effects is the higher solubility of cyclosporine at colder temperatures. Those findings indicate that any change of solubility (e.g. increase of dissolution volume, addition of compounds which increase the solubility, increase or decrease in temperature) will lead to changes of the systems in a way not comparable to their origin anymore.
The observations from microscopy are also confirmed by laser diffractometry, but only when the optimised method was used. Figure 6-20 shows the comparison of the LD 50s obtained at d0 and d1 from all suspensions produced.

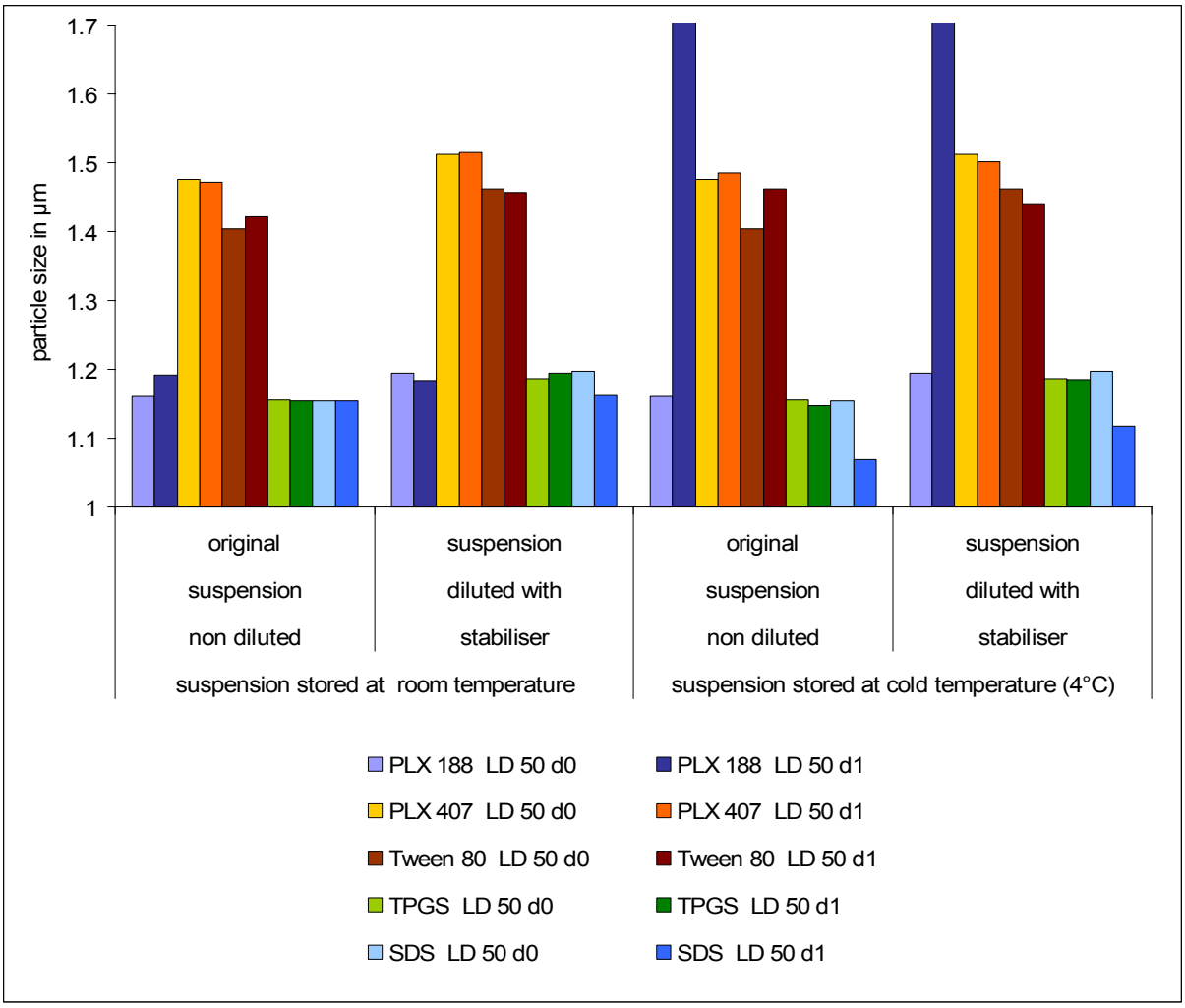


Figure 6-20: Comparison of LD 50 at day of production and d1 for all nanosuspensions non diluted and diluted with stabiliser solution

Suspensions were also diluted with glycerol. Glycerol is no dissolution medium for cyclosporine; therefore no dissolution was expected for the suspensions stored at room temperature, whereas little changes were expected within the suspensions stores at cold temperature. However, from microscopy some dissolution effects were observed for the suspensions stabilised with SDS and stored at room temperature. The storage of suspensions at cold temperatures showed some decreases in particle size for the suspensions stabilised with TPGS and SDS. However, the LD measurements show tremendous changes in the LD 50 after 24 hours of storage. The question, if those changes are contributed to dissolution, can be answered by comparing the results obtained with the images obtained from microscopy. It gives evidence that the increase in particle size measured is due to crystal growth and the appearance of agglomerates in case of Poloxamer 188. The increase in size within the suspensions stabilised with Poloxamer 407 is contributed to air bubbles, visible as black dots in the picture. The increase of the particle size (LD 50) within the suspension stabilised with

TPGS is not directly observed by microscopy, indicating that only growth of the main population without the appearance of large agglomerates is hardly to see by microscopy. However by comparing the LD 90, 99 and 100, really no large particles are found in these suspensions. Suspensions stabilised with glycerol did not change, neither in microscopy nor in LD analysis. Suspensions stabilised with SDS contained air bubbles, which do not influence the LD 50.

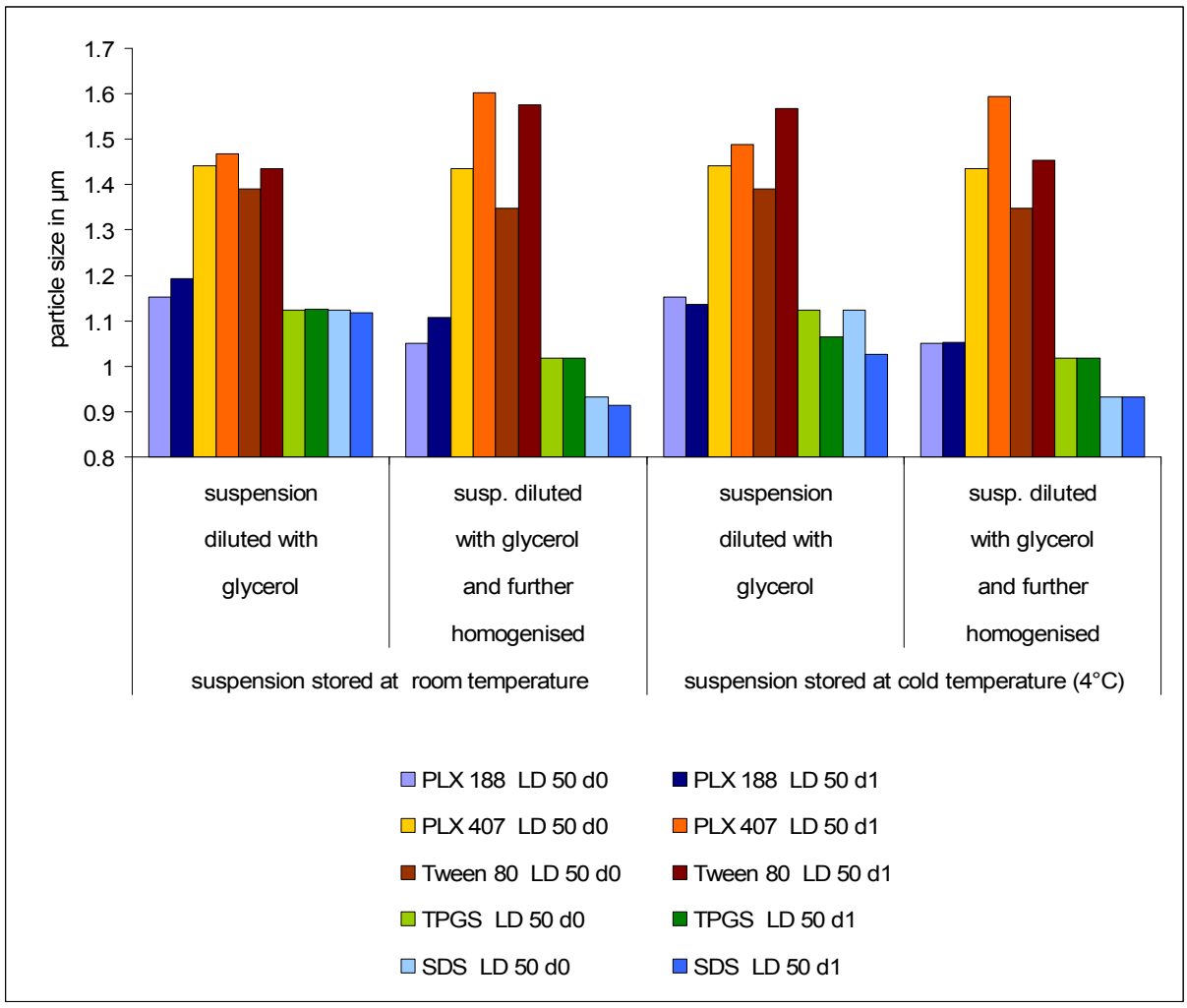


Figure 6-21: Comparison of LD 50 at day of production and d1 for all nanosuspensions diluted with glycerol with and without further homogenisation

In summary, as expected, dilution with glycerol did not lead to distinct changes within the nanosuspensions. Only storage at cold temperatures led to some dissolution effects. The increase in particle size in some of the cases (PLX 188, PLX 407) obtained from laser diffractometry could by explained by the appearance of air bubbles or agglomerates seen in microscopy analysis. Microscopic imaging in parallel to laser diffractometry could avoid misinterpretation of the results obtained from laser diffractometry (i.e. increase of particle size

due to dissolution of particles), which again shows the importance of a combined particle size characterisation method.

Further storage of the suspensions led to further changes of the systems. After only one week (d7) of storage, remarkable differences of the suspensions could be observed.

Poloxamer 188 seems to be not sufficient for stabilising cyclosporine nanocrystals, as large crystals were observed in each suspension, which could also be proven by LD measurements (Figure 6-22). Beside this, nanosuspensions diluted with glycerol seem to be better than those without glycerol. Diluted suspensions with aqueous stabiliser solution are worst, whereas storage at room temperature proved to be better than storage at cold temperatures.

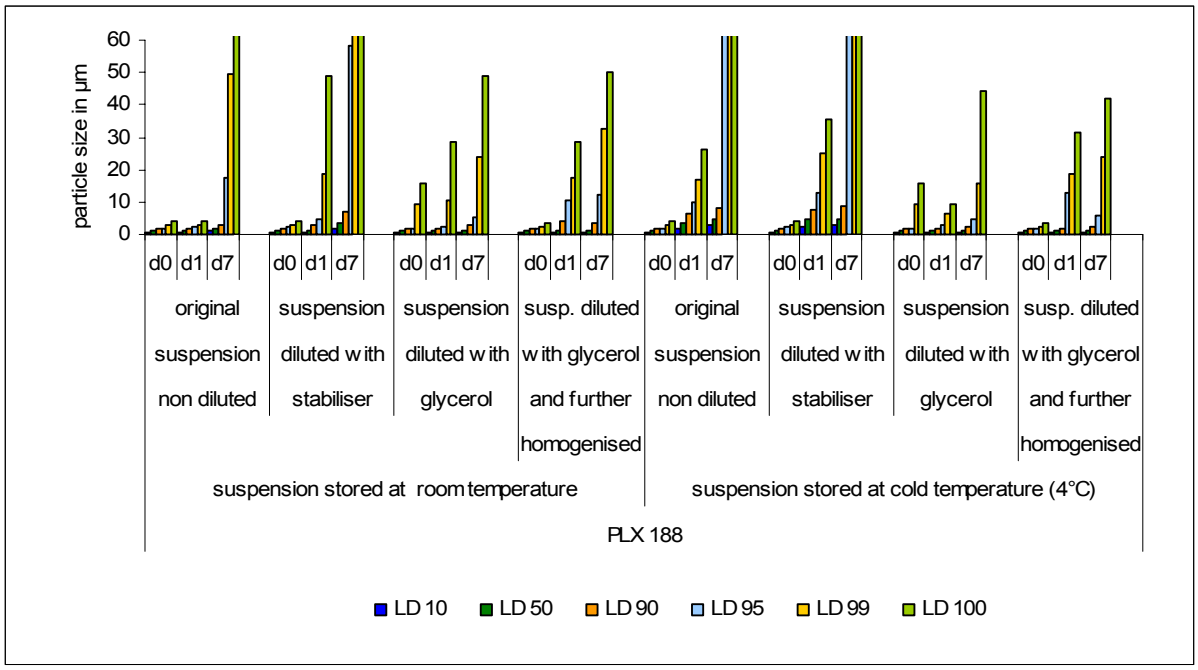
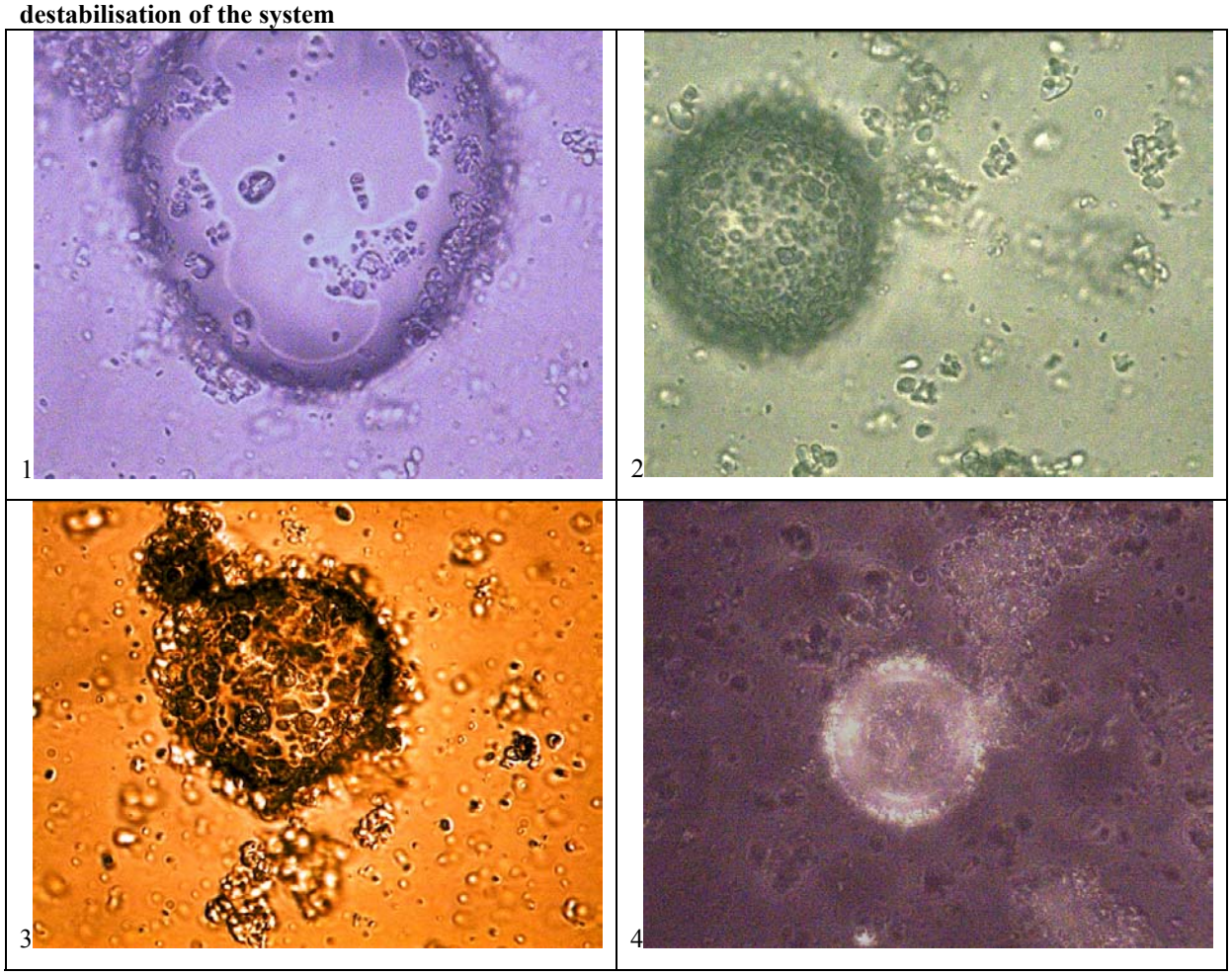


Figure 6-22: LD data of cyclosporine nanosuspensions stabilised with Poloxamer 188 at d0, d1 and d7

Poloxamer 407 seems to be more efficient in the stabilisation of cyclosporine nanocrystals. However only the suspensions diluted with glycerol but not further homogenised seem to be physically stable. Further homogenisation led to the incorporation of air bubbles, which led to the absorption of cyclosporine nanocrystals (Table 6-26, picutres 1-3). Microscopy with polarised light gives evidence that the absorption of cyclosporine nanocrystals will lead to aggregation of the particles and the growth of larger crystals further on (Table 6-26, picture 4).

Table 6-26: Cyclosporine nanosuspension stabilised with Poloxamer 407- air bubbles were incorporated during the homogenisation with glycerol leading to the adsorption of cyclosporine nanocrystals and destabilisation of the system



Non diluted suspensions showed agglomerated crystals. Here the suspensions stored at room temperature seemed to be less stable than those stored at cold temperatures, where suspensions diluted with aqueous stabiliser solution were worst. Figure 6-23 shows the corresponding LD data of d0, d1 and d7 for the suspensions stabilised with Poloxamer 407. Also here the LD data give the same results as obtained from microscopy.

Suspensions stabilised with Tween 80 seem to be more stable than those stabilised with Poloxamers. Non diluted suspensions and suspensions diluted with glycerol stored at room temperature seem to be stable. Also the suspension diluted with glycerol and further homogenised stored at cold temperatures remains stable after a storage of 7 days. All other suspensions (suspensions stored at cold temperatures and the suspension diluted with water and stored at room temperature) show agglomerates, where the one mentioned last was worst. However, the results from laser diffractometry (Figure 6-24) do not detect some of the agglomerates, which indicates the loose agglomeration of the particles, weak enough to get de-aggregated during the measurement in the laser diffractometer.

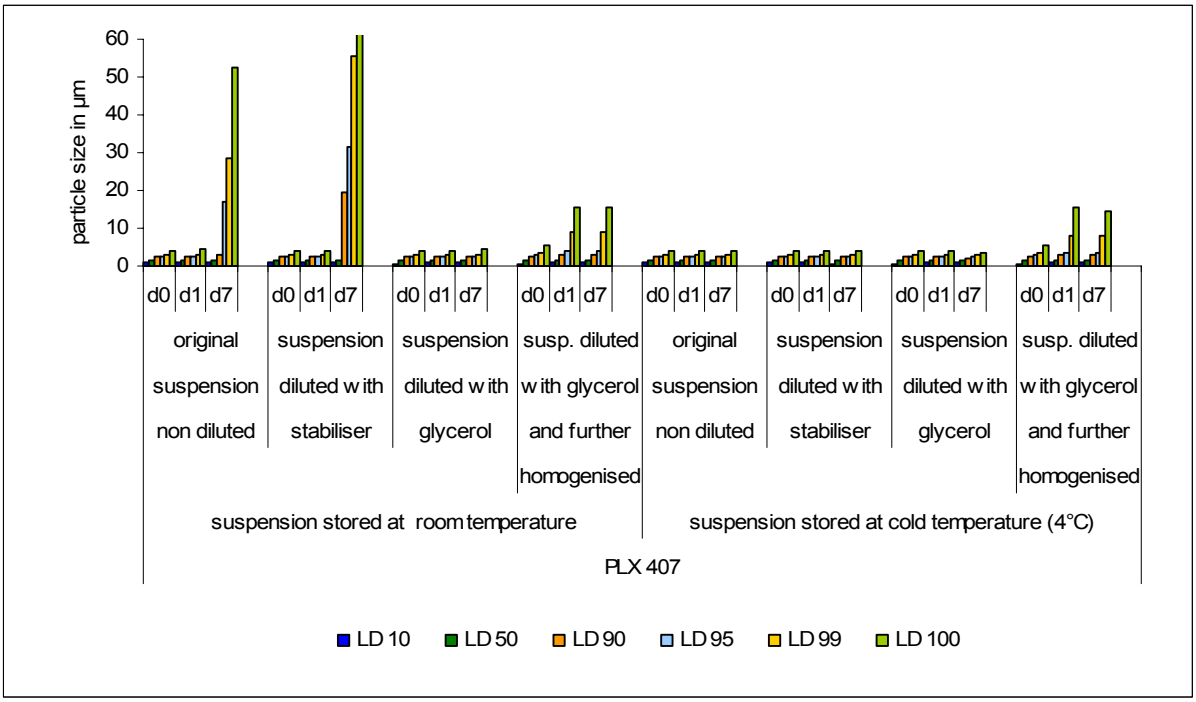


Figure 6-23: LD data of cyclosporine nanosuspensions stabilised with Poloxamer 407 at d0, d1 and d7

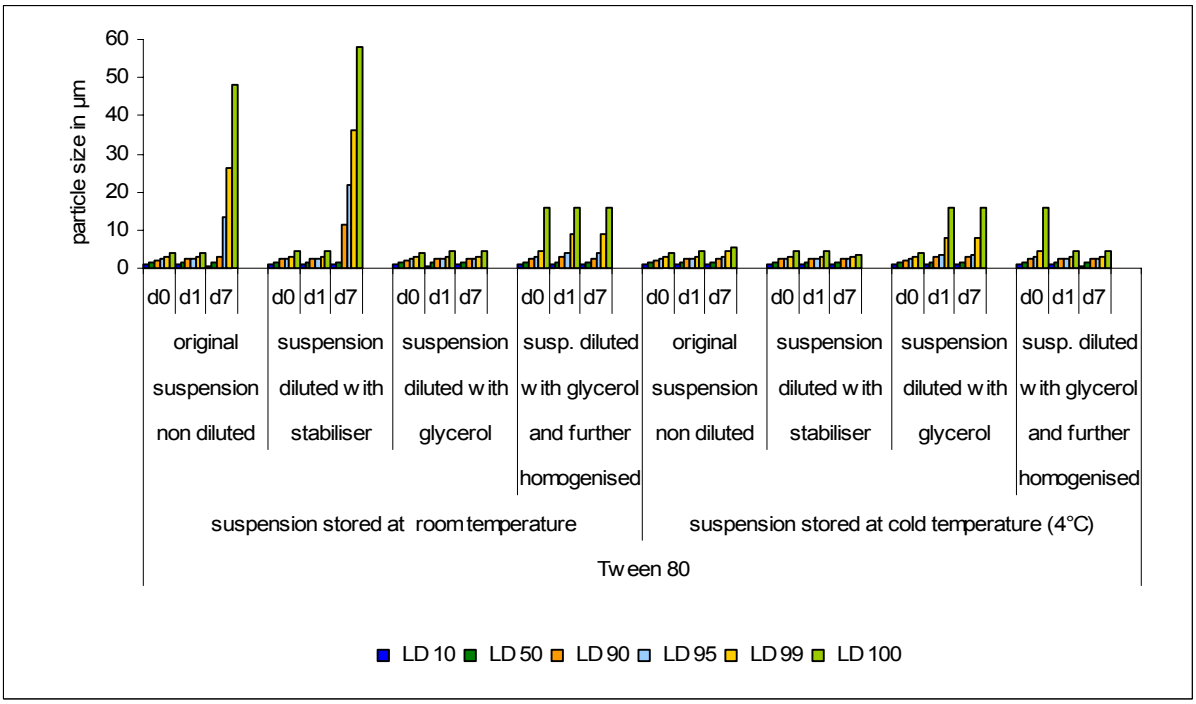


Figure 6-24: LD data of cyclosporine nanosuspensions stabilised with Tween 80 at d0, d1 and d7

Stabilisation of cyclosporine nanocrystals with TPGS seems to be sufficient. Suspensions diluted with glycerol are stable. Suspensions none diluted show some agglomerates (not detected with laser diffractometry, Figure 6-25), whereas more agglomerates were found in the suspension stored at cold temperatures. Only the suspensions diluted with aqueous stabiliser solution show larger crystals. Therefore, also here it could be seen that dilution can

cause destabilisation of a system. In conclusion, if the conventional stabiliser screening is performed, where the nanosuspension is subsequently diluted with stabiliser solution, observed instabilities can also derive from dissolution effects and not only from a poor choice of the stabiliser tested. If dissolution effects are not considered, the interpretation of the data would lead to the false exclusion of a stabiliser tested.

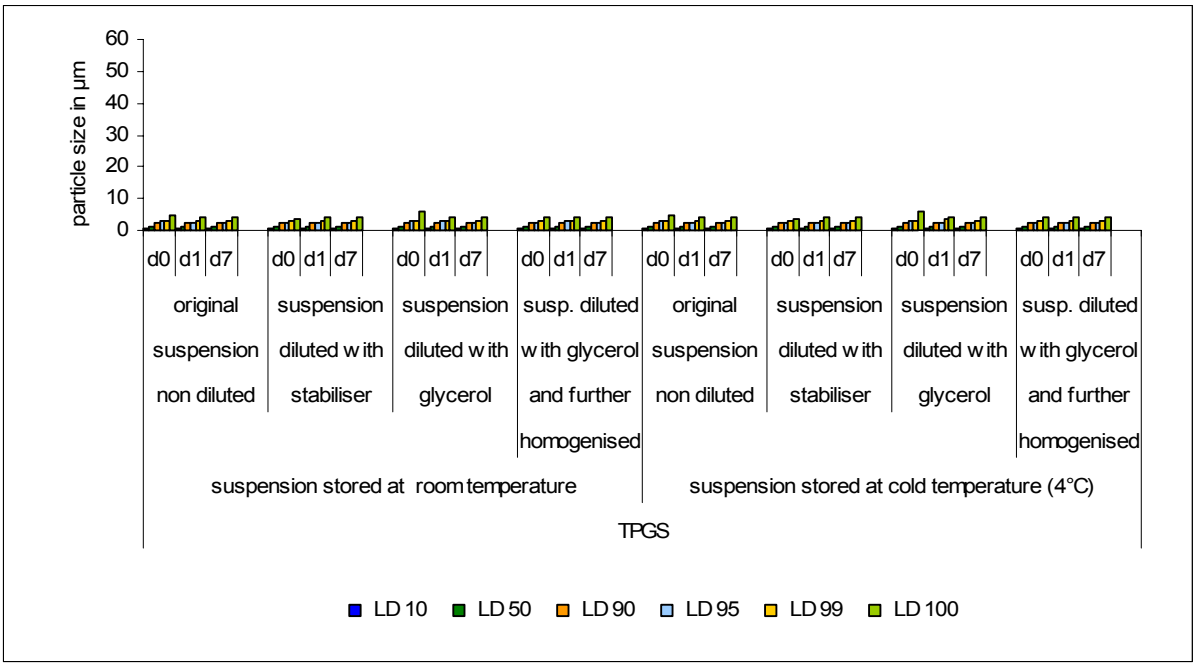


Figure 6-25: LD data of cyclosporine nanosuspensions stabilised with TPGS at d0, d1 and d7

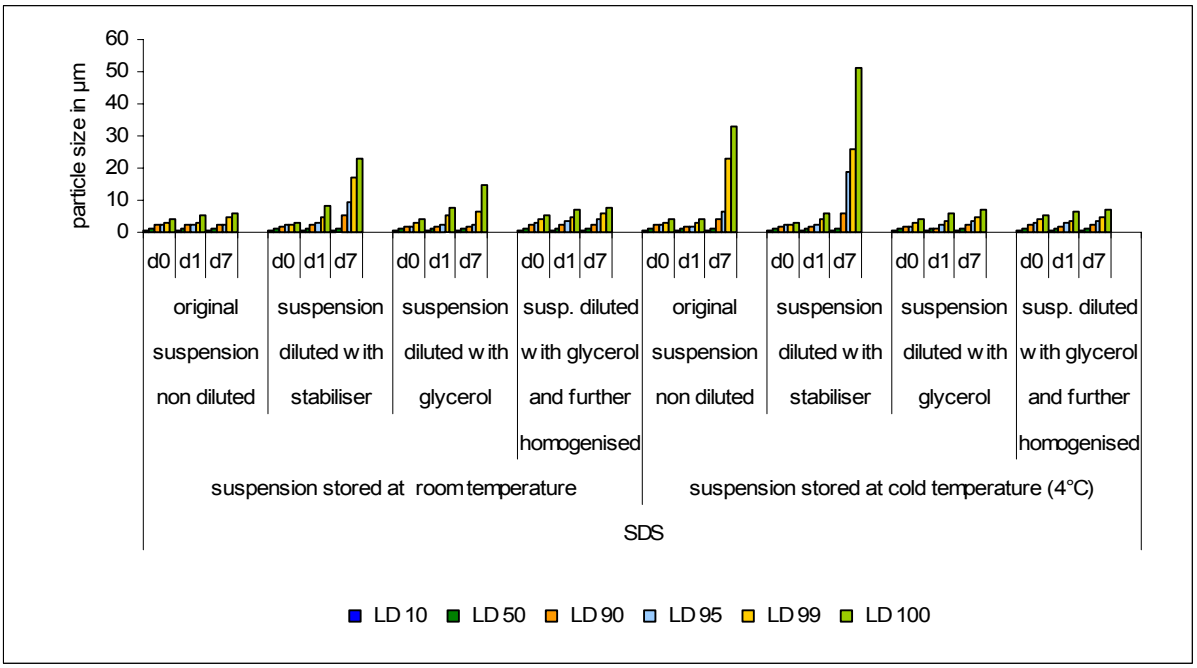


Figure 6-26: LD data of cyclosporine nanosuspensions stabilised with SDS at d0, d1 and d7

SDS was the last stabiliser tested. Only the suspension diluted with glycerol and further homogenisation stored at cold temperatures seems to be physically stable. Suspensions without further homogenisation include air bubbles, which led to the same effect as observed from the suspensions stabilised with Poloxamer 407. However, all other suspensions produced contain crystals and agglomerates. As expected, most of the crystals are found in the suspensions which were diluted with aqueous stabiliser solution (Figure 6-26).

In conclusion, after 7 days of storage most of the suspensions produced contained agglomerates and crystals. TPGS was found to be the most sufficient stabiliser, followed by SDS. However, it was found that the addition of glycerol seems to stabilise the systems. Only if air bubbles are incorporated it destabilises the suspension, as nanocrystals adhere onto the surface of the bubbles. These adsorbed particles form aggregates, which will transfer into large crystals over time. Air bubbles were found within the suspension stabilised with Poloxamer 407 and further homogenised and in the suspension stabilised with SDS not further homogenised. The finding may indicate that the physical properties of the stabiliser used may play a role for the appearance of bubbles. Poloxamer 407 is a relatively hydrophobic stabiliser, whereas SDS is a very hydrophilic compound. Hydrophobic compounds tend to be aerophilic, which means they easily adhere onto air. High pressure homogenisation as high energy process may therefore enhance the incorporation of those air bubbles observed. On the opposite, SDS is an easily foaming surfactant. The addition of glycerol increases the viscosity of the system, which probably avoids the escape of the air bubbles being already in the system. Shear forces may be able to destroy the bubbles during the high pressure homogenisation process.

The most important result found here was, that truly the dilution of suspensions after homogenisation destabilises the system and promotes crystal growth and agglomeration in these suspensions. The results obtained from none diluted and from diluted suspensions with aqueous stabiliser solution give different results over the time of storage. Some agglomerates seen from microscopy could not be detected by laser diffractometry, even though the optimised method was used. If a lack of detection due to low sensitivity of the instrument can be excluded, the explanation is that agglomerates are only loose and are de-aggregated by the stirrer of the laser diffractometer during the measurement.

6.1.2.2.4 Comparison of suspensions after 4 weeks of storage

The Table 6-27 shows the images which were obtained at day 28 (d28) from the suspensions none diluted and diluted with aqueous stabiliser solution and stored at room temperature. For comparison the table below (Table 6-28) shows all the suspensions none diluted and diluted with water but stored at cold temperature.

Table 6-27: Microscopic images obtained after 4 weeks of storage from the non diluted and with stabiliser solution diluted suspensions stored at room temperature

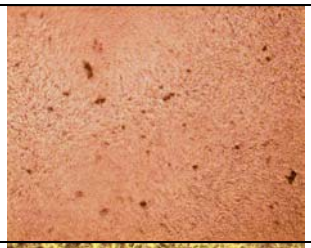
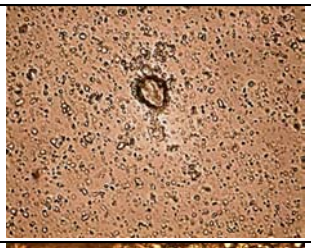
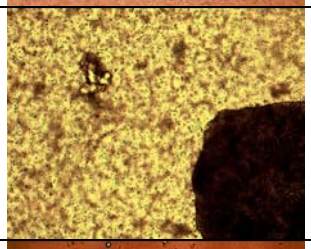
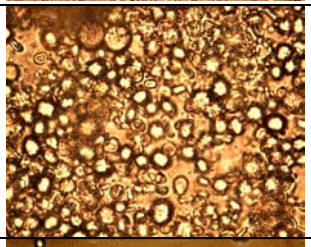
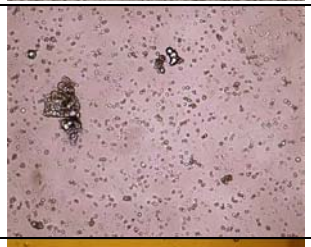
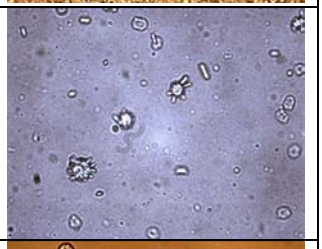
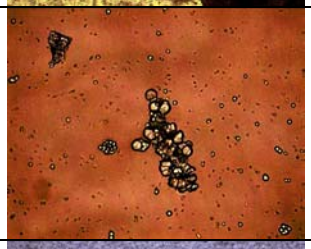
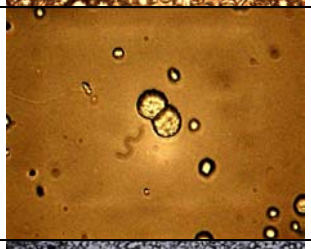
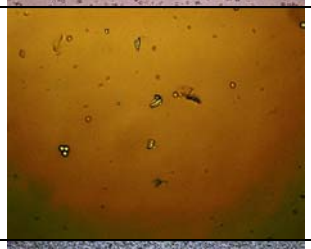
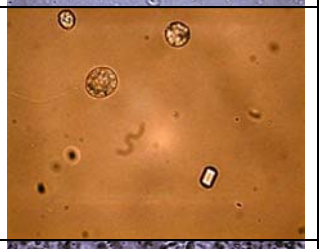
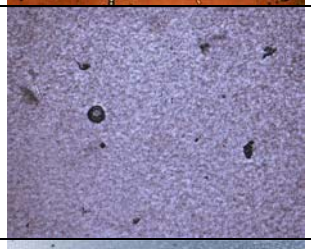
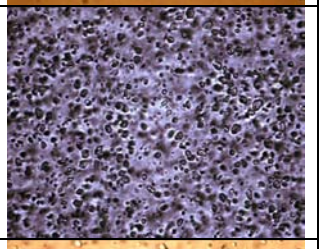
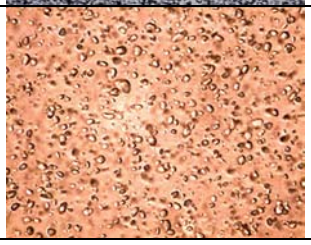
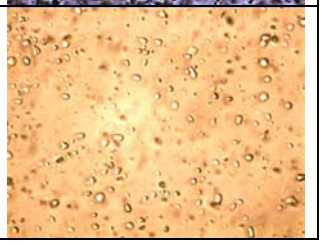
	original suspension - non diluted		suspension diluted with stabiliser solution 1:1	
	160x	1000x	160x	1000x
Poloxamer 188				
Poloxamer 407				
Tween 80				
TPGS				
SDS				

Table 6-29 and Table 6-30 show all the suspensions which were diluted with glycerol. The images are differently arranged than before. Suspensions should directly be compared between each other. Therefore the first two columns show the original non diluted suspensions, the third and fourth column show the suspensions diluted with aqueous stabiliser solution. The first and third columns show images with a magnification of 160x to give an overview of the consistency of the suspensions. The second and fourth columns show the suspensions with a magnification of 1000x for detailed information.

Table 6-28: Microscopic images obtained after 4 weeks of storage from the non diluted and with stabiliser solution diluted suspensions stored at cold temperature

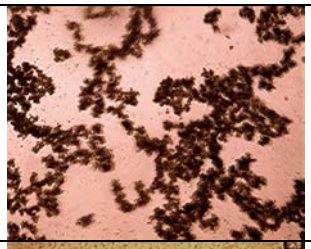
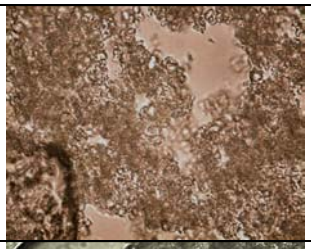
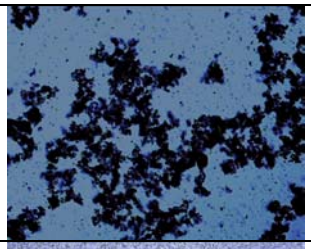
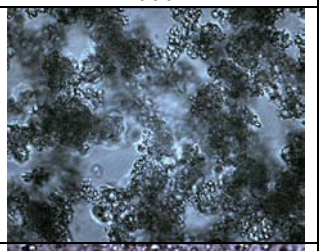
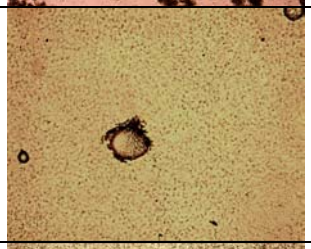
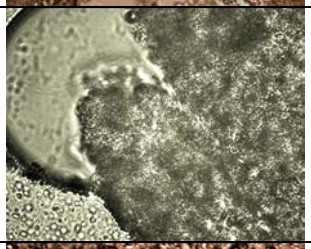
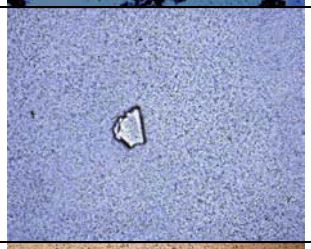
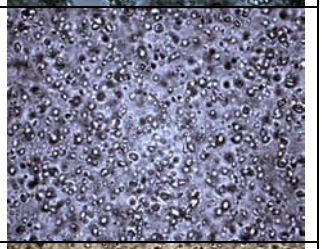
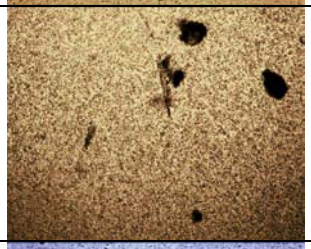
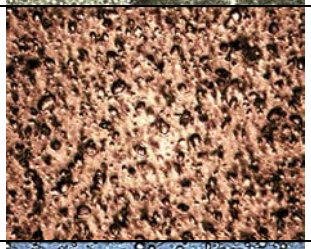
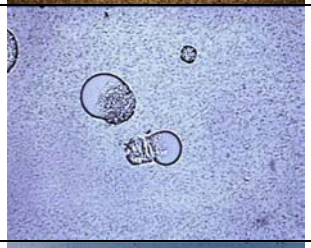
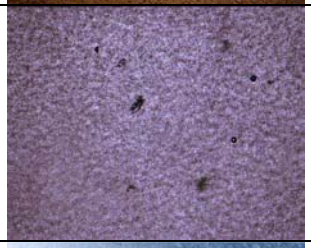
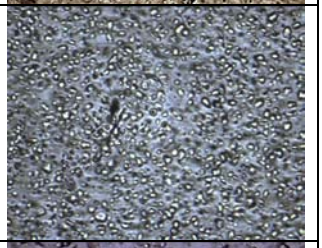
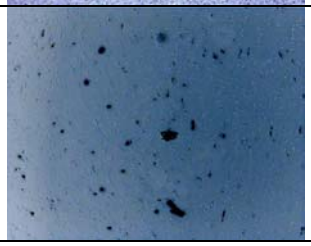
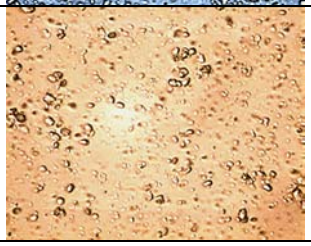
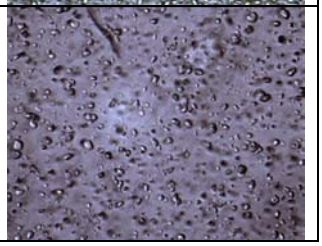
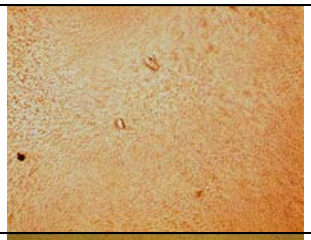
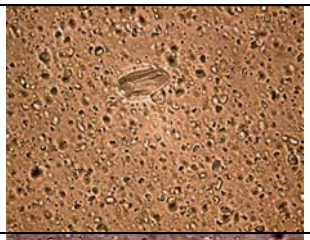
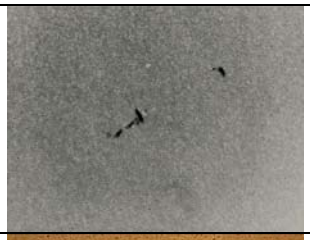
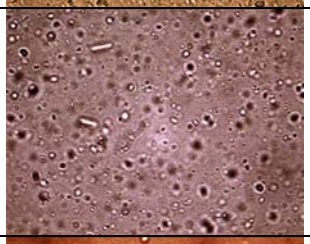
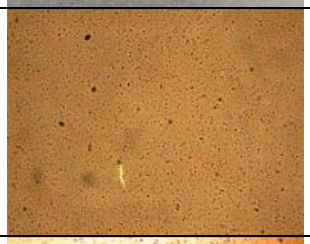
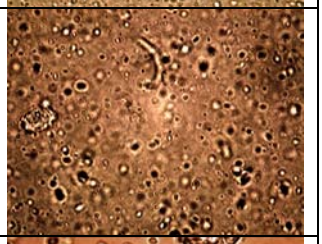
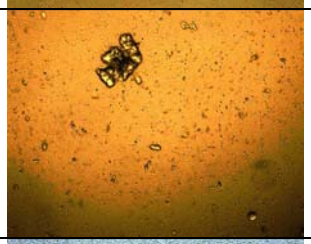
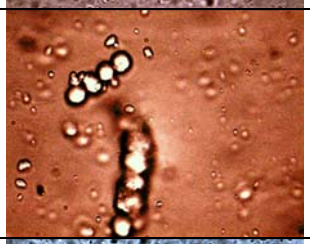
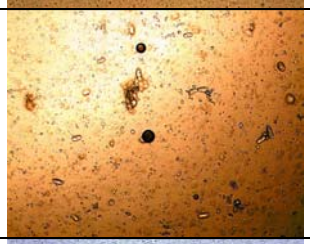
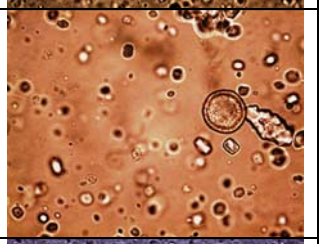
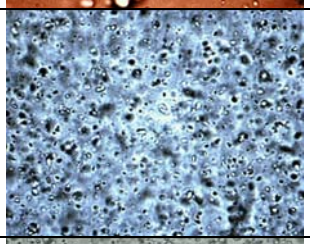
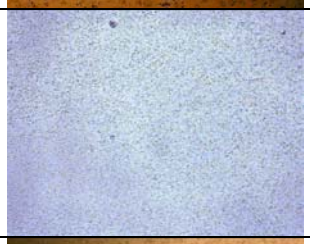
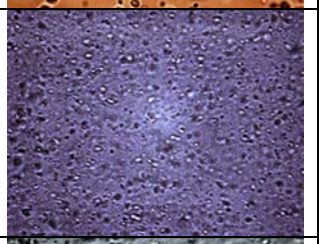
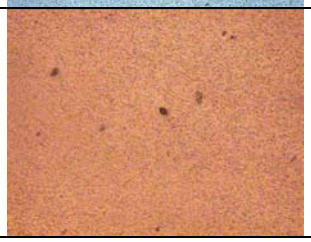
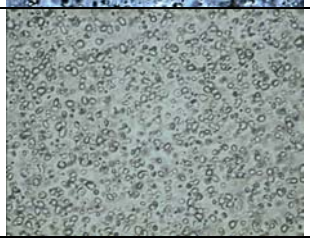
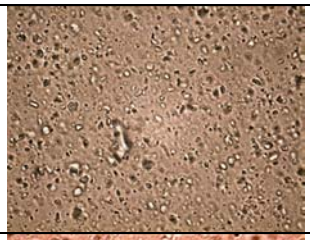
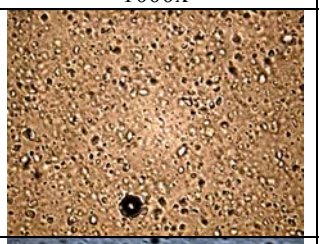
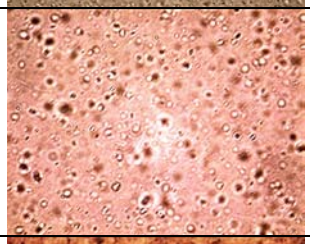
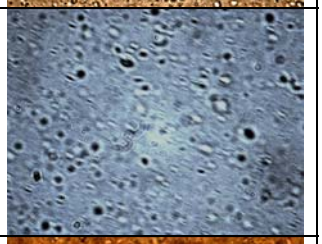
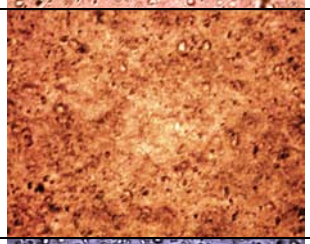
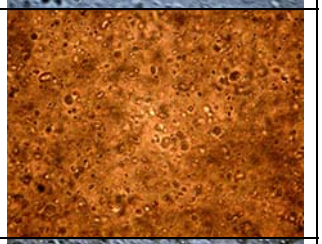
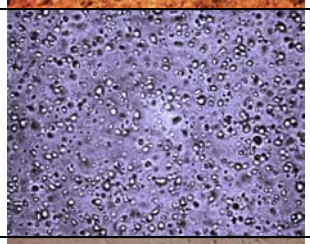
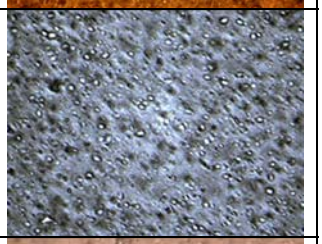
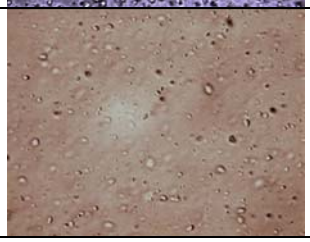
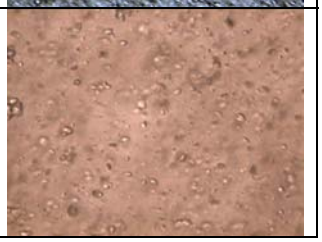
	original suspension - non diluted		suspension diluted with stabiliser solution 1:1	
	160x	1000x	160x	1000x
Poloxamer 188				
Poloxamer 407				
Tween 80				
TPGS				
SDS				

Table 6-29: Microscopic images obtained after 4 weeks of storage from suspensions diluted with glycerol with and without further homogenisation stored at room temperature

	suspension diluted with glycerol 1:1		suspension diluted with glycerol 1:1 and further homogenised	
	160x	1000x	160x	1000x
Poloxamer 188				
Poloxamer 407				
Tween 80				
TPGS				
SDS				

In Table 6-29 and Table 6-30 the suspensions diluted with glycerol are compared between each other. The first two columns show the suspensions only diluted with glycerol. The third and the fourth columns show the suspensions which were further homogenised. The comparison of the images obtained after four weeks of storage show an unexpected result for all the suspension stabilised with Tween 80 stored at room temperature. Those suspensions underwent a transformation into a droplet like form (Table 6-29). Nanocrystals are not present in these suspensions anymore. Storage at cold temperatures could avoid the transformation (Table 6-30). The same observation was done for the suspensions stabilised with Poloxamer 407, even though to a much lesser extent.

Table 6-30: Microscopic images obtained after 4 weeks of storage from suspensions diluted with glycerol with and without further homogenisation stored at cold temperature

	suspension diluted with glycerol 1:1		suspension diluted with glycerol 1:1 and further homogenised	
	160x	1000x	160x	1000x
Poloxamer 188				
Poloxamer 407				
Tween 80				
TPGS				
SDS				

For the nanosuspensions stabilised with PLX 188, TPGS and SDS storage at room temperature was better than storage at cold temperature. However, for the suspensions stabilised with Tween 80 and PLX 407 the trend was opposite. Suspensions cooled seemed to be more stable than those stored at room temperature, which could already be seen at day seven of storage. Tween 80 is a liquid. When cyclosporine is added to pure liquid Tween 80 it dissolves. Poloxamer is solid; therefore dissolution in pure PLX 407 cannot be investigated. However, dissolved in water it forms micelles, similar to Tween 80. Therefore the transformation seen might be due to dissolution within the micelles of the stabilisers. Because of the extraordinary behaviour of the suspensions stabilised with Tween 80 and PLX 407 they are excluded from the further considerations and discussions.

For the comparison to the microscopic images the results from LD analysis are shown. Figure 6-27 corresponds to the data for the suspensions stabilised with Tween 80, which only gives the impression that suspensions stabilised with Tween 80 and stored at room temperature are less stable than those stored at cold temperature. Furthermore dilution either with aqueous stabiliser solution or glycerol seems to destabilise the system tremendously when stored at room temperature.

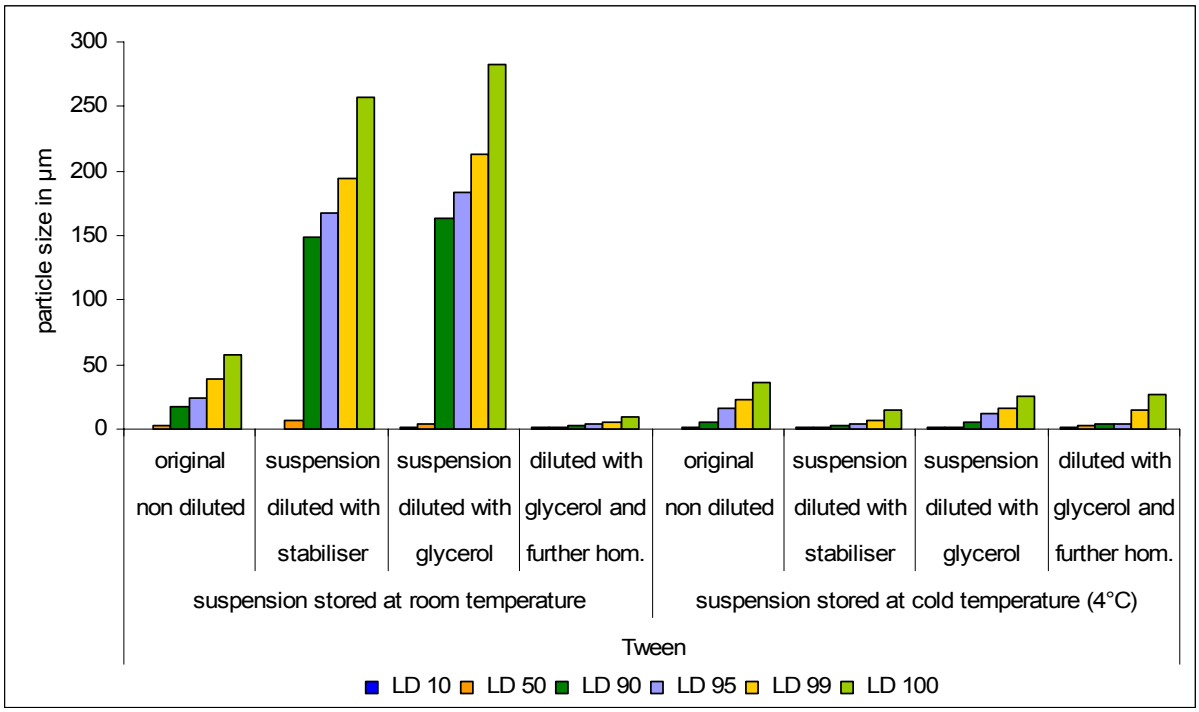


Figure 6-27: Results of LD analysis of suspensions stabilised with Tween 80 at day 28 (d28) of storage

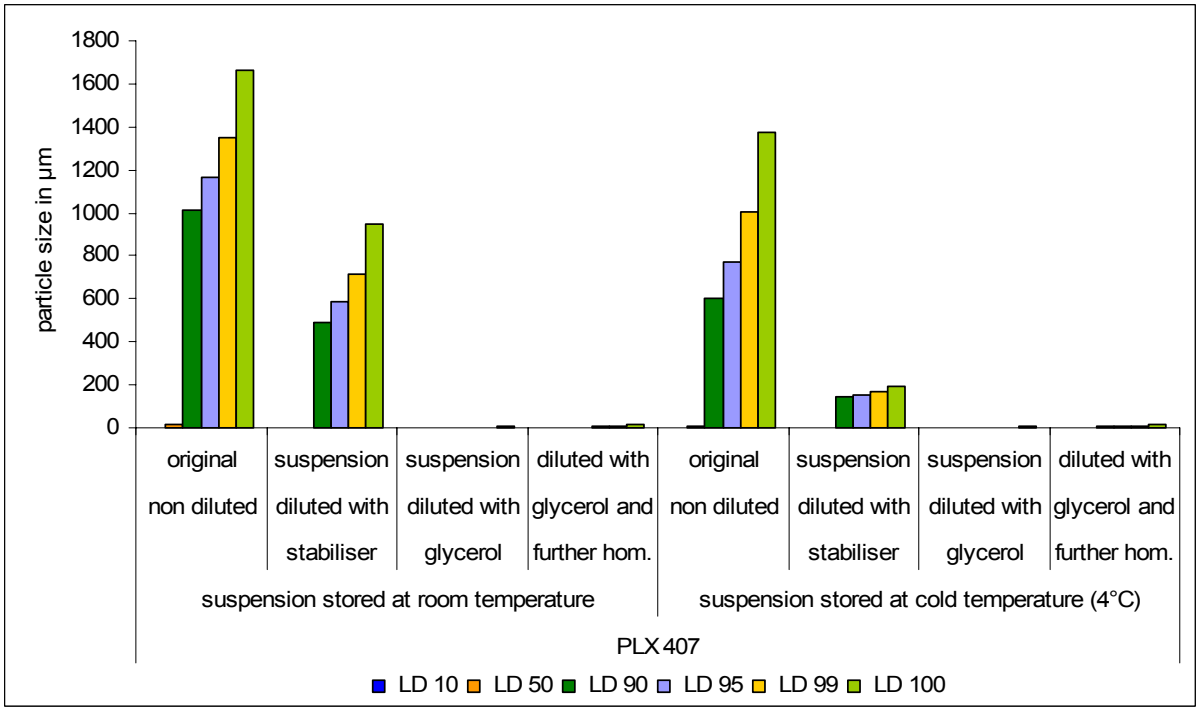


Figure 6-28: Results of LD analysis of suspensions stabilised with PLX 407 at day 28 (d28) of storage

LD analysis of the suspensions stabilised with Poloxamer 407 (Figure 6-28) indicates the enhanced stability of the suspensions diluted with glycerol. However no detailed information, e.g. the observed transformation of the particles into droplets can be drawn from LD measurements alone.

Suspensions stabilised with Poloxamer 188 developed agglomerates and large crystals. The best suspensions remained those diluted with glycerol and subsequent homogenisation. Suspensions diluted with aqueous stabiliser solution are worse when compared to those which remained non diluted. None of the suspensions is physically stable. Therefore also Poloxamer 188 is not an appropriate stabiliser for the stabilisation of cyclosporine nanosuspensions.

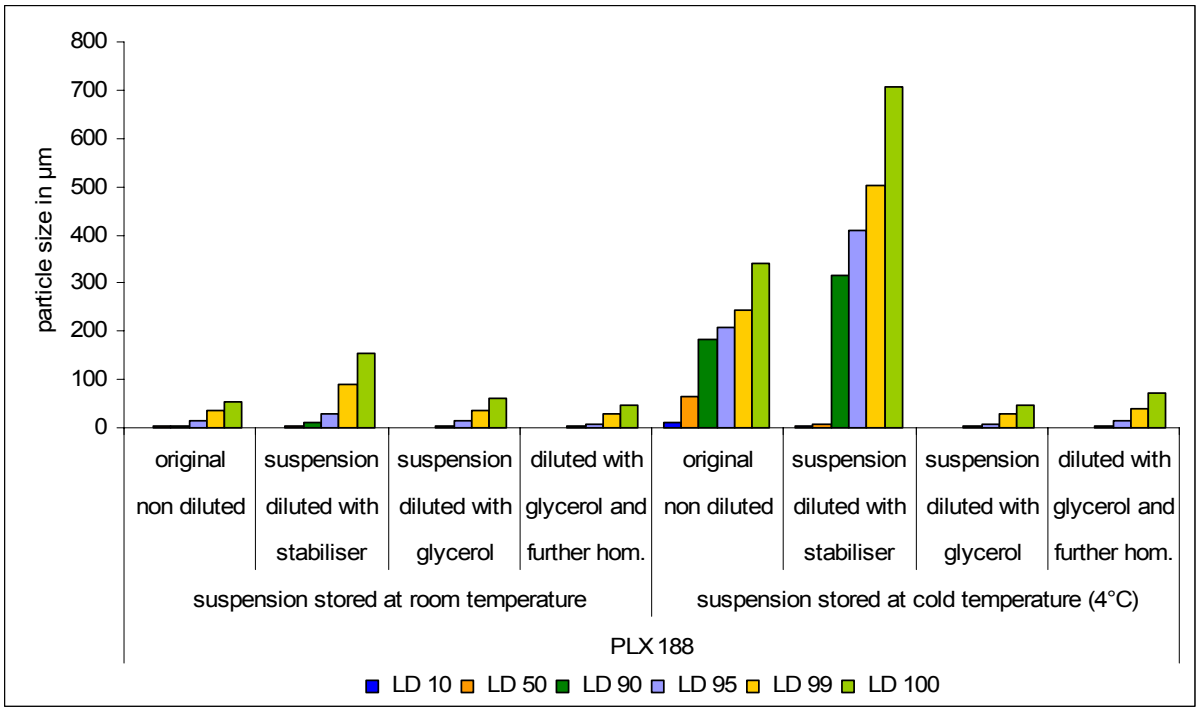


Figure 6-29: Results of LD analysis of suspensions stabilised with PLX 188 at day 28 (d28) of storage

The suspensions stabilised with TPGS seem to be the best of all suspensions produced. However, also suspensions diluted with glycerol seem to be more stable than those without glycerol. Storage at room temperature gives fewer agglomerates than storage at cold temperatures. The original suspension stored at room temperature now contained air bubbles. On their interface a beginning re-crystallisation can be observed. The suspensions diluted with aqueous stabiliser solution and the original non diluted suspension show a few crystals. In conclusion, only the suspensions diluted with glycerol seem to be physically stable after a storage time of 28 days. From laser diffractometry analysis the size of all suspensions was analysed to be smaller than 5µm, with only one exception - the original non diluted suspension stored at cold temperatures. Larger air bubbles with crystals were observed from microscopy, however probably to less in volume for the detection in LD analysis.

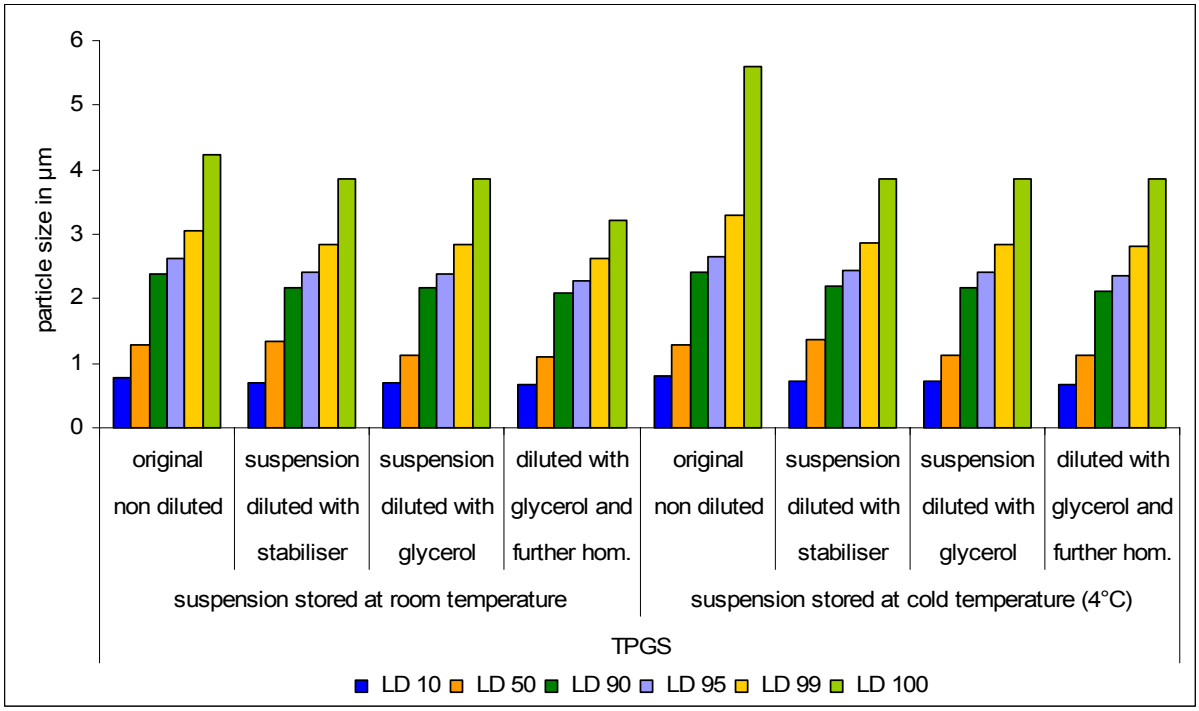


Figure 6-30: Results of LD analysis of suspensions stabilised with TPGS at d28 of storage

Agglomerates were not detected from laser diffractometry, indicating loose agglomeration. In conclusion after a time of strorge of 28 days only the suspensions diluted with glycerol are physically stable.

The suspensions stabilised with SDS show the same trend as observed for the suspensions stabilised with TPGS.

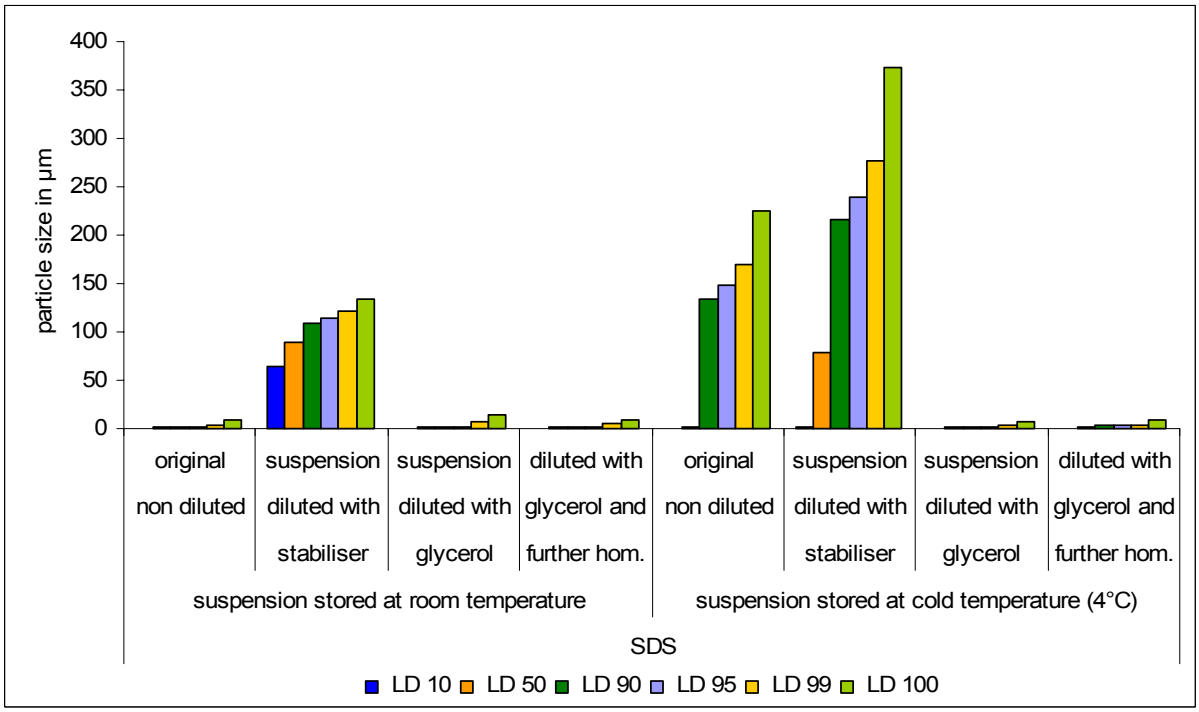


Figure 6-31: Results of LD analysis of suspensions stabilised with SDS at d28 of storage

Nevertheless, they are not as homogeneous as the suspensions stabilised with TPGS. Also here laser diffractometry did not detect the agglomerates found from microscopy. Therefore, a correct conclusion can only be drawn if the images from light microscopy are taken into consideration (c.f. Table 6-29 and Table 6-30). From this it is clear, that only the suspension stabilised with SDS, diluted with glycerol and further homogenisation and stored at cold temperatures remained physically stable over the period of 28 days of storage.

6.1.2.2.5 Comparison of LD data obtained at day 28 of storage

Finally all results of laser diffractometry obtained at d28 of storage are compared between each other, in order to draw the final conclusion of the study performed. Some attention should be drawn that diagrams showing original suspensions and the suspensions diluted with aqueous stabiliser solution have a y-scale ranging from 0-1600µm (Figure 6-32 and Figure 6-34), whereas the diagrams of the suspensions diluted with glycerol have a y-scale which only ranges from 0-100µm (Figure 6-33 and Figure 6-35).

The comparison of all data shows, that suspensions obtained from this study change differently. Cyclosporine nanosuspensions stabilised with Tween 80 and Poloxamer 407 transfered into oil droplet like systems, which cannot be seen from laser diffractometry, it can only be conducted, that the storage at room temperature is worse than storage at cold temperatures. The other suspensions which were stabilised with Poloxamer 188, TPGS and SDS confirm the expectations from theory. Nanosuspensions diluted with aqueous stabiliser solution are less stable than non diluted suspensions, whereas dilution with glycerol can stabilise the suspension. Additionally it was found that dilution with glycerol may lead to an incorporation of air bubbles, which actually destabilises the system.

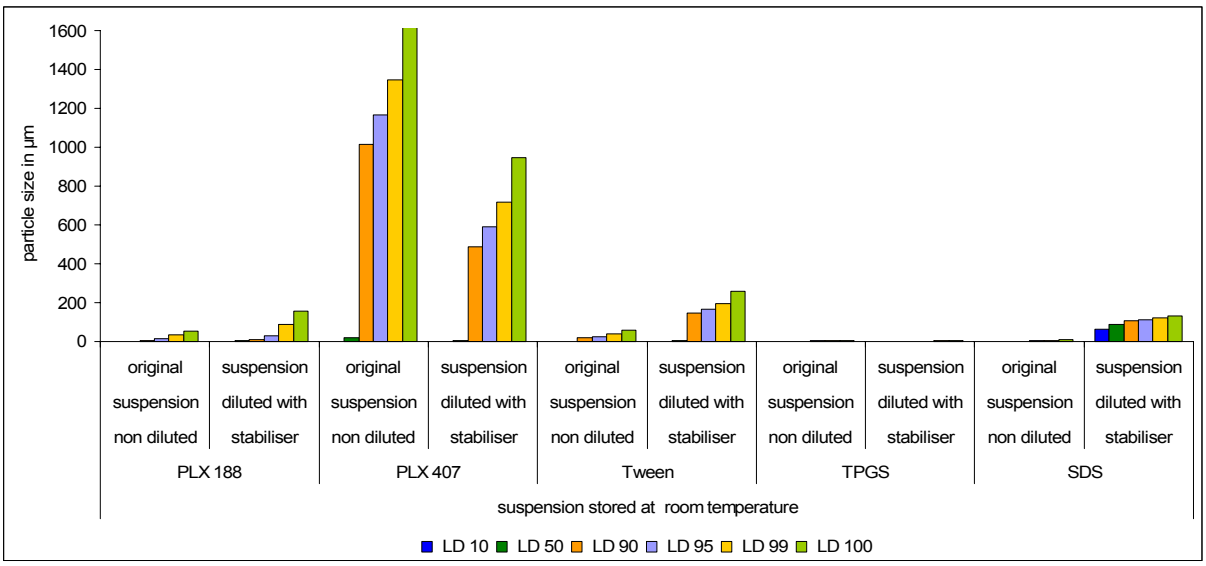


Figure 6-32: Results of LD analysis at day 28 of original non diluted suspensions and suspensions diluted with aqueous stabiliser solution, stored at room temperature

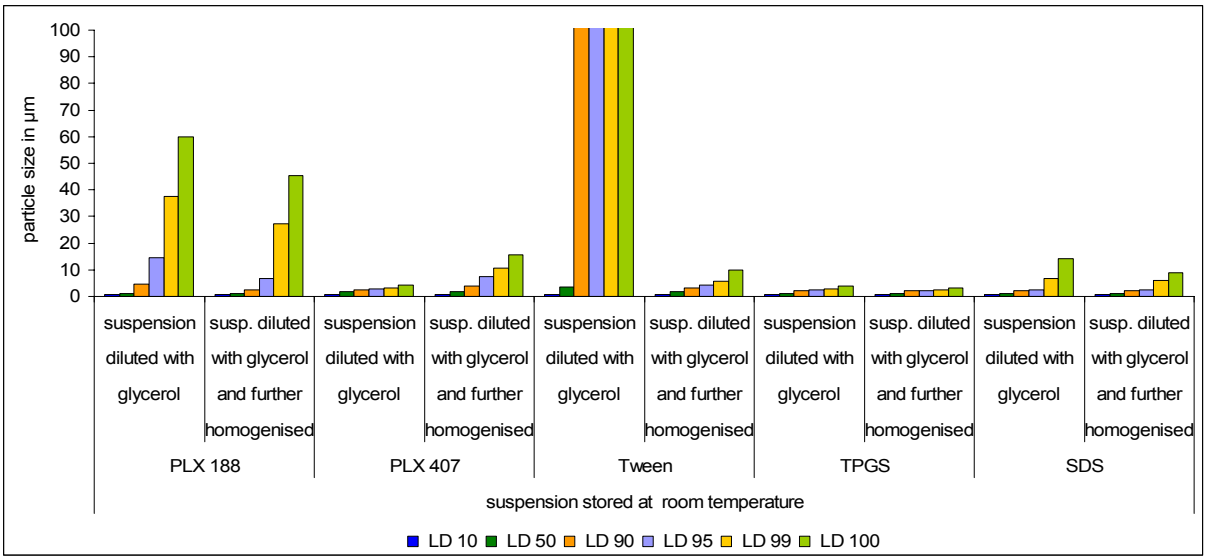


Figure 6-33: Results of LD analysis at day 28 of suspensions diluted with glycerol with and without further homogenisation, stored at room temperature

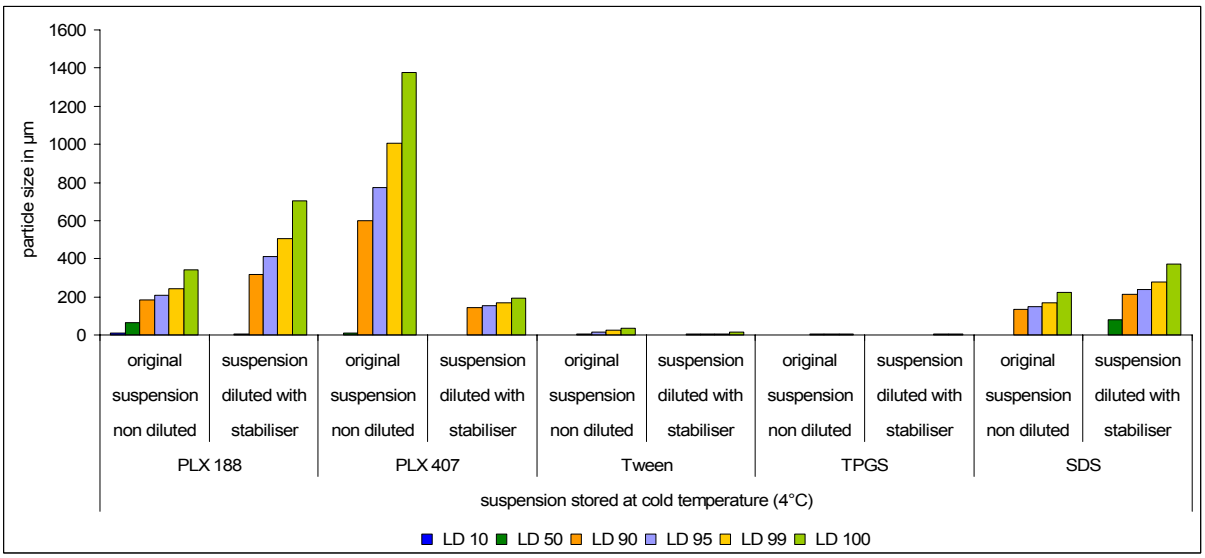


Figure 6-34: Results of LD analysis at day 28 of original non diluted suspensions and suspensions diluted with aqueous stabiliser solution, stored at cold temperature (4°C)

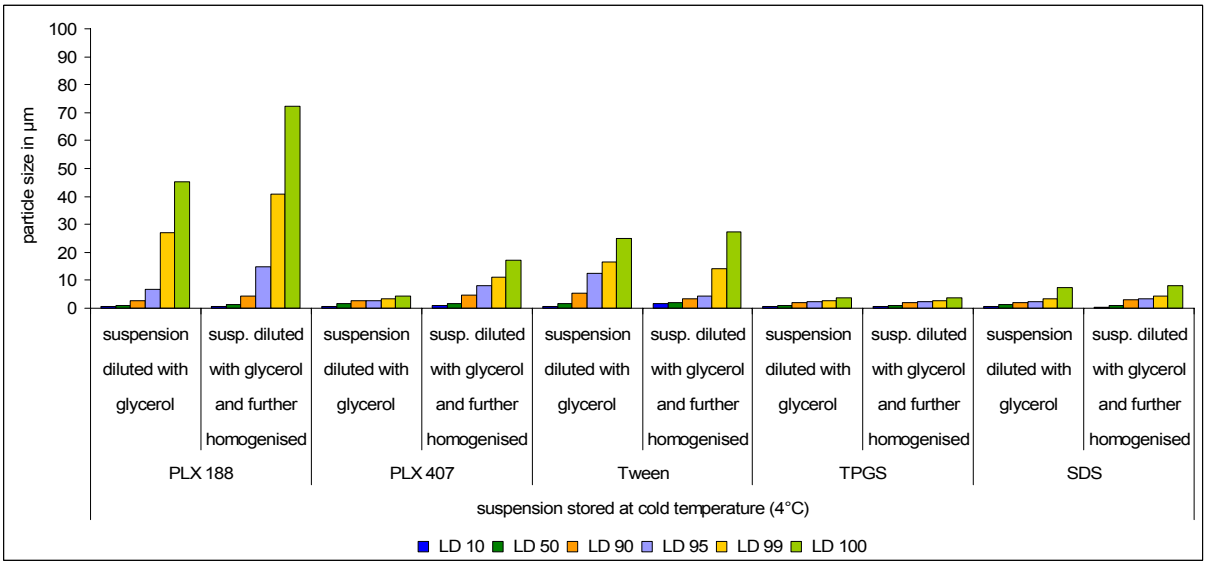


Figure 6-35: Results of LD analysis at day 28 of suspensions diluted with glycerol with and without further homogenisation, stored at cold temperature (4°C)

6.1.2.2.5 Results of the study obtained by particle size characterisation via the conventional method of particle size characterisation

The analysis of the study with the data obtained by using the newly established and optimised LD measuring procedure was shown before. The results from that study gave clear and detailed information about the changes of the different suspensions produced. The influencing parameters, e.g. temperature of storage, dilution and the kind of stabiliser used, on the stability could be identified by this set of data. Below the same suspensions were analysed by using the "conventional method" of particle size characterisation. The aim of this study was to prove the usefulness of the new method established within this thesis. The conventional method corresponds to the way of characterisation normally performed. It includes laser diffractometry and photon correlation spectroscopy (PCS). Light microscopy is not included.

Laser diffractometry was performed by using the LS 230. PIDS technology is always included when small particles are expected within the sample analysed. The analysis mode used for nanosuspensions is Fraunhofer approximation. Saturation of the measuring medium is not performed.

The data shown here were measured and analysed in this way. All data obtained here were obtained from the same suspensions and were measured in parallel to the measurements with the applied optimised standard at the day of production, at d1, d7 and d28.

The following four pages show the diagrams obtained from the static light measurements. The first page shows the data from the original, non diluted suspensions. The second page shows the results for the suspensions which were diluted with stabiliser solution, the third page the data for the suspensions diluted with glycerol, whereas the fourth page gives the overview of the data obtained from the suspensions diluted with glycerol and further homogenisation. The scaling of the abscissa of the different diagrams was kept as small as possible for more detailed information, but is different for each diagram. The LD 10, 50, 90, 95, 99 and 100 are used as characterisation parameters. For those the following symbols are used in the diagrams:

For more detailed information please it is referred to the appendix, were the complete set of data is listed.

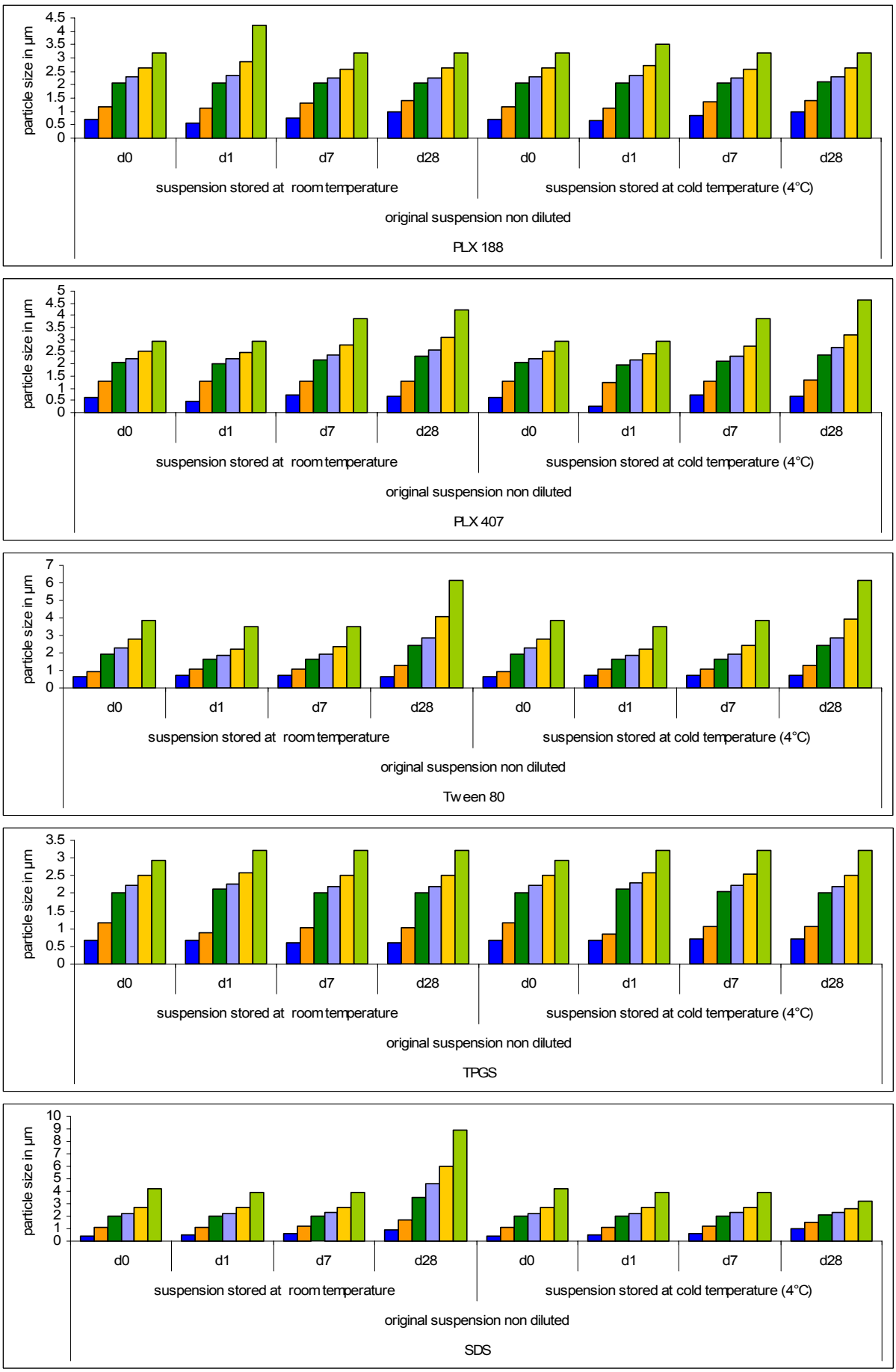
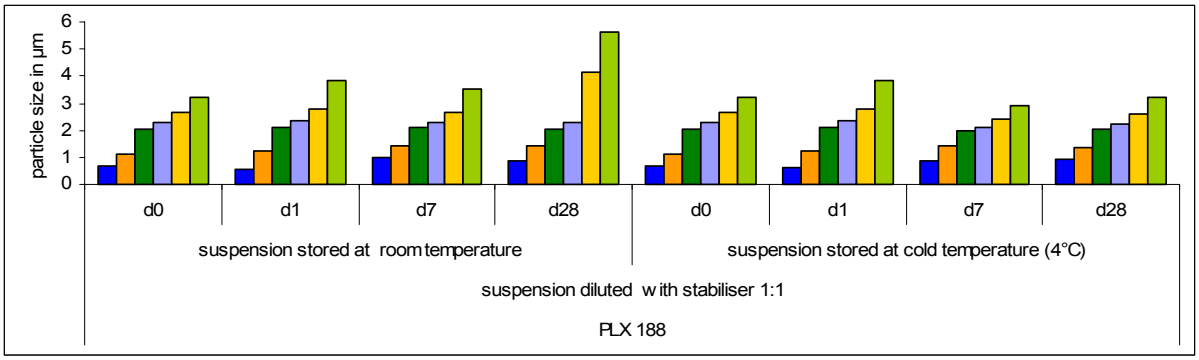
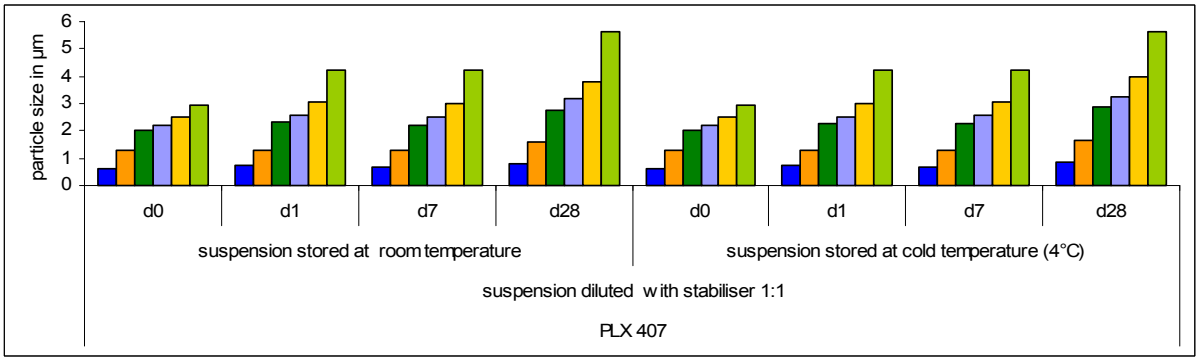
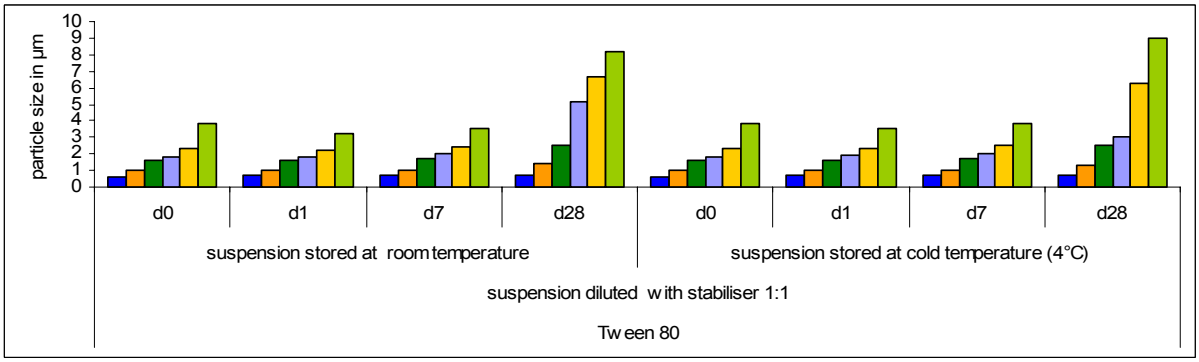
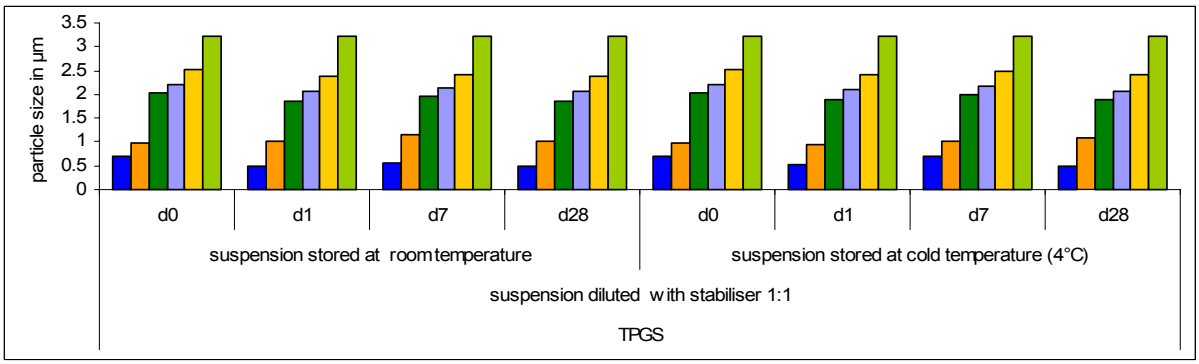


Figure 6-36: LD data of original non diluted nanosuspensions









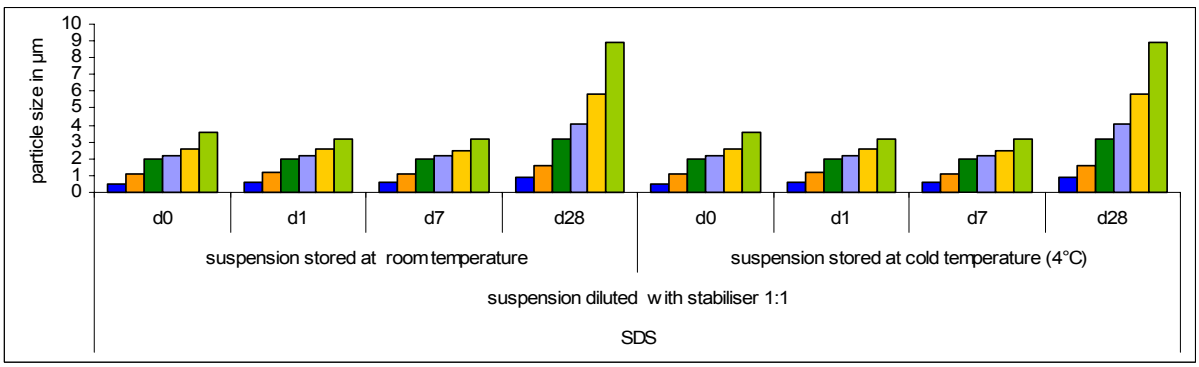


Figure 6-37: LD data of nanosuspensions diluted with stabiliser 1:1 (v/v)

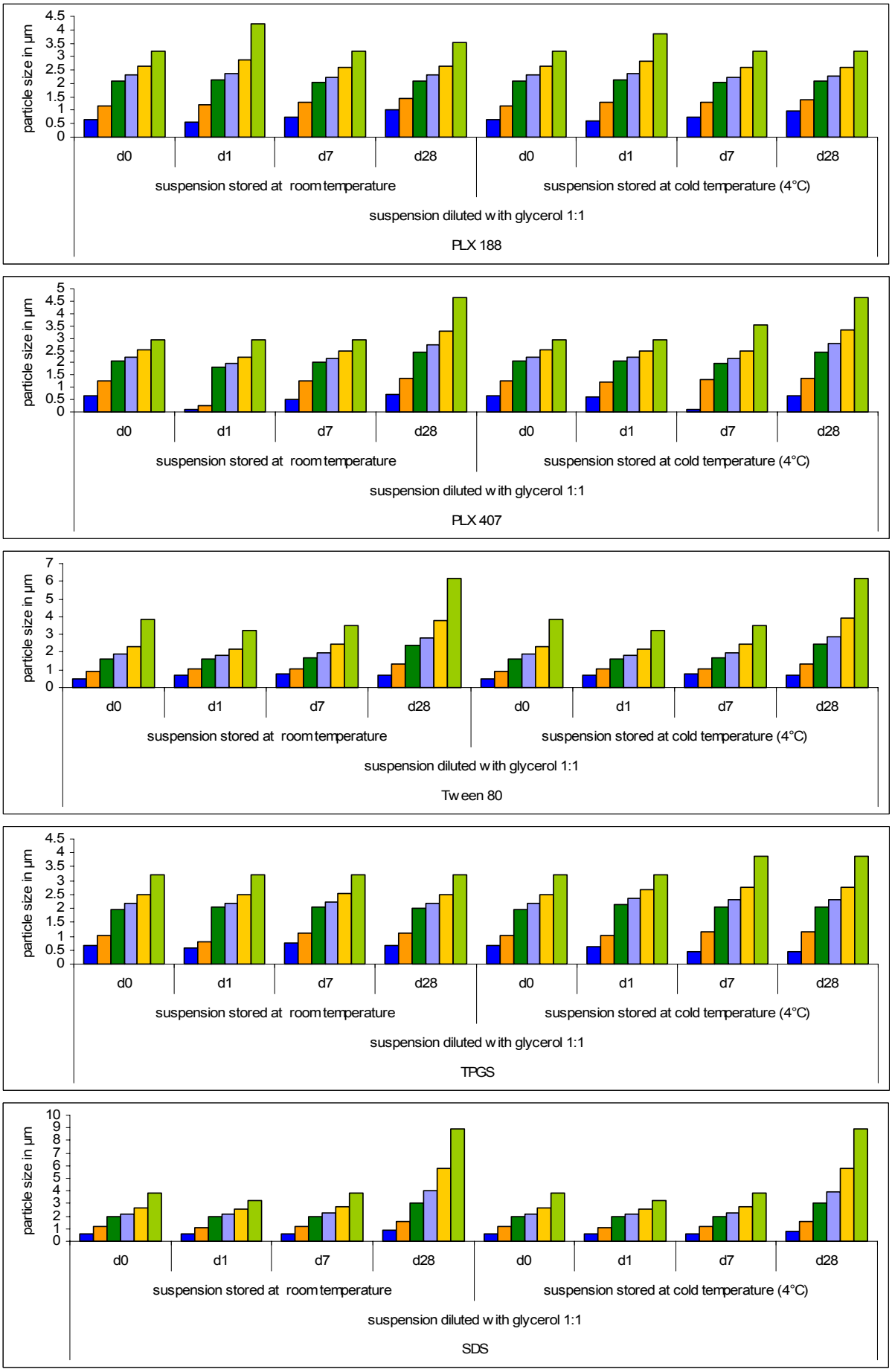


Figure 6-38: LD data of nanosuspensions diluted with glycerol 1:1 (v/v)

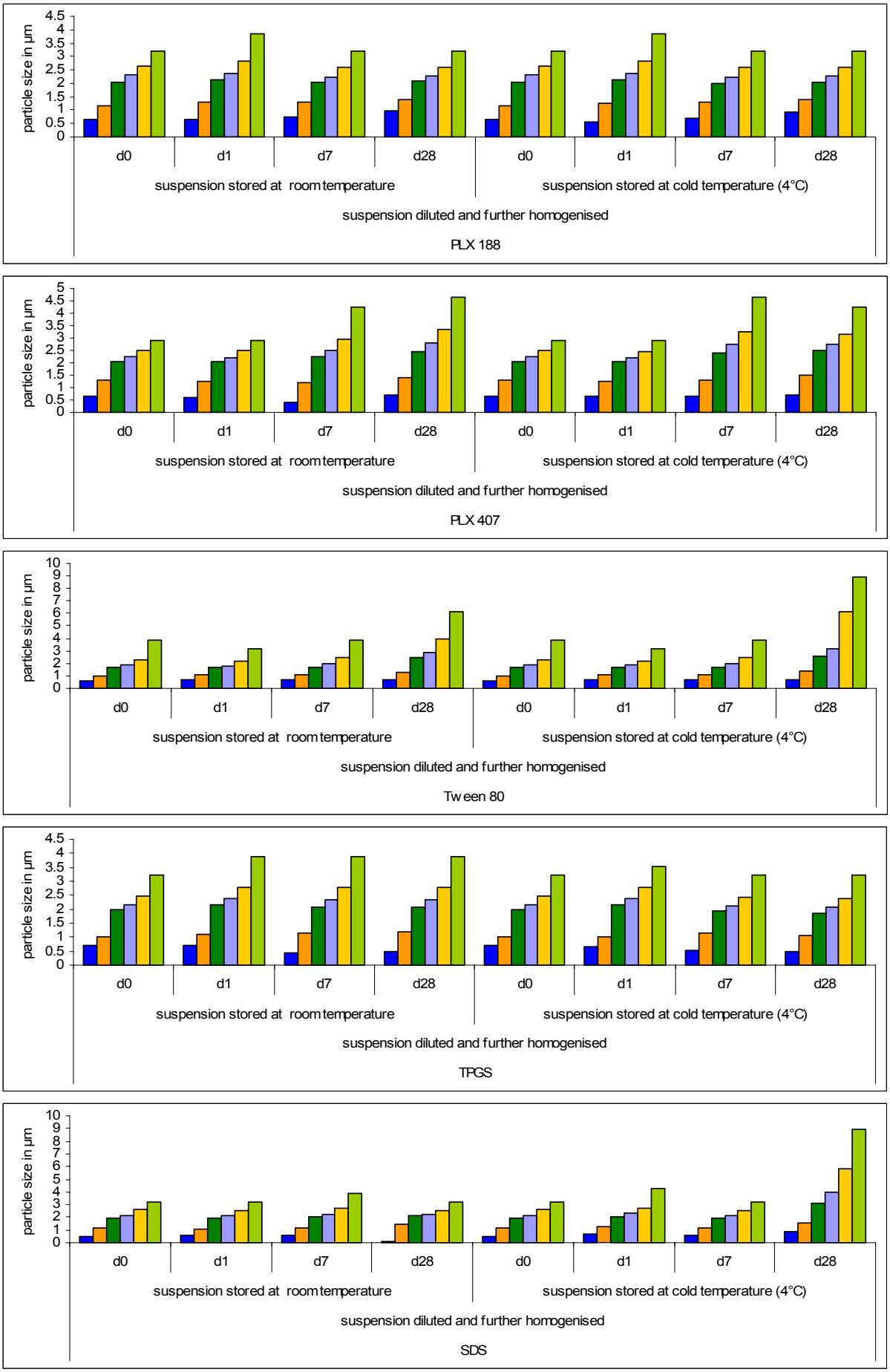


Figure 6-39: LD data of nanosuspensions diluted with glycerol 1:1 (v/v) and further homogenisation

In contrast to the optimised method were particles were found well above 400µm for most of the suspensions here, no particles larger than 9µm were found in any suspension analysed. These results clearly show once again, how important analysis without PIDS analysis can be. Measurements with included PIDS technology put focus on small particles and cancel out signals from larger particles, leading to too small particle size distributions. However, the main interest of laser diffractometry is the detection of larger particles which can occur due to agglomeration phenomena or crystal growth over a longer time of storage. Therefore measurements with included PIDS technology fail to analyse complex systems correctly and should not be applied for those investigations anymore.

The LD 50 is the parameter used to characterise the main particle size of the suspensions (=bulk population). Therefore it is the parameter observed to investigate particle growth within the suspensions analysed. In this study applying the conventional LD analysis with included PIDS, no temperature control and without saturation, tremendous changes could be observed for the LD 50, indicating a large change in particle size (Figure 6-40).

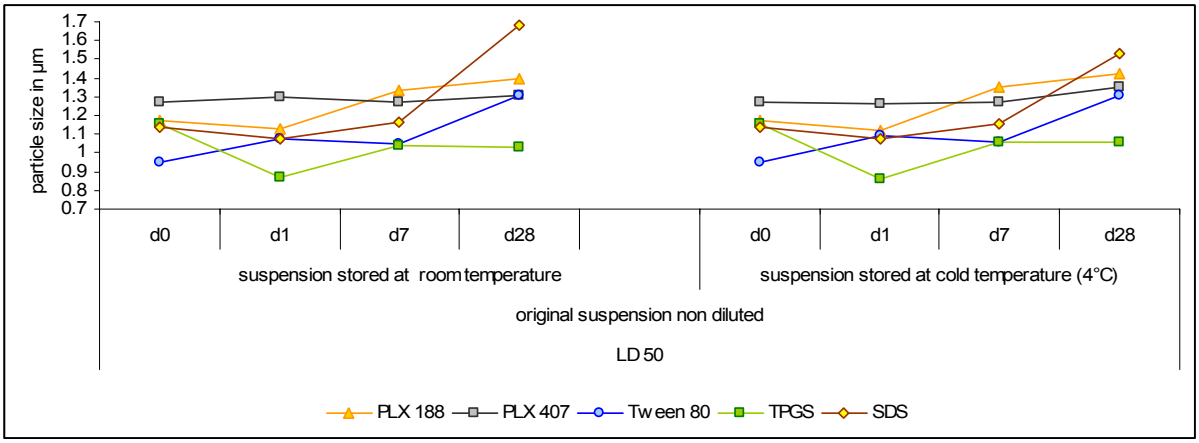
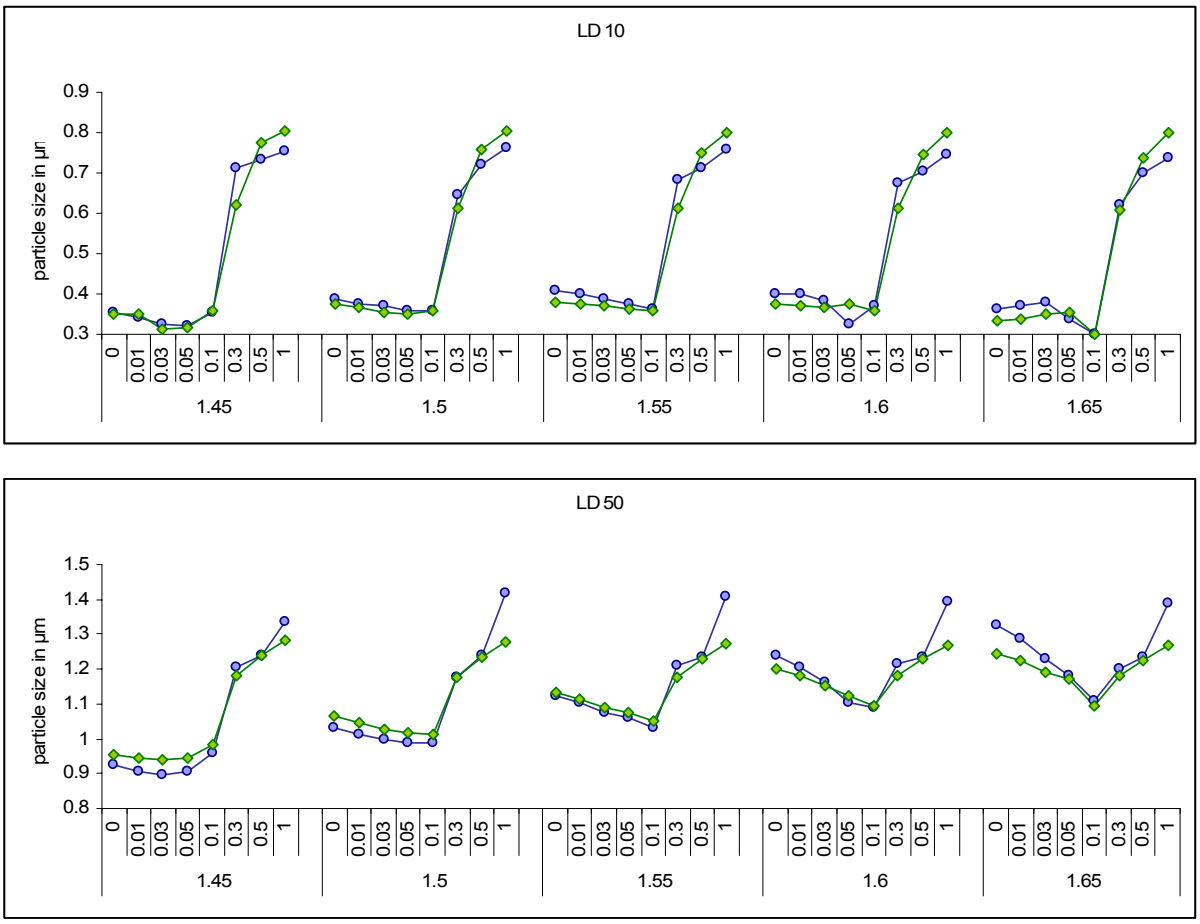


Figure 6-40: LD 50 of all non diluted suspensions, obtained by LD measurement using the conventional method with included PIDS and no saturation of the measuring medium

However the measuring medium was not saturated within these measurements performed according to the previous "conventional method". In chapter 4.7.1.4 the influences of dissolution on the particle size analysed were shown. The particle size easily increased 500nm when the measuring medium was unsaturated, depending on the time of the measurement, the particle size and the dissolution velocity on the particular drug analysed. Here none of these parameters could be controlled, because the time of the measurement changes within every measurement, because time of the measurement also includes the time needed for adding the sample to the measuring medium, as well as the set up of the measurement. This also includes the input of the name and details about the sample to be analysed, which is usually done after the sample is added. Depending on those parameters the suspension will dissolve more or

less. Therefore, also these data remain meaningless for a correct characterisation. Additionally here no correct index of refraction was used. In chapter 4 and 5 it was shown how sensitive the results are in respect to changes of the optical parameters used. Changes in the optical parameters directly change the particle size analysed. However, beside the fact that the particle size analysed will be incorrect if the wrong optical parameters are used, also the comparison of results from different measurements can lead to different interpretations depending on the optical model used. For example, sample 1 is smaller than sample 2 when the optical parameters x and y are used (e.g. Mie mode, RI = 1.456, IRI = 0.01), but if the optical parameters are changed (e.g. Mie mode, RI = 1.49, IRI 0.03) it is possible that now sample 2 is calculated to be smaller than sample 1. This phenomenon could also be observed in this study. In the following diagrams it is demonstrated for the two suspensions of the study stabilised with PLX 188 non diluted and diluted with satbiliser solution. The data shown were obtained 24 hours (d1) after the production of the nanosuspensions. In the diagrams the LD 10, LD 50, LD 99 and LD 100 of both suspensions calculated with different optical parameters were compared (Figure 6-41). The blue line corresponds to the original non diluted suspension and the green line corresponds to the suspension diluted with stabiliser solution.



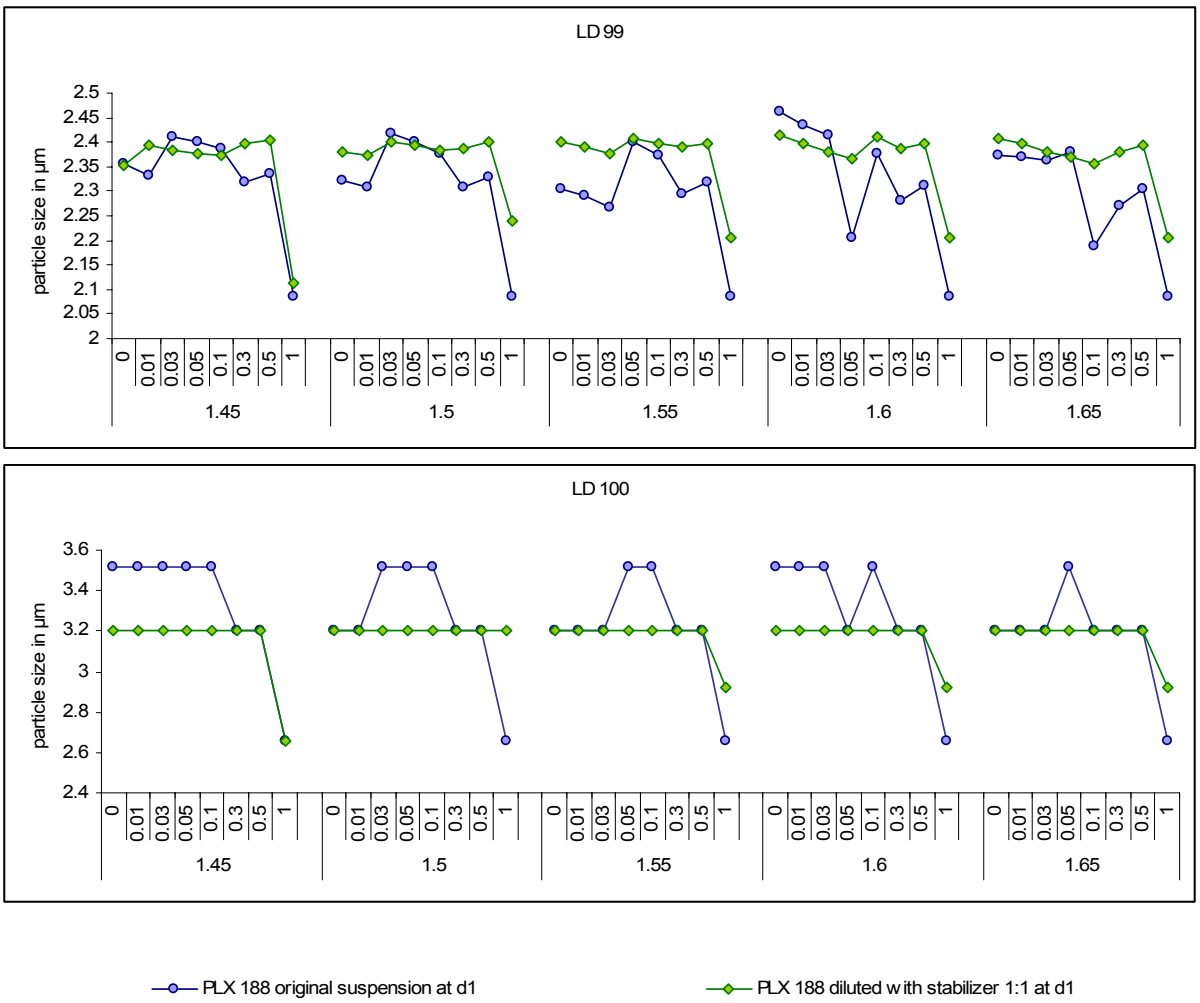


Figure 6-41: Comparison of LD data (LD 10, LD 50, LD 99 and LD 100) for two different nanosuspensions analysed with different optical parameters (RI was kept constant and varied from 1.45-1.65; IRI was varied for every RI, ranging from 0-1); the size trend changes, depending on the optical model used sample 1 (blue) is larger than sample 2 (green) and vice versa

The data clearly show the influence of the optical parameters on the comparison of the data of different suspensions. It can be seen that the LD 50 is larger for the suspension diluted with stabiliser solution (diluted suspensions) if the optical parameters are 1.5 for the real refractive index (RI) and 0 for the imaginary refractive index (IRI). Both LD 50 are calculated to be identical if RI is 1.55 and IRI is 0. If the RI would be 1.6 the diluted suspension would have a higher LD 50 than the non diluted suspension. Changes in the size trend occur also if the RI is kept constant and only the IRI is changed. This can be seen in case of the LD 99 for RI=1.5. If the IRI is 0 the LD 99 of the diluted suspension is calculated larger than the LD 99 of the non-diluted suspension, but if the IRI of 0.03 is used the LD 99 of the diluted suspension is smaller than the LD 99 of the non diluted sample. In conclusion, without correct optical parameters the comparison of LD data from different systems can be the opposite of the truth.

6.1.3 Conclusion

From all this it can be concluded, that particle sizing via the "old method" is not advisable. Only saturation of the measuring medium, the correct input of the optical parameters, standardisation of the measuring time and exclusion of PIDS technology will lead to results useful for the characterisation of nanosupensions. Further more, it could be proven that laser diffractometry alone does not give enough information for the complete characterisation of nanosuspensions. Agglomerates are often destroyed during laser diffractometry measurement. However, early agglomerates indicate instability of the system, as well as they change the properties of the suspensions (e.g. dissolution velocity (Keck et al. 2004)) Therefore they need to be taken into consideration for the interpretation of the systems. Also such important changes, as seen from the suspensions stabilised with Tween 80 and Poloxamer 407 in the stabiliser screening without subsequent dilution of the sample cannot be detected without microscopic imaging. In conclusion, particle size characterisation of nanosuspensions without microscopic monitoring in parallel is not sophisticated and can lead to failures in the interpretation of the systems investigated.

The results from this study would have been useless if the conventional method of characterisation would have been applied alone. Only by applying the new optimised standards this study gave detailed and useful information. It was found that the production of cyclosporine nanosuspensions needs to be performed with temperature control. The temperature during the production should not exceed 30°C.

It was shown that subsequent dilution of nanosuspension causes changes, e.g. further dissolution of the particles and/or agglomeration of the systems. If a conventional stabiliser screening is performed, stabilisers are investigated only by subsequent addition to a stock suspension. For those cases systems change not only due to an insufficient stabiliser but also due to the dilution itself. The selection of the appropriate stabiliser might fail therefore. In this study cyclosporine was formulated as a nanosuspension. The conventional stabiliser screening could not identify a suitable stabiliser, whereas the stabiliser screening without subsequent addition of stabiliser solution gave five stable suspensions in total. The most stable suspensions found are the four suspensions stabilised with TPGS and diluted with glycerol and the suspension stabilised with SDS, diluted with glycerol, further homogenised and stored at cold temperatures.

6.2 Stability enhancement of cyclosporine nanosuspensions by increased viscosity of the dispersion medium

Cyclosporine nanosuspensions did not possess long time stability when stored in their original composition. Agglomeration and crystal growth occurred over time. An increase of the viscosity decreases the dissolution velocity (Noyes-Whitney equation), the sedimentation velocity, and the all over motion of the particles (Stokes Law), thus minimising phenomena e.g. Ostwald ripening, crystal growth and agglomeration. The increase of the viscosity of the dispersion medium by glycerol already showed an increase in stability. Therefore it is expected to yield further stabilisation by a further increase in the viscosity of the dispersion medium. The increase in viscosity of the dispersion medium can be achieved by adding gel forming excipients. The following excipients were investigated in this study in order to identify the agent capable to increase the long time stability of cyclosporine nanosuspensions (see Table 6-31).

Table 6-31: Gel forming excipients used in this study

Aerosil 200	10%
polyacrylic acid	10%
methylcellulose (MC)	7%
hydroxyethyl cellulose (HEC)	7%
hydroxypropyl cellulose (HPC)	5%,7%,10%
gelatine A	5%,7%,10%
gelatine B	5%,7%,10%

In order to identify the efficiency of stabilisation for the different gelling agents, one stable and one physically unstable cyclosporine nanosuspensions have been investigated. Nanosuspension 1 was stabilised with Poloxamer 188 and was produced without temperature control. From the experience of the previous study, this was expected to be an unstable system, as agglomeration and crystal growth occurred due to the choice of an inappropriate stabiliser and too high temperatures during the production process. Nanosuspension 2 was stabilised with TPGS and was produced under conditions were the temperature was kept below 30°C during the production process. This suspension was known to be stable, as found in the previous study.

Both of the suspensions were produced by high pressure homogenisation (pre-cycles, 40 cycles at 1500bar), the concentration of cyclosporine was 2%, the concentration of the

stabilisers was 1%, respectively. Directly after their production the nanosuspensions were transformed into gels. For this the yield of one production was divided into 11 equal parts of 3.0g each. The remaining suspension was kept as original sample. Gels have been obtained by directly adding the gel former to the nanosuspension in case of HEC, HPC, MC and Aerosil 200. Gels obtained with gelatine were produced by adding the gelatine to the nanosuspension, followed by a hydration process for 20min. Afterwards the suspensions were heated to 50°C and cooled until gelling started. Suspensions stabilised with polyacrylic acid were added to the polyacrylic acid, which was already neutralised with sodium hydroxide solution (5%). All samples were carefully homogenised using small sized mortars and pistils until full gelling was obtained.

Each gel obtained was divided into two parts, were one was stored at room temperature and the other was stored at cold temperatures (6°C). The study was repeated twice. In the second repetition the water used was exchanged by aqua conservans.

The obtained samples were characterised by light microscopy at the day of production (d0) and 24 hours after production. Long-term stability was investigated by characterising the samples after 7, 14 and 28 days.

6.2.1.1 Results

Figure 6-42 shows the images obtained from the suspensions stabilised with Poloxamer 188 at the day of production and after four weeks of storage at a magnification of 160. The first row shows the images of the original aqueous suspension. As expected, the suspension agglomerated after a short period of time, and only a few single nanoparticles remained after 4 weeks of storage. The gels which were obtained by the addition of Aerosil 200, polyacrylic acid, methylcellulose, hydroxyethyl cellulose and gelatine B could not increase the stability of the nanosuspensions. However, gels containing gelatine A or hydroxypropyl cellulose could enhance the stability of the cyclosporine nanosuspension. This was seen for all suspensions studied, indicating that the type of stabiliser had no influence on the stability of the gels obtained. If agglomerates occurred they directly appeared within or shortly after the manufacturing process of the gel (d1), further changes of the systems over time were little.

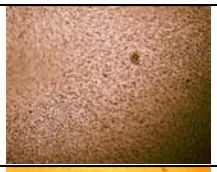
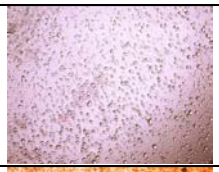
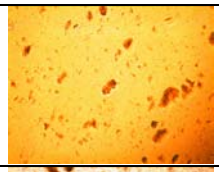
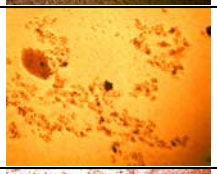
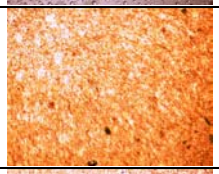
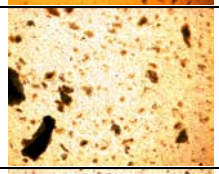
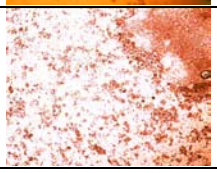
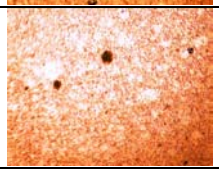
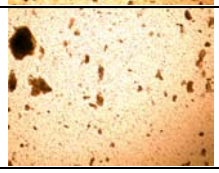
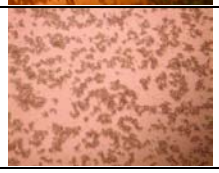
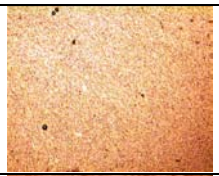
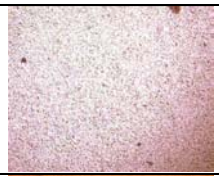
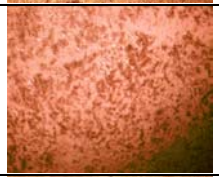
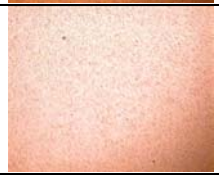
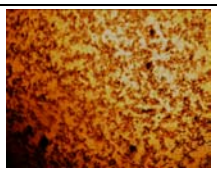
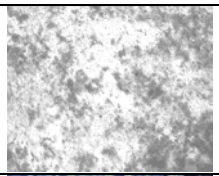
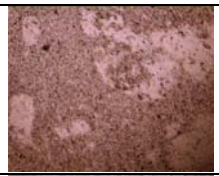
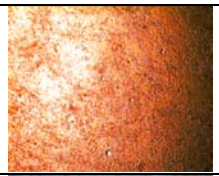
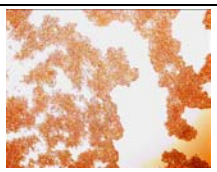
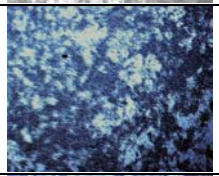
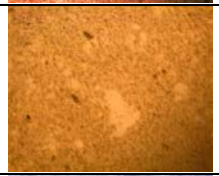
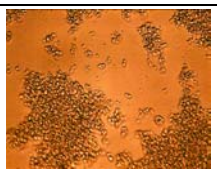
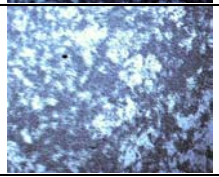
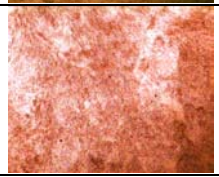
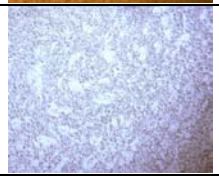
	original	Aerosil 10%	polyacrylic acid	HEC 7%
day of production				
d28 stored at room temperature				
d28 stored at cold temperature				
	HPC 7%	gelatine A 5%	gelatine A 7%	gelatine A 10%
day of production				
d28 stored at room temperature				
d28 stored at cold temperature				
	MC 7%	gelatine B 5%	gelatine B 7%	gelatine B 10%
day of production				
d28 stored at room temperature				
d28 stored at cold temperature				

Figure 6-42: Gels from cyclosporine nanosuspension stabilised with poloxamer 188 at day 0 (upper row) and after 28 days of storage (lower rows); magnification 160x

Figure 6-43 shows the samples obtained from the suspension stabilised with TPGS, only for the gels obtained from gels with HPC and Gelatine A, as all the other gels agglomerated in the same manner as already seen for the gels containing the suspension stabilised with PLX 188. The original TPGS stabilised aqueous suspension is smaller and contained no agglomerates, therefore all gels obtained from this suspension were better than those obtained from the suspension stabilised with Poloxamer 188. Suspensions produced at temperatures below 30°C and stabilised with TPGS are more stable than those stabilised with PLX 188, therefore no agglomeration occurred within the original suspension over the time of storage (first column). However, also the other images obtained from gels of gelatine A and hydroxylpropyl cellulose proved to be stable over the time of observation.

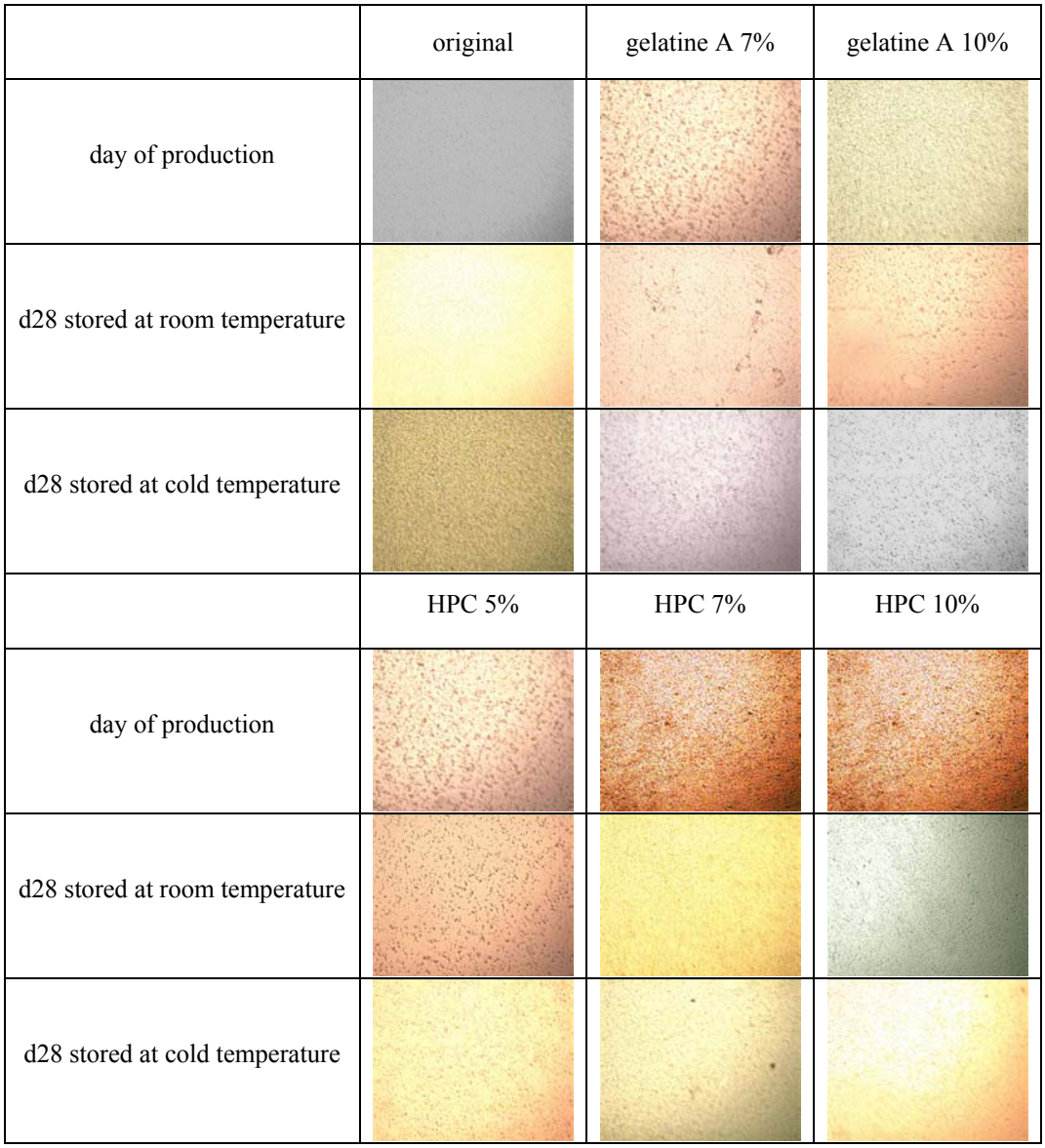


Figure 6-43: Gels from cyclosporine nanosuspension stabilised with TPGS at d0 (upper row) and after 28 days of storage (lower rows); magnification 160x

By comparing the different gels only varying in the concentration of the gel former used, it can be seen that the stability increases with an increase in concentration of the gel forming agent. In addition it appears that storage at cold temperatures was more advantageous when compared to the storage at room temperatures. Both observations can be explained, as the viscosity is concentration and temperature dependent. The higher the concentration and the lower the temperature, the higher is the viscosity of the system and the stability therefore.
Moreover it was found that crystal growth, which was observed in the original suspension stabilised with Poloxamer 188, did not occur within the gelled suspensions (Figure 6-44).

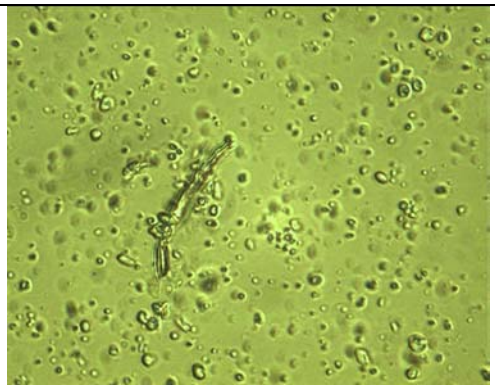
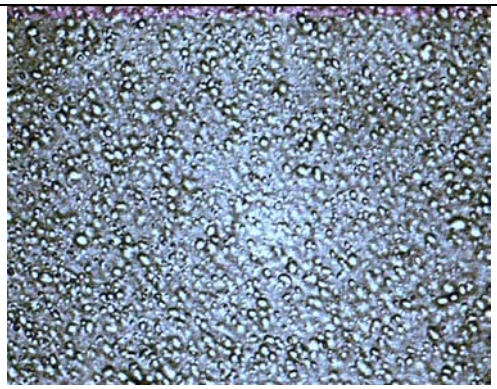
original suspension stabilised with PLX 188	gelled suspension with gelatine A 7%
	

Figure 6-44: Images taken at day28 of storage; left: original aqueous suspension stabilised with Poloxamer 188 - only a few nanocrystals are left in the suspension but a large crystal (25µm) is visible in the in the non gelled suspension; right: gelled suspension 1 with gelatine A 7% - no crystal growth can be observed, the suspension is physically stable (magnification 1000x)

6.2.1.2 Conclusion

These results prove that also physically instable suspensions can be stabilised by the addition of gel building agents. The appearance of agglomerates and crystal growth could be avoided by the addition of appropriate gel forming agents.
The most effective agents for the stabilisation of cyclosporine nanosuspensions were found to be gelatine A and hydroxypropyl cellulose.