8 Further investigations of cyclosporine for oral delivery

Cyclosporine nanosuspensions were produced and characterised for their physically stability and resistance against agglomeration in the chapters above. In vivo studies could not be performed within this work. However, in one previous study of Runge et. al cyclosporine nanosuspensions showed only a very poor bioavailability (Runge 1998; Muller and Keck 2004). Nanosuspensions are aqueous systems, containing no fat. However in the literature it is reported that the bioavailability of cyclosporine is dependent on the amount of fat available during the absorption (Fahr and Seelig 2001) and the particle size of the drug carrier, e.g. size of emulsion droplet (Tarr and Yalkowsky 1989). Also there is evidence that cyclosporine is absorbed with the fat by the normal fat uptake, as cyclosporine dissolved in indigestible fat shows a strongly reduced bioavailability when compared to formulations where it was dissolved in digestible oil (Benmoussa et al. 1994). Therefore a delivery system where aspects such as a better absorption in the presence of digestible, apolar substances and the p-gp mediated efflux are considered might be more promising than the administration of nanosuspensions.

The motivation of this study therefore was the development of an alternative dosage form to the cyclosporine nanosuspensions. Focus was put on the inhibition of p-glycoprotein to increase the oral bioavailability of cyclosporine, on small particle sizes of the drug carrier and the use of an apolar compound for further increase of the uptake of cyclosporine. The aim was to include only surfactants, known to inhibit the function of the efflux pump p-glycoprotein. Further literature research brought up some controversy about the inhibition capability of surfactants. In some recent studies it was shown that the addition of surfactants, even though the are capable to inhibit p-gp, decreased the bioavailability due to the micellation of the drug (Chiu et al. 2003). The effect of drug entrapment was also shown from (Bermejo et al. 1999; Ruiz-Garcia et al. 1999; Sanchez-Castano et al. 2000; Bermejo et al. 2004) and simply refers to the Levi gold fish test (Voigt 2006). Therefore further investigations were extended to two different approaches. The first approach was the development of a small sized formulation, containing surfactants or other compounds known to inhibit p-glycoprotein. The second alternative approach was the development of an emulsifier free formulation, also inhibiting p-glycoprotein. Both formulations should contain an apolar compound as drug carrier. Further literature screening for suitable apolar compounds brought the finding that peppermint oil shows inhibitory effects on p-glycoprotein (Wacher et al. 2002), making it an interesting compound as drug carrier for cyclosporine.

Solubility studies were performed and showed an excellent high solubility of cyclosporine in peppermint oil (33% w/w). From this peppermint oil was chosen as the main compound for the following studies performed.

8.1 Development of small sized emulsions containing cyclosporine

The production of emulsions via high pressure homogenisation yields particle sizes as small as 200nm, making this method suitable for the production of small sized emulsions containing cyclosporine. Different emulsifiers and oils (mainly peppermint oil) were studied in order to obtain a physically stable emulsion suitable for further investigations, e.g. in vivo studies.

The whole study consisted of six different approaches (A1-A6). TPGS is known to be an efficient inhibitor of p-gp. In A1 different oils with TPGS used as emulsifier were investigated. The production was performed at 80°C. A2 investigated the same formulations, but at production temperatures at only 25°C. A3-A6 investigated formulations containing only peppermint oil as p-gp inhibiting compound; various stabilisers were used in order to find the optimal formulation. The production temperatures in A3 were 80°C and 25°C in A4. A5 investigated the stability of systems containing mixtures of peppermint oil with other oils. In A6 systems containing mixtures of stabilisers were investigated. All systems investigated, including the parameters of production are listed in Table 8-1. Only the formulations in A1 contained cyclosporine, all other formulations (A2-A6) were drug free, because the aim was to develop a physically stable small sized emulsion based on peppermint oil first. This was done in order to save expensive cyclosporine.

Table 8-1: Formulations investigated

	formulation	concentration cyclosporine	oil/ concentration	stabiliser/ concentration	homogenisation pressure	homogenisation temperature
A1	1	5%	olive oil 33%	TPGS 0.5%	1x 150bar 3x500bar	80°C
	2	5%	Miglyol 812 15%, 33%	TPGS 0.5%	1x 150bar 3x500bar	80°C
	3	5%	soy bean oil 15%, 33%	TPGS 0.5%	1x 150bar 3x500bar	80°C
	4	5%	corn oil 15%, 33%	TPGS 0.5%	1x 150bar 3x500bar	80°C
	5	5%	saflor oil 15%	TPGS 0.5%	1x 150bar 3x500bar	80°C
	6	5%	peppermint oil 15%	TPGS 0.5%	1x 150bar 3x500bar	80°C
	7	5%	peppermint oil 15%	TPGS 0.5%	1x 150bar 3x500bar 3x 1500bar	80°C

	formulation	concentration cyclosporine	oil/ concentration	stabiliser/ concentration	homogenisation pressure	homogenisation temperature
A2	8	-	olive oil 5%	TPGS 0.75%	1x 150bar 3x500bar	25°C
	9	-	Miglyol 5%	TPGS 0.75%	1x 150bar 3x500bar	25°C
	10	-	soy bean oil 5%	TPGS 0.75%	1x 150bar 3x500bar	25°C
	11	-	saflor oil 5%	TPGS 0.75%	1x 150bar 3x500bar	25°C
	12	-	peppermint oil 5%	TPGS 0.75%	1x 150bar 3x500bar	25°C
A3	13	-	peppermint oil 5%	Tween 20 0.75%	1x 150bar 3x500bar	80°C
	14	-	peppermint oil 5%	Tween 80	1x 150bar 3x500bar	80°C
	15	-	peppermint oil 5%	PLX 407	1x 150bar 3x500bar	80°C
	16	-	peppermint oil 5%	SDS 0.75%	1x 150bar 3x500bar	80°C
	17	-	peppermint oil 5%	SDS 0.25%	1x 150bar 3x500bar	80°C
	18	-	peppermint oil 5%	Lipoid E80 0.9%	1x 150bar 3x500bar pH 5.5	80°C
	19	-	peppermint oil 5%	Lipoid E80 0.9%	1x 150bar 3x500bar pH 10.4	80°C
	20	-	peppermint oil 5%	Lipoid S75 0.9%	1x 150bar 3x500bar	80°C
	21	-	peppermint oil 5%	phosphlipid mix 0.9%	1x 150bar 3x500bar	80°C
A4	22	-	peppermint oil 5%	SDS 0.25%	1x 150bar 3x500bar	25°C
	23	-	peppermint oil 5%	Lipoid E80 0.9%	1x 150bar 3x500bar	25°C
	24	-	peppermint oil 5%	Lanette N 0.25%	1x 150bar 3x500bar	25°C
	25	-	peppermint oil 5%	Lanette N 0.25%	1x 150bar 5x500bar	25°C
	26	-	peppermint oil 5%	Chremophor EL 0.75%	1x 150bar 3x500bar	25°C
	27	-	peppermint oil 5%	saponin 1%	1x 150bar 3x500bar	25°C
	28	-	peppermint oil 5%	saponin 1%	1x 150bar 5x500bar	25°C
	29	-	peppermint oil 2.5% soy bean oil 2.5%	Lanette N 0.25%	1x 150bar 3x500bar	25°C
A5	30	-	peppermint oil 2.5% saflor oil 2.5%	Lanette N 0.25%	1x 150bar 3x500bar	25°C
	31	-	peppermint oil 2.5% olive oil 2.5%	Lanette N 0.25%	1x 150bar 3x500bar	25°C
	32	-	peppermint oil 2.5% Miglyol 812 2.5%	Lanette N 0.25%	1x 150bar 3x500bar	25°C

	formulation	concentration cyclosporine	oil/ concentration	stabiliser/ concentration	homogenisation pressure	homogenisation temperature
A6	33	-	peppermint oil 5%	Xanthan 0.25%	1x 150bar 3x500bar	25°C
	34	-	peppermint oil 5%	Xanthan 0.25% SDS 0.25%	1x 150bar 3x500bar	25°C
	35	-	peppermint oil 5%	Xanthan 0.25% Lanette N 0.25%	1x 150bar 3x500bar/ 25°C	25°C
	36	-	peppermint oil 5%	Xanthan 0.25% Chremophor 0.25%	1x 150bar 3x500bar	25°C
	37	-	peppermint oil 5%	Lanette N 0.25% Lanette O 0.1%	1x 150bar 3x500bar	25°C
	38	-	peppermint oil 5%	Lanette N 0.25% Lanette O 0.1%	1x 150bar 5x500bar	25°C

8.1.1 Production and characterisation

8.1.1.1 A1 - Emulsions containing different oils, stabilised with TPGS, produced at 80°C

8.1.1.1.1 Observation prior high pressure homogenisation

Cyclosporine was not soluble in a concentration of 33% (w/w) in Miglyol 812, olive oil, soy bean oil and corn oil. Only when it was diluted to 16.5% (w/w) cyclosporine dissolved in those media. During the homogenisation, when the temperature was increased, cyclosporine precipitated in all oils but not in peppermint oil. The precipitation was irreversible for Miglyol, soy bean oil and corn oil. Emulsions containing Miglyol, soy bean oil and corn oil could not be homogenised under those conditions therefore. For the emulsions containing olive oil and saflor oil the precipitation was reversible after cooling down to room temperature. Interestingly, a repeated increase in temperature did not lead to a repeated precipitation. The investigation might be related to a change in the conformation of cyclosporine, but remains unclear.

8.1.1.1.2 Characterisation of emulsions produced

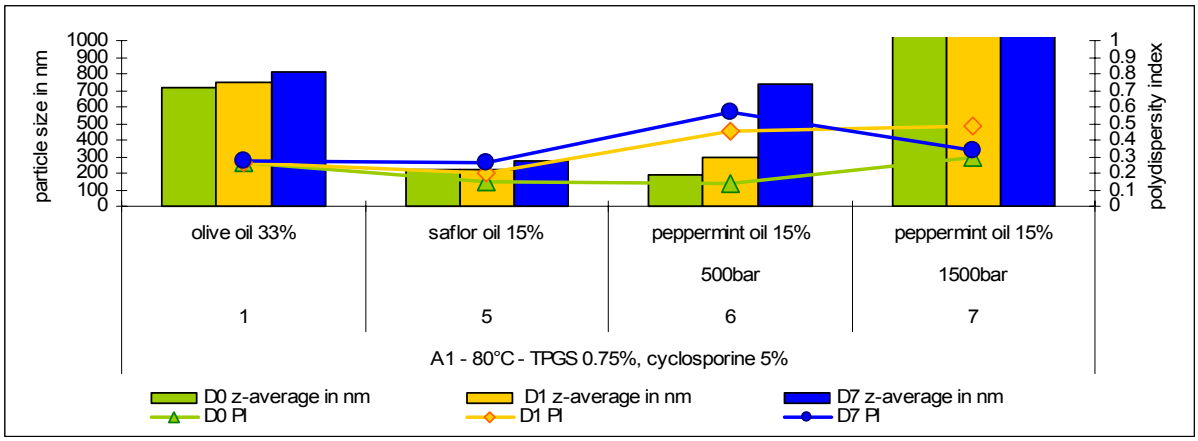


Figure 8-1: PCS data (z-average and polydispersity index (PI)) from all formulations of experiment A1 (emulsions 1, 5, 6 and 7) - only emulsions containing saflor oil proved to be stable (d0-d7)

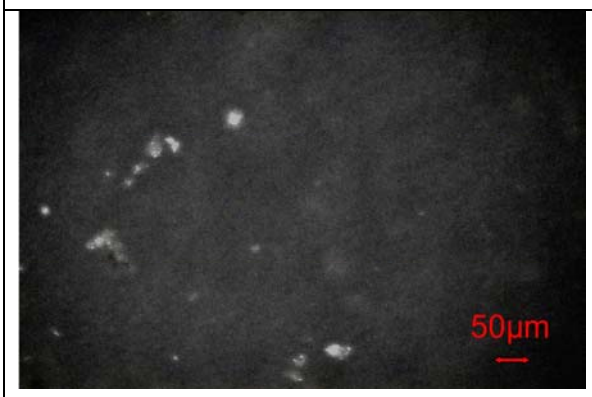
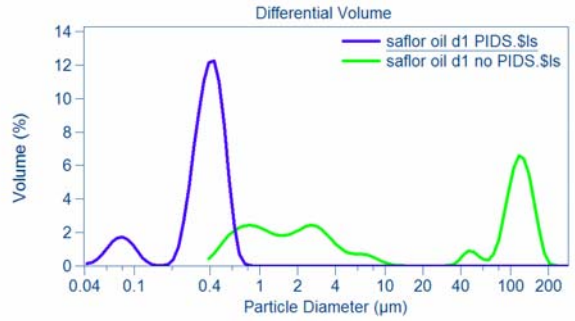
A	B
	

Figure 8-2: Saflor oil emulsion at day1 A: microscopic image under polarised light, magnification 160x, large crystals from approx. 30- 100µm were observed. They could be detected by Laser diffractometry (B) but only when PIDS was not included in the measurement. With included PIDS large crystals were not detected (independent on which optical model used (simulation not shown). The data shown were calculated using Mie mode with optical parameters 1.467 (RI) and 0.01 (IRI)

None of the emulsions produced at 80 °C was suitable to emulsify cyclosporine efficiently. Even though saflor oil yielded a suitable particle size in PCS (Figure 8-1), from microscopy (Figure 8-2 A (right)) it was seen, that cyclosporine already re-crystallised one day after production. Laser diffractometry could prove the results from microscopy, however only when PIDS technology was not included (Figure 8-2 B (left)).

8.1.1.2 A2 - Emulsions containing different oils stabilised with TPGS, produced at 25°C

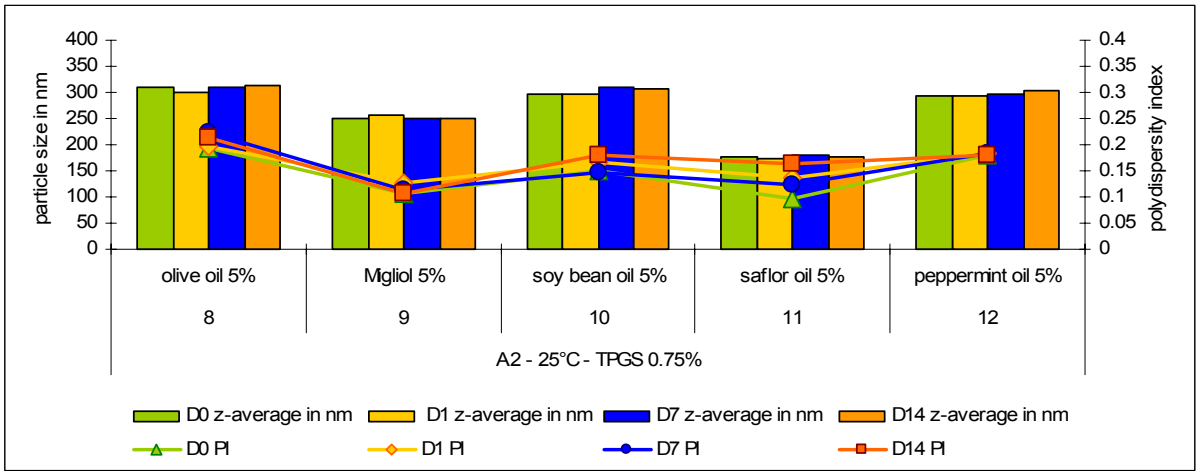


Figure 8-3: PCS data (z-average and polydispersity index) from A2 (emulsions 8-12), production at room temperature led to an increase in the stability of the emulsions (d0-d14)

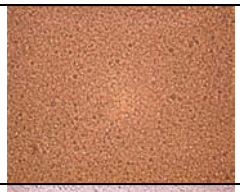
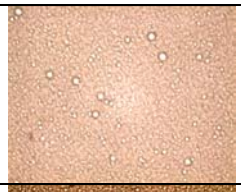
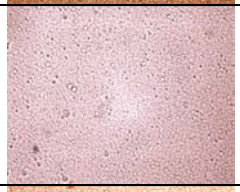
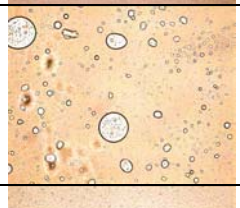
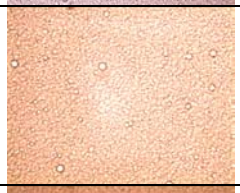
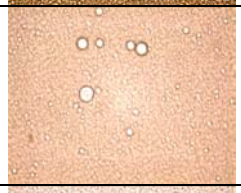
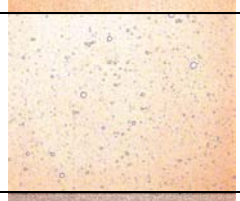
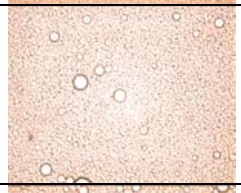
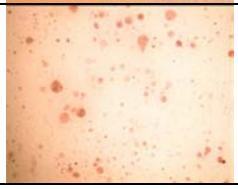
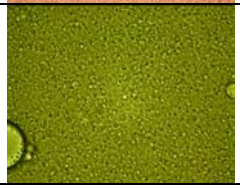
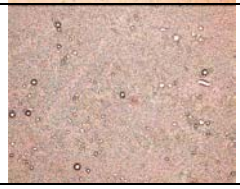
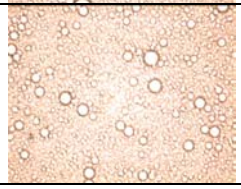
formulation	day 0		day 14	
	enlargement 160x	1000x	enlargement 160x	1000x
8 olive oil				
9 Migliol 912				
10 soy bean oil				
11 saflor oil				
12 pepper mint oil				

Figure 8-4: Microscopic images from A2 (emulsions 8-12) at day 0 and day 14, results from PCS are not in agreement with light microscopy, all systems obtained contain larger droplets after a short period of time and are not physically stable

The production at room temperature could improve the stability of the emulsions as concluded from the PCS data (Figure 8-3).

However microscopic imaging shows that all emulsions obtained from this trail showed coalescence after at least 14 days of storage. Oil droplets were sometimes hardly to distinguish from air bubbles. To prove the observation of oil droplet instead of air bubbles, the systems were coloured using Sudan red III solution. Additionally the observation of the Becke line (c.f. 5.2.2.) was used (Figure 8-4). Also from this trial no stable emulsion could be obtained.

8.1.1.3 A3 - Emulsions containing peppermint oil and different stabilisers, produced at 80°C

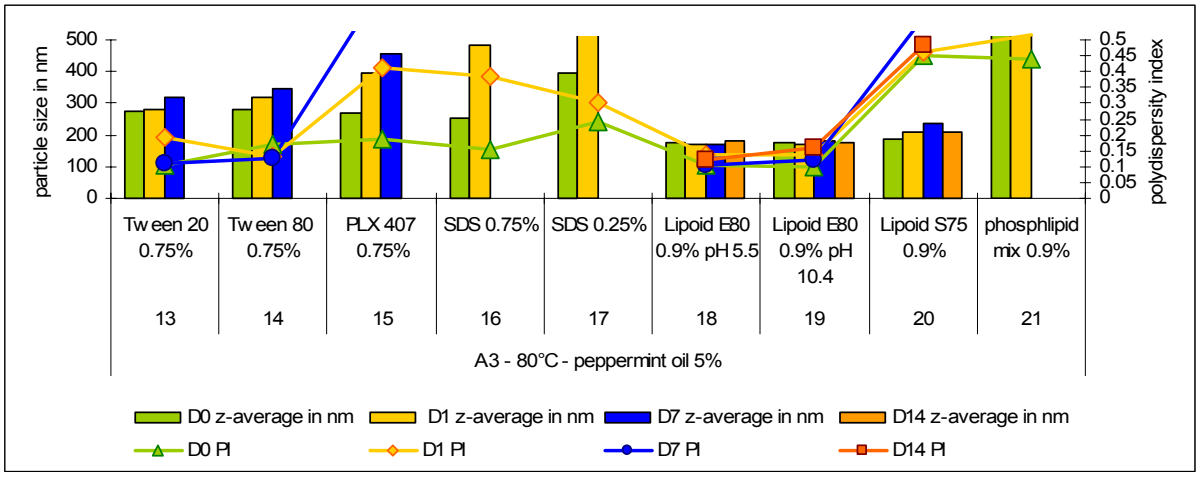


Figure 8-5: PCS data from A3 (emulsions 13-21), the data show that emulsification of peppermint oil by using several stabilisers was only successful when Lipoid E80 was used

Beside Lipoid E80, none of the stabilisers investigated was able to stabilise peppermint oil in a satisfying way. Lipoid E80 showed a phase shift and turned into a water-in-oil emulsion during the pre-homogenisation with the Ultra Turrax. This was proven by conductivity measurements. After one cycle at 150bar again a phase inversion back to an oil-in-water emulsion was observed. From the theory it is suggested, that emulsions produced via the continental method (phase inversion) are more stable, than those produced via the english method (no phase inversion). From this it might be explainable, that Lipoid E80 was the best stabiliser in regard to stabilise a peppermint oil emulsion. Lipoid E80 is also used as a stabiliser for intravenous fat emulsions, here the pH is adjusted to 9-10 prior autoclaving to enhance the stability of the emulsions (Rote Liste, 2003). Also in this study different pH values were investigated for this formulation, but it was found that emulsions having a pH of 5.5 were more stable than those adjusted to pH 10.

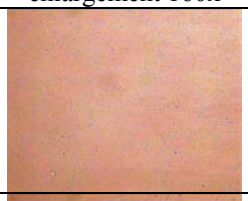
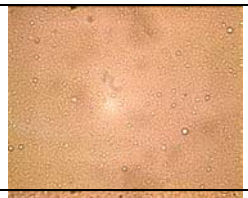
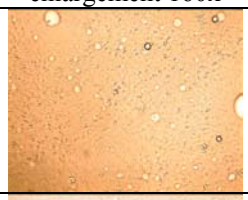
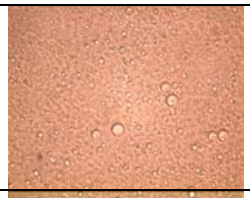
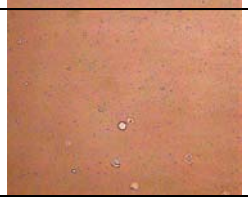
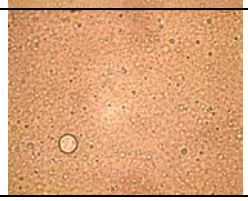
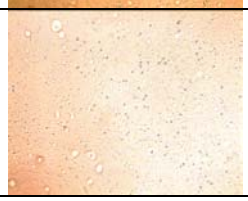
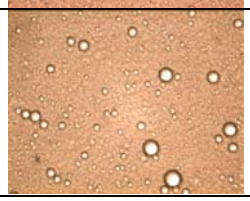
formulation	day 0		day 14	
	enlargement 160x	1000x	enlargement 160x	1000x
18 Lipoid E80 pH 5.5				
19 Lipoid E80 pH 10.4				

Figure 8-6: Microscopic images from A3 (emulsion 18 and 19), results from PCS are not in agreement with light microscopy, also all systems containing Lipoid E80 contained larger droplets after a short period of time

Additional analysis by light microscopy gave evidence, that all Lipoid emulsions obtained also contained larger droplets, which were not detected from PCS analysis (Figure 8-6). Results from laser diffractometry are shown in Figure 8-7. Larger particles were detected with and without PIDS, but were almost doubled in size, when PIDS was not included in the measurement. Measurements without PIDS are therefore more related to the results by microscopy, where also oil droplets larger than 40µm were found.

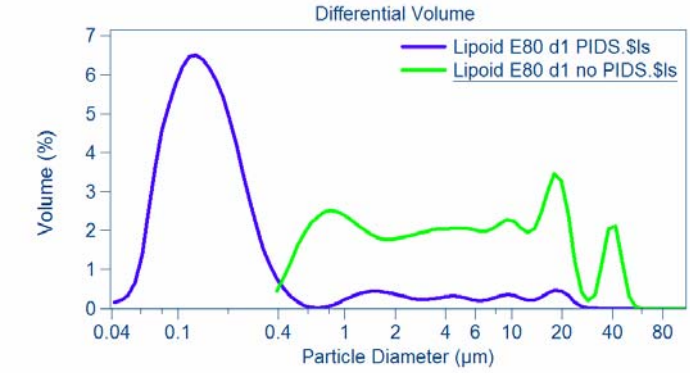


Figure 8-7: LD data from A3, showing that also emulsions containing Lipoid E80 contained large particles, only measurements without PIDS are meaningful. (Data analysed with Mie mode (RI 1.467, IRI 0.01)

8.1.1.4 A4, A5 and A6 - Emulsions containing peppermint oil and different stabiliseres, produced at 25°C

The production at room temperature and several emulsifiers (A4), as well as the combination of peppermint oil and other oils (A5) did not lead to physically stable emulsions. Also the addition of a quasi emulsifier (Xanthan) (A6) did not lead to stable emulsions. Results from LD and microscopy (data not shown) confirmed the results obtained by PCS (Figure 8-8 for A4, Figure 8-9 for A5 and Figure 8-10 for A6)

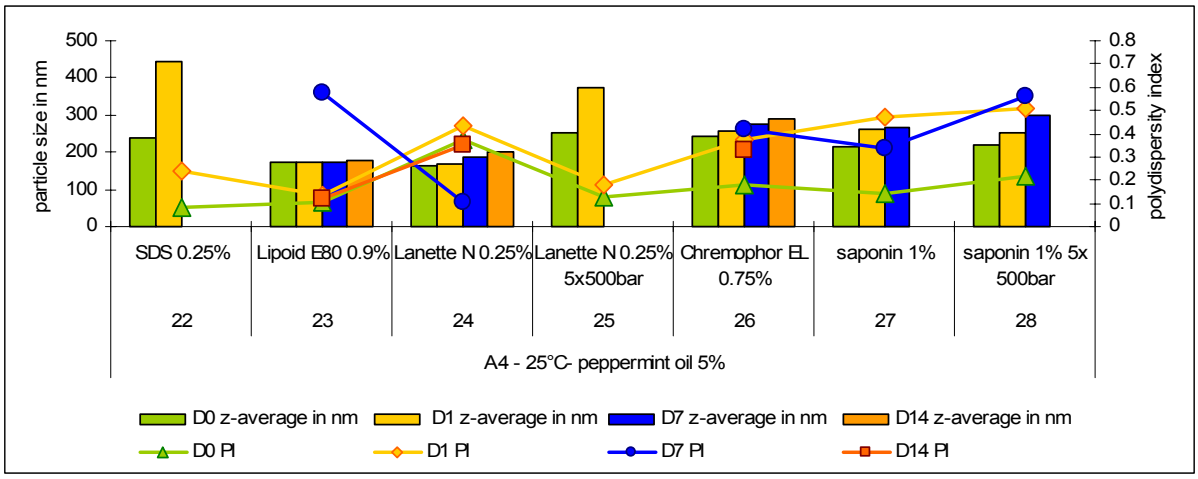


Figure 8-8: PCS data from A4, no stable emulsion could be obtained

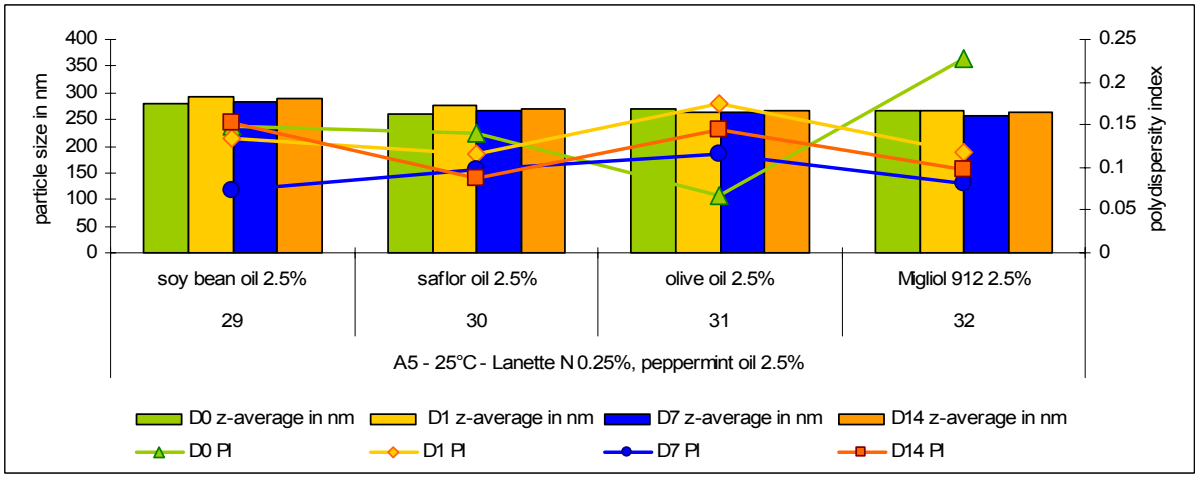


Figure 8-9: PCS data from A5, no stable emulsion could be obtained, LD and microscopy showed large droplets

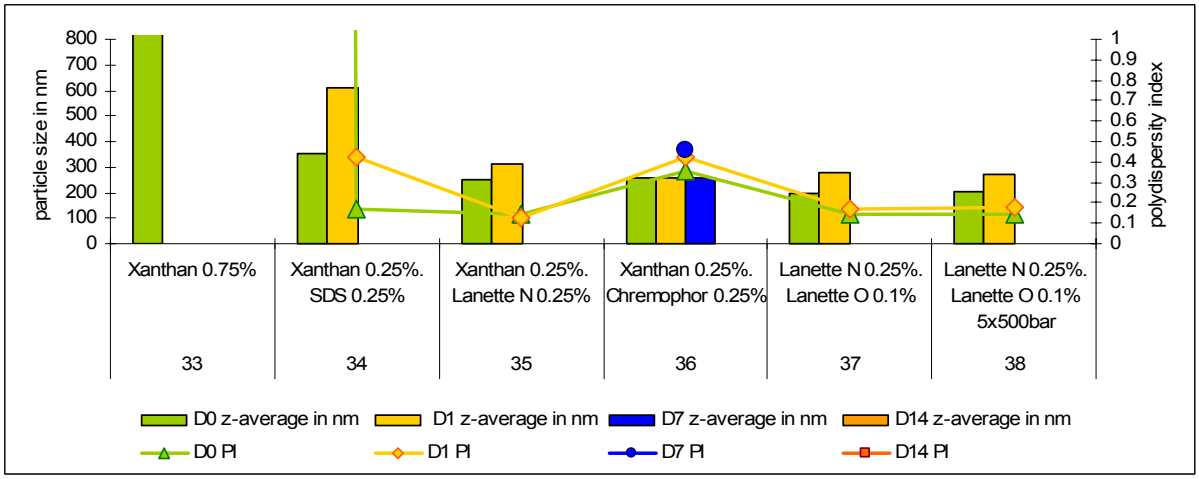


Figure 8-10: PCS data from A6, no stable emulsion could be obtained, LD and microscopy showed large droplets

8.1.2 Conclusion

The attempt to produce physically stable emulsions containing dissolved cyclosporine failed. With regard to the analytics it could be shown, that PCS alone does not necessarily detect the instability and formation of larger particles, when the bulk population remains small. In combination with laser diffractometry via the "conventional method (= measurements with included PIDS)", all the systems containing larger particles were not analysed correctly, emphasising again the importance of light microscopy and optimised LD measurements (= measurements without PIDS and correct optical models).

8.2 Development of a self-emulsifying drug delivery system (SEDDS)

The production of small sized and physically stable emulsions containing cyclosporine failed, as rapid particle growth could not be avoided by the stabilisers investigated. As an alternative to the emulsions the development of SEDDS seemed to be more appropriate. SEDDS are small sized systems. The advantage in comparison to the emulsions is the way of production. SEDDS are produced only by gentle agitation. This way of production is simple and does not require any technical aids. Therefore SEDDS are often stored and delivered as SEDDS pre-concentrate, containing only the lipid phase and the emulsifier. The formation itself (adding water and shaking or stirring the system) is performed shortly prior to application. Therefore a long-term stability of these systems is not required, making this SEDDS interesting for peppermint oil and cyclosporine. Therefore the aim of this study was the development of SEDDS, containing dissolved cyclosporine in peppermint oil as lipid phase. The emulsifier chosen was Tagat TO, because of its self-emulsifying properties, capable for the formation of SEDDS (Charman et al. 1992) and additionally because of its inhibitory effects on p-glycoprotein (Szu-Wen Wang 2004). The whole study was performed as follows:

1. Solubility studies of cyclosporine in peppermint oil were performed before, additionally solubility studies were performed in Tagat TO. Cyclosporine was subsequently added to Tagat TO until saturation was reached. The solution was stirred during cyclosporine addition using a magnetic stirrer (oblong 6x3.5mm).

2. The influence of the time and the power of shaking and mixing on the particle size obtained was investigated and standardised for the further investigations. A reference SEDDS after (Charman et al. 1992) containing Miglyol instead of peppermint oil was used for this (Table 8-2) The system was produced be varying the time of shaking (10, 20, 30 and 60s) and the power input (shaking by hand with a frequence of approx. 120 shakes/min, stirring by using a magnetic stirrer (oblong 6x3.5mm) at 60 and 120 rpm and by homogenising using an Ultra Turrax at 5000rpm).

3. SEDDS containing Tagat TO were successfully produced before when Miglyol 912 was used as lipid phase (Charman et al. 1992). Thus this formulation was re-produced and taken as the reference. A screening of peppermint oil SEDDS containing no cyclosporine was performed in order to investigate the possibility to formulate SEDDS containing peppermint oil and to identify the optimal composition of peppermint oil and Tagat TO (formulations investigated are listed in Table 8-3)

4. Next SEDDS containing cyclosporine were produced and characterised. First a screening was performed by using peppermint oil containing 33% (w/w) of dissolved cyclosporine as lipid phase. The volume of the lipid phase was kept constant, whereas the concentration of Tagat TO was varied. The concentration of Tagat TO found from the first screening was used to start with and was than slightly decreased or increased, as the optimal composition was expected around this concentration also for SEDDS containing cyclosporine (the formulations are listed in Tabe 8-4)

5. Second SEDDS were produced containing a fixed concentration of Tagat TO, whereas the concentration of cyclosporine within the peppermint oil phase was varied. The total volume of the lipid phase was also kept constant (Table 8-5)

6. Also it was approached to study differences of the interfacial tension within the different systems produced. However, due to the self-emulsifying properties, no clear definite interface could be obtained by adding water to the mixtures. Therefore the surface tension was analysed instead (Table 8-6)

7. Because of the results obtained from the surface measurements, SEDDS containing much lower concentrations of TAGAT TO were produced and characterised (Table 8-7).The aim was to draw a conclusion about the interfacial effects.

Table 8-2: Composition of reference SEDDS, used to study the influence on formation parameters on the droplet size obtained

	formulation	Miglyol 912 in g	Tagat T0 in g	water in g	concentration of Tagat TO in ratio to peppermint oil in w%	concentration of Tagat in pre-concentrate in w%
influence of production on particle size	reference after (Charman et al. 1992)	1.4	0.6	2.0	30.0	42.9

Table 8-3: Composition of SEDDS screening for optimal concentration of Tagat TO (the total amount of peppermint oil was kept constant (2.0g) and the Tagat TO concentration was steadily increased (0.24 to 2.0g); i.e. the lipophilc phase (oil+Tagat TO) contained an increasing concentration of Tagat TO from 10.7% to 50.0%)

	formulation	peppermint oil in g	Tagat T0 in g	water in g	concentration of Tagat TO in ratio to peppermint oil in w%	concentration of Tagat in pre-concentrate in w%
screening of SEDDS containing no cyclosporine	1	2.0	0.24	10.0	12.0	10.7
	2	2.0	0.30	10.0	15.0	13.0
	3	2.0	0.36	10.0	18.0	15.3
	4	2.0	0.40	10.0	20.0	16.7
	5	2.0	0.46	10.0	23.0	18.7
	6	2.0	0.50	10.0	25.0	20.0
	7	2.0	0.56	10.0	28.0	21.9
	8	2.0	0.60	10.0	30.0	23.1
	9	2.0	0.66	10.0	33.0	24.8
	10	2.0	0.70	10.0	35.0	25.9
	11	2.0	0.76	10.0	38.0	27.5
	12	2.0	0.80	10.0	40.0	28.6
	13	2.0	0.86	10.0	43.0	30.1
	14	2.0	0.90	10.0	45.0	31.0
	15	2.0	0.96	10.0	48.0	32.4
	16	2.0	1.00	10.0	50.0	33.3
	17	2.0	1.06	10.0	53.0	34.6
	18	2.0	1.10	10.0	55.0	35.5
	19	2.0	1.16	10.0	58.0	36.7
	20	2.0	1.20	10.0	60.0	37.5
	21	2.0	1.26	10.0	63.0	38.7
	22	2.0	1.30	10.0	65.0	39.4
	23	2.0	1.34	10.0	67.0	40.1
	24	2.0	1.40	10.0	70.0	41.2
	25	2.0	1.50	10.0	75.0	42.9
	26	2.0	2.00	10.0	100.0	50.0

Table 8-4: Composition of SEDDS containing cyclosporine and various concentrations of Tagat TO

	formulation	peppermint oil containing dissolved cyclosporine 33 w%	Tagat T0 in g	water in g	concentration of Tagat TO in ratio to peppermint oil in w%	concentration of Tagat in pre-concen-trate in w%t
screening of SEDDS containing cyclosporine 33% (w/w) in ratio to peppermint oil	27	2.0 (1.33+0.66)	0.66	10.0	33	24.8
	28	2.0 (1.33+0.66)	0.70	10.0	35	25.9
	29	2.0 (1.33+0.66)	0.76	10.0	38	27.5
	30	2.0 (1.33+0.66)	0.80	10.0	40	28.6
	31	2.0 (1.33+0.66)	0.86	10.0	43	30.1
	32	2.0 (1.33+0.66)	0.90	10.0	45	31.0
	33	2.0 (1.33+0.66)	0.96	10.0	48	32.4
	34	2.0 (1.33+0.66)	1.00	10.0	50	33.3
	35	2.0 (1.33+0.66)	1.06	10.0	53	34.6
	36	2.0 (1.33+0.66)	1.10	10.0	55	35.5
	37	2.0 (1.33+0.66)	1.16	10.0	58	36.7
	38	2.0 (1.33+0.66)	1.20	10.0	60	37.5
	39	2.0 (1.33+0.66)	1.26	10.0	63	38.7
	40	2.0 (1.33+0.66)	1.30	10.0	65	39.4
	41	2.0 (1.33+0.66)	1.34	10.0	67	40.1
	42	2.0 (1.33+0.66)	1.40	10.0	70	41.2
	43	2.0 (1.33+0.66)	1.50	10.0	75	42.9
	44	2.0 (1.33+0.66)	2.00	10.0	100	50.0

Table 8-5: Composition of SEDDS containing various concentrations of cyclosporine and 38.7% Tagat TO

	formulation	peppermint oil containning cyclosporine 33w% in g	peppermint oil with out cyclosporine in g	Tagat TO in g	water in g	concentration of Tagat in ratio to peppermint oil in w%	concentration of Tagat in preconcentrate in w%	concentration of cyclosporine in peppermint oil in w%	concentration of cyclosporine in preconcentrate in w%
screening of SEDDS containing cyclosporine with various concentrations	45	0.15	1.85	1.26	10.0	63.0	38.7	2.5	1.5
	46	0.30	1.70	1.26	10.0	63.0	38.7	5.0	3.1
	47	0.46	1.54	1.26	10.0	63.0	38.7	7.7	4.7
	48	0.62	1.38	1.26	10.0	63.0	38.7	10.2	6.2
	49	0.77	1.23	1.26	10.0	63.0	38.7	12.7	7.8
	50	0.92	1.08	1.26	10.0	63.0	38.7	15.2	9.3
	51	1.07	0.93	1.26	10.0	63.0	38.7	17.7	10.8
	52	1.23	0.77	1.26	10.0	63.0	38.7	20.3	12.5
	53	1.38	0.62	1.26	10.0	63.0	38.7	22.8	14.0

Table 8-6: Composition of systems for surface tension measurements

surface tension measurements		cyclosporine in g	Tagat TO in g	peppermint oil in g	concentration of Tagat TO in w%
	Tagat TO in peppermint oil	0	0.00	5.00	0.00
		0	0.45	4.55	9.09
		0	1.15	3.85	23.08
		0	1.77	3.23	35.48
		0	1.88	3.13	37.50
		0	1.93	3.07	38.65
		0	1.97	3.03	39.39
		0	2.22	2.78	44.44
		0	2.50	2.50	50.00
	cyclosorine in peppermint oil without Tagat TO	cyclosporine in g	peppermint oil containing 33% cyclosporine (w/w) in g	peppermint oil without cyclosporine in g	concentration of cyclosporine in w%
		0.00	0.00	5.00	0.00
		0.25	0.76	4.24	5.00
		0.50	1.52	3.48	10.00
		0.63	1.89	3.11	12.50
		0.75	2.27	2.73	15.00
		1.00	3.03	1.97	20.00
		1.25	3.79	1.21	25.00
		1.65	5.00	0.00	33.00
	cyclosporine in peppermint oil and Tagat 38.7%	cyclosporine in g	m peppermint oil containing 33% cyclosporine (w/w) in g	Tagat TO/ peppermint oil in g	w% Tagat TO / w% cyclosporine
		0.00	0.00	1.94/ 3.07	38.7/ 0.0
		0.19	0.58	1.94/ 2.49	38.7/ 3.80
		0.39	1.17	1.94/ 1.89	38.7/ 7.73
		0.48	1.46	1.94/ 1.61	38.7/ 9.63
		0.58	1.75	1.94/ 1.32	38.7/ 11.53
		0.77	2.34	1.94/ 0.72	38.7/ 15.46
		0.87	2.63	1.94/ 0.43	38.7/ 17.36
		1.01	3.07	1.94/ 0.00	38.7/ 20.23

Table 8-7: Composition of SEDDS containing cyclosporine and various but low concentrations of Tagat TO*

	formulation	peppermint oil /cyclosporine 033% in g	Tagat T0 in g	water in g	concentration of Tagat in ratio to peppermint oil in w%	concen-tration of Tagat in pre-concen-trate in w%
screening of SEDDS containing cyclosporine 33% (w/w) in ratio to peppermint oil	61	2.0 (1,33+0.66)	0.24	10.0	12.0	10.7
	62	2.0 (1,33+0.66)	0.30	10.0	15.0	13.0
	63	2.0 (1,33+0.66)	0.36	10.0	18.0	15.3
	64	2.0 (1,33+0.66)	0.40	10.0	20.0	16.7
	65	2.0 (1,33+0.66)	0.46	10.0	23.0	18.7
	66	2.0 (1,33+0.66)	0.50	10.0	25.0	20.0
	67	2.0 (1,33+0.66)	0.56	10.0	28.0	21.9
	68	2.0 (1,33+0.66)	0.60	10.0	30.0	23.1

*the total amount of oil was kept constant and cyclosporine was kept constant (1.33 + 0.66=2.0g), Tagat was added in increasing amounts, i.e. the total volume of the lipophilic phase (oil + cyclosporione + Tagat) increased from 2.24 to 2.60g, water was added constant at 10.0g)

For producing the SEDDS, the pre-concentrate (lipophilic phase, emulsifier) was mixed and stirred for 10min, to ensure homogeneity. Water was added rapidly using a 10.0ml pipette. After adding the water, the obtained system was stirred using a magnetic stirrer (oblong 6mm x 3.5mm), time and speed were controlled. Cyclosporine used was dissolved prior usage in peppermint oil. This concentrate, containing 33% (w/w) of cyclosporine was diluted if required for the different formulations investigated (see formulation tables). All systems obtained were analysed by light microscopy and photon correlation spectroscopy.

8.2.1 Solubility of cyclosporine

Up to 33% (w/w) of cyclosporine can be dissolved in peppermint oil. The dissolution velocity is approx. 6h under stirring conditions (magnetic stirrer). The obtained solution remains clear over a period of at least 6 month. For cyclosporine in Tagat TO also a high saturation solubility of 27.5% (w/w) was found. The dissolution velocity is 3h under stirring conditions. However re-crystallisation occurs within 24 hours after the dissolution (see figure below). In conclusion a solution of cyclosporine in Tagat TO cannot be used for further investigations. The approach to increase the total concentration of cyclosporine in the final SEDDS formulation by dissolving the drug in both compounds can not be realised therefore. Only cyclosporine concentrations up to 33% in ratio to the peppermint oil content of the total formulation can by used. Otherwise crystal growth would occur.

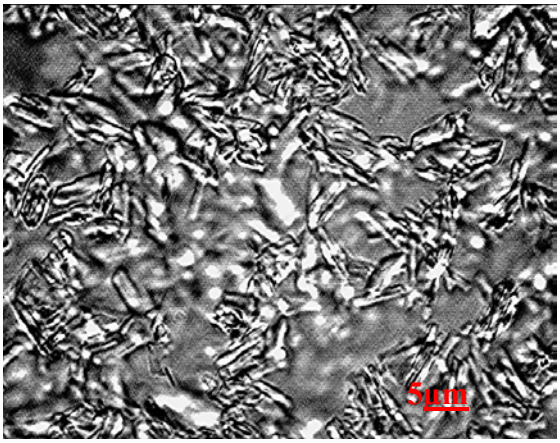


Figure 8-11: Re-crystallised cyclosporine (25µm) in Tagat TO 24h after dissolution (magnification 1000x)

8.2.2 Influence of production parameters on particle size

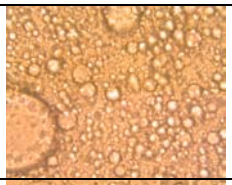
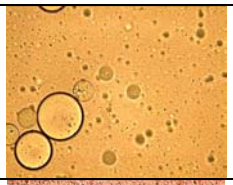
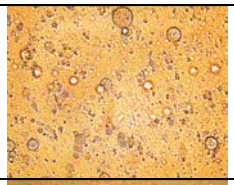
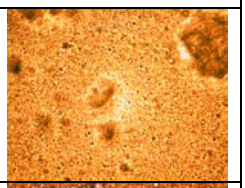
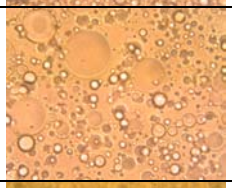
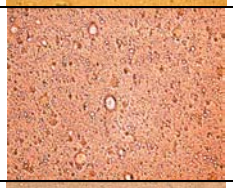
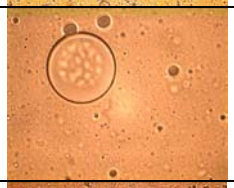
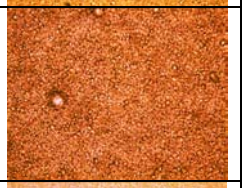
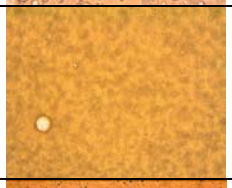
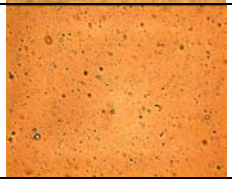
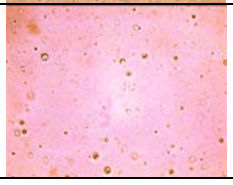
produced by	time → / speed ↓	10sec.	20sec.	30sec.	60sec.
hand	approx. 120rpm				
magnetic stirrer	30rpm				
	60rpm				
Ultra Turrax	5000rpm				

Figure 8-12: Microscopic images of SEDDS reference produced under various production parameters (explanation c.f. text below)

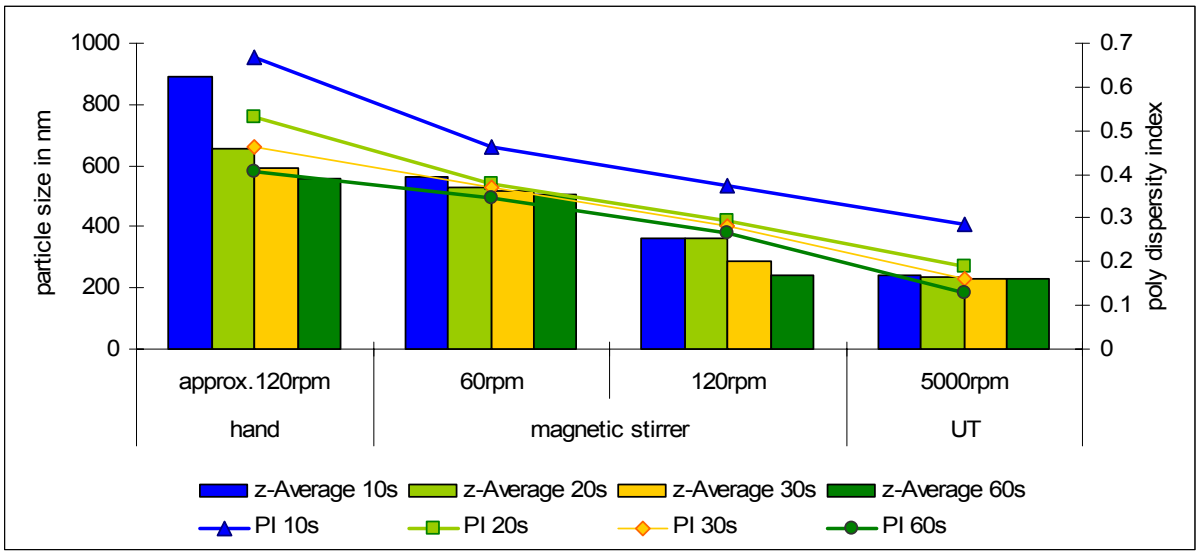


Figure 8-13: PCS data of SEDDS reference produced under various production parameters (explanation c.f. text below)

From the data obtained (Figure 8-12 and Figure 8-13) it is concluded, that the parameters of production strongly influence the particle size and size distribution of the SEDDS produced. The longer the system is shaken/stirred or homogenised and the more forces are applied to it, the smaller is the particle size and the narrower the size distribution. Shaking by hand and the stirring via a magnetic stirrer (30rpm) were not efficient to form a small sized and homogeneous SEDDS. Stirring with the magnetic stirrer with 60rpm could from a small sized SEDDS. The best SEDDS was obtained when the Ultra Turrax was used with a homogenisation time of 30 or 60s (c.f. Figure 8-12 and Figure 8-13).

For the rest of this study the production via magnetic stirrer was selected. Shaking by Hand is not reproducible and was excluded therefore. Homogenisation of SEDDS using an Ultra Turrax was excluded, as SEDDS should be obtained by only applying gentle agitation.

The speed was chosen to be 120rpm, as the movements of the fluid caused by the magnetic stirrer at 60 rpm was observed to be too poor for the viscous pre-concentrates studied. Depending on the viscosity of the formulation the stirrer showed a delayed motion, which would not be reproducible. The time of stirring was chosen to be 30s.

8.2.3 Screening for optimal concentration of Tagat TO

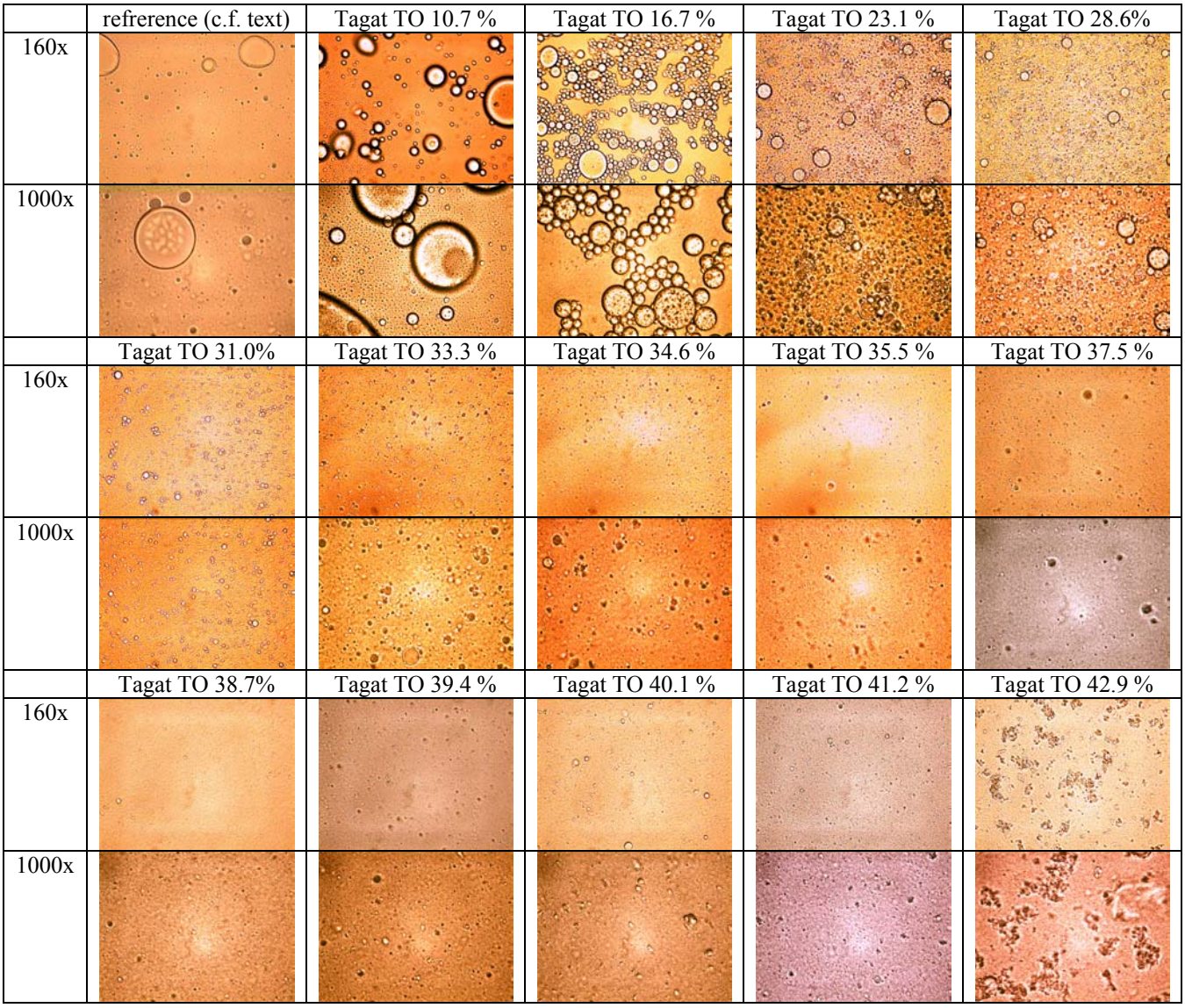


Figure 8-14: Microscopic images of SEDDS screening, SEDDS containing 37.5-37.8% Tagat TO are best, systems did not contain cyclosporine

Microscopic imaging (Figure 8-14, first image) shows the SEDDS containing Miglyol and Tagat TO (21.3%) as reference from (Charman et al. 1992). Beside very small particles, large droplets could be observed here. In the following images of the SEDDS containing peppermint oil the decrease in particle size and size distribution of the systems with an increase in the concentration of Tagat TO can be seen. The best SEDDS were obtained with concentrations of Tagat from 37.5-39.4%. A further increase in Tagat concentration led to agglomeration of the oil droplets. PCS analysis proved the results obtained by microscopy, also here the smallest size were obtained for these systems (Figure 8-15 and Table 8-8).

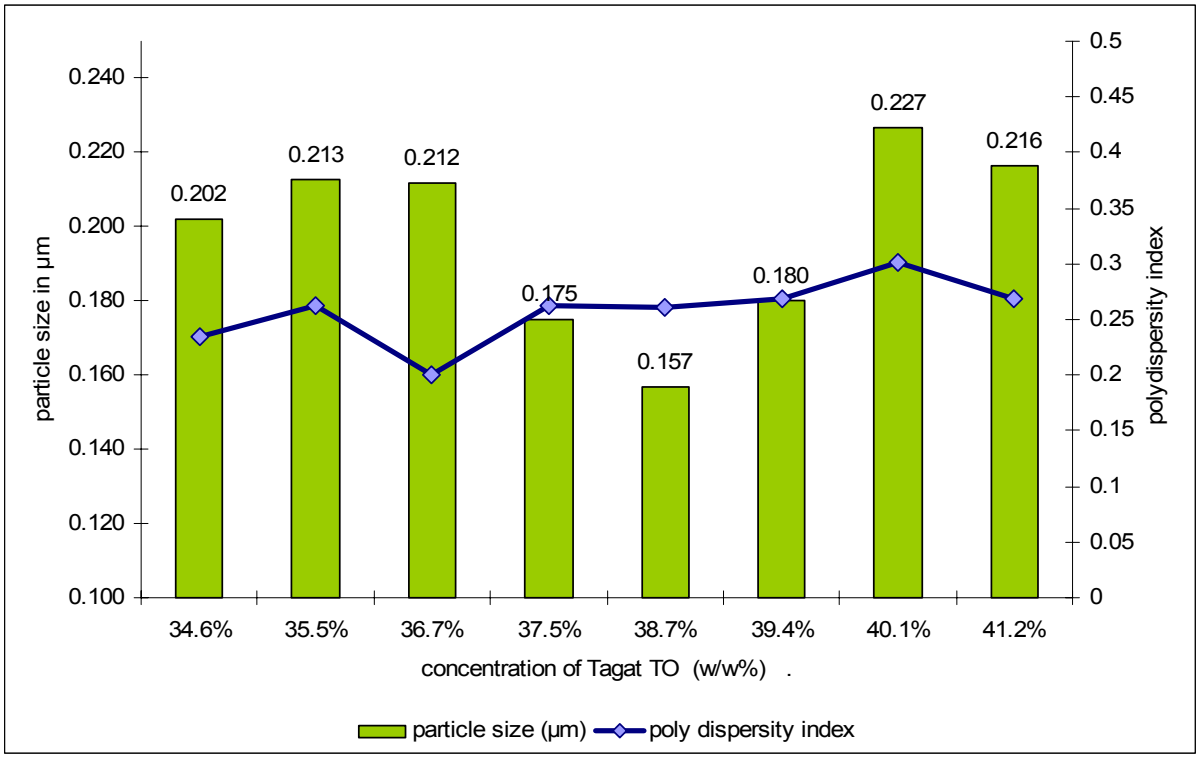


Figure 8-15: PCS data of screening

Table 8-8: PCS data of screening (formulations containing peppermint oil 2.0g various concentrations of Tagat TO and 10.0g water and no cyclosporine)

concentration of Tagat TO in w%		particle size (z-average) in µm	polydispersity index
in the pre-concentrate	in ratio to peppermint oil		
34.6	50	0.202	0.235
35.5	53	0.213	0.263
36.7	55	0.212	0.2
37.5	60	0.175	0.262
38.7	**63**	**0.157**	**0.261**
39.4	65	0.18	0.268
40.1	67	0.227	0.302
41.2	70	0.216	0.268

The formulation containing 38.7% Tagat TO was chosen as reference for further investigations.

8.2.4 Characterisation of SEDDS containing cyclosporine

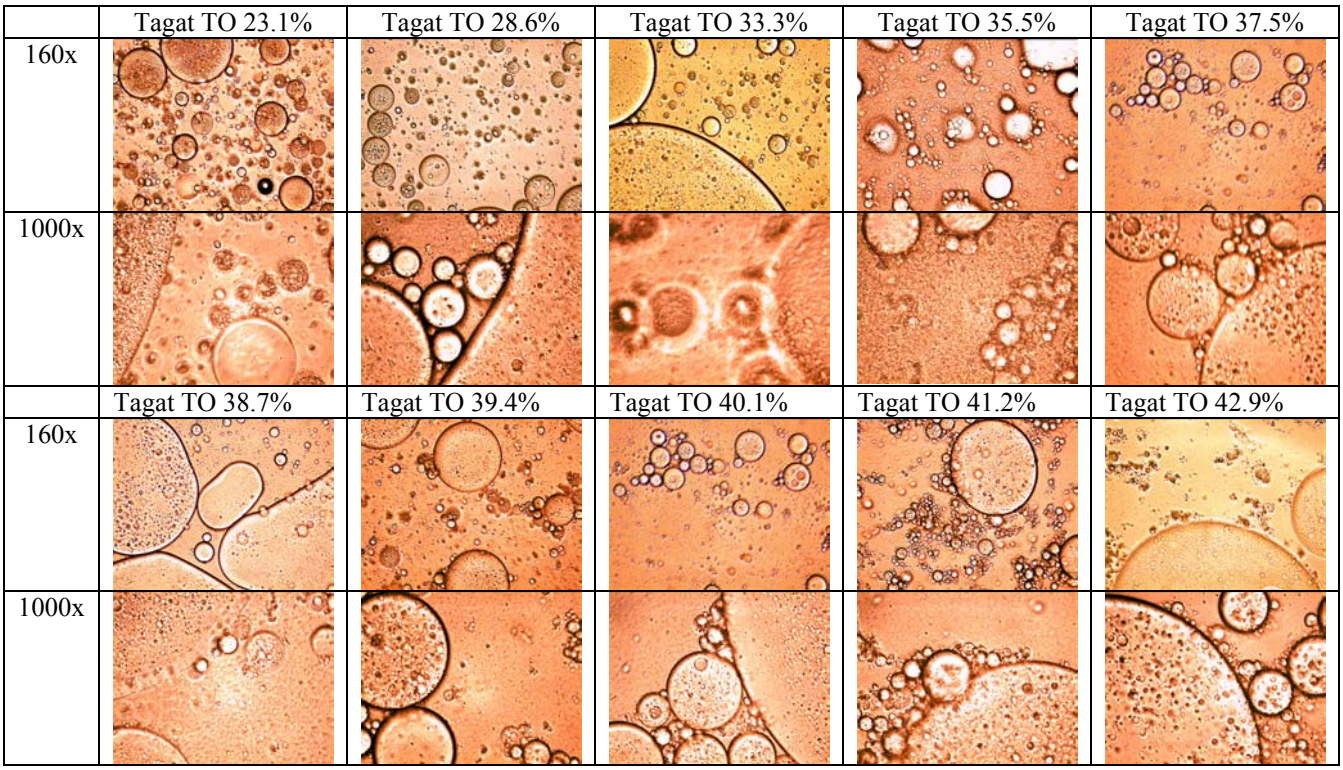


Figure 8-16 microscopic images of SEDDS containing cyclosporine with various concentrations of Tagat TO

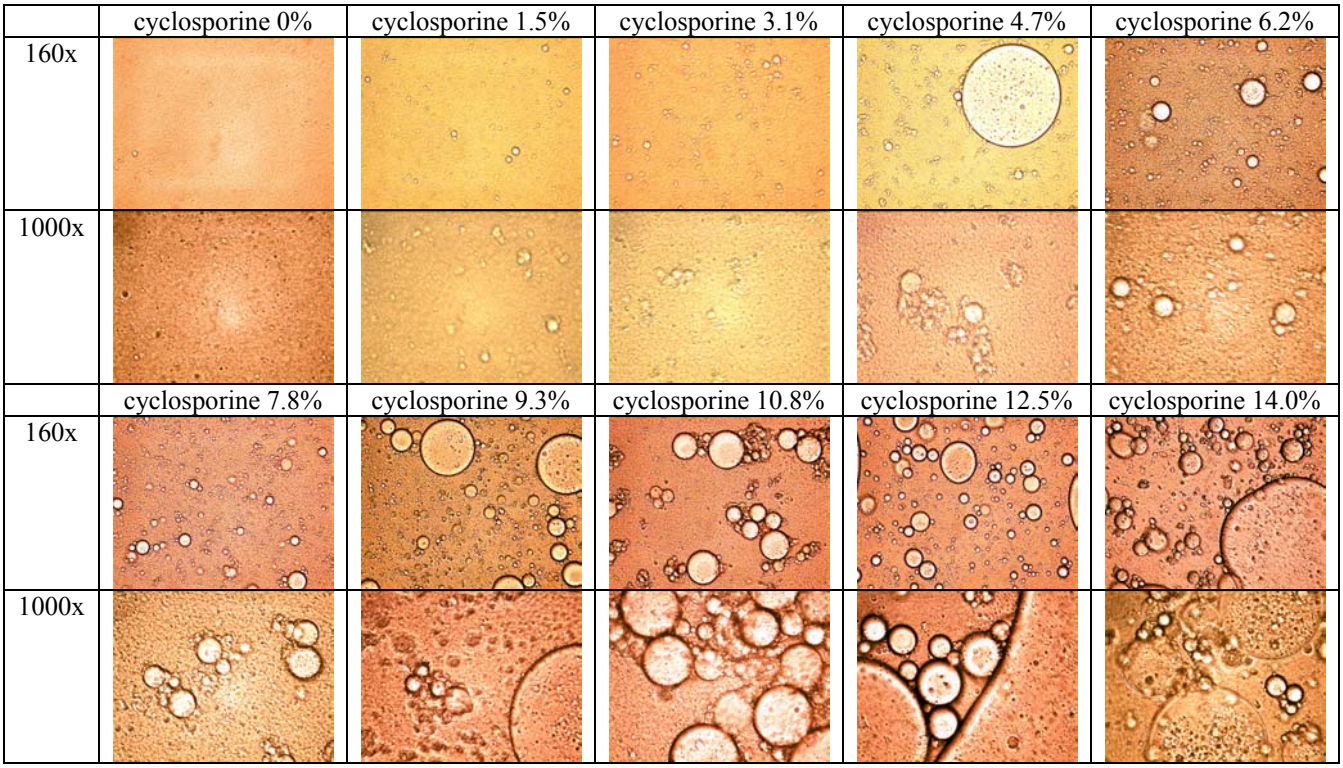


Figure 8-17: Microscopic images of SEDDS containing cyclosporine with varying concentrations and 37.8% of Tagat TO

The optimal concentration of Tagat TO in pure peppermint oil was found to be 38.7%. However small sized homogeneous SEDDS containing cyclosporine and concentrations of Tagat TO near the optimum found from the previous screening could not be obtained (Figure 8-16). PCS measurements were hardly to perform due to the large particles within the system. None of the formulations investigated had a polydispersity index smaller 1 indicating, also from this analysis method, the broad size distribution of the systems. The smallest system analysed was the formulation containing 38.7% Tagat TO. The particle size was analysed to be 1679 nm. SEDDS produced with a constant concentration of Tagat and increasing concentrations of cyclosporine clearly showed the influence of the cyclosporine concentration on the quality of the SEDDS obtained. The quality is the better the less cyclosporine the SEDDS contains (Figure 8-17). The best SEDDS containing cyclosporine was the SEDDS with only 1.5 w% of cyclosporine and a concentration of Tagat TO of 37.8%.

8.2.5 Measurement of surface tension

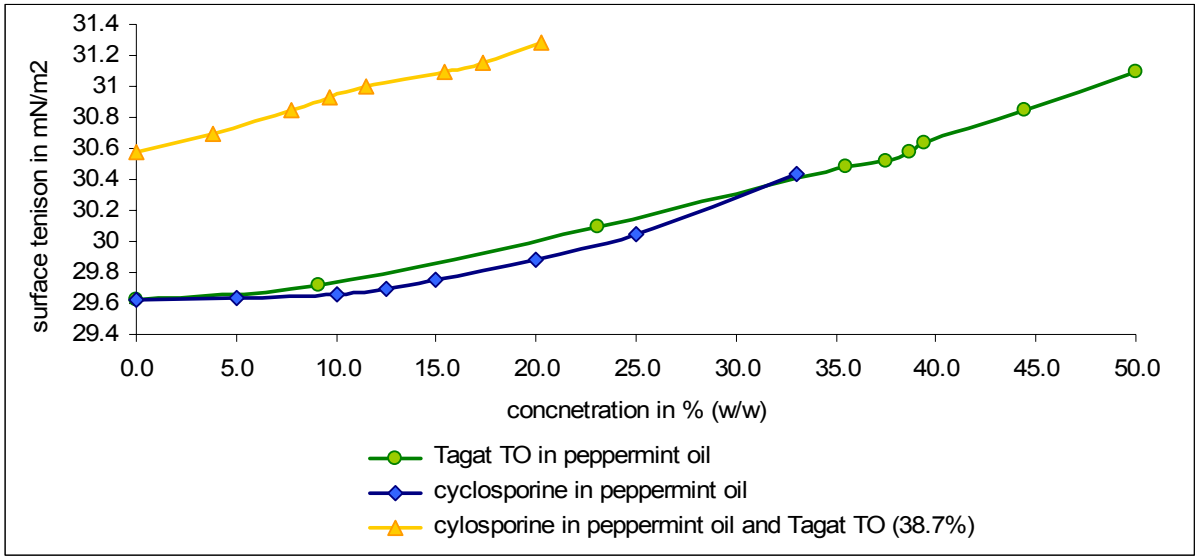


Figure 8-18: Results of surface tension measurements, the surface tension increases with an increase in concentration of Tagat TO and/or cyclosporine

The analysis of the results (Figure 8-18) shows that an increase in concentration either of Tagat TO (green curve) or cyclosporine (blue curve) increases the surface tension of the system. Systems containing Tagat TO in a fixed concentration and various concentrations of cyclosporine also showed an increase with an increasing concentration of cyclosporine (orange curve). The total surface tension of the systems containing cyclosporine and Tagat TO is higher, than Tagat TO alone and increases with an increase in cyclosporine concentration. From this it was assumed that the concentration dependent instability of the SEDDS containing cyclosporine might arrive from an increase of the surface tension. Therefore it was expected to find better results for SEDDS by reducing the surface tension of

the systems containing cyclosporine. As it was found that the surface tension depends on the concentration of Tagat TO and cyclosporine, it is expected that the surface tension of SEDDS containing cyclosporine can also be reduced by reducing the concentration of Tagat TO and keeping the concentration of cyclosporine constant. A further screening of SEDDS containing much lower concentrations of Tagat TO should prove this theoretical expectation. From the results it could be calculated that a content of 15.43% (w/w) Tagat TO in peppermint oil containing cyclosporine 33% (w/w) should lead to SEDDS having the same surface tension than SEDDS containing 38.7% Tagat TO but no cyclosporine.

8.2.6 SEDDS containing lower concentrations of Tagat TO

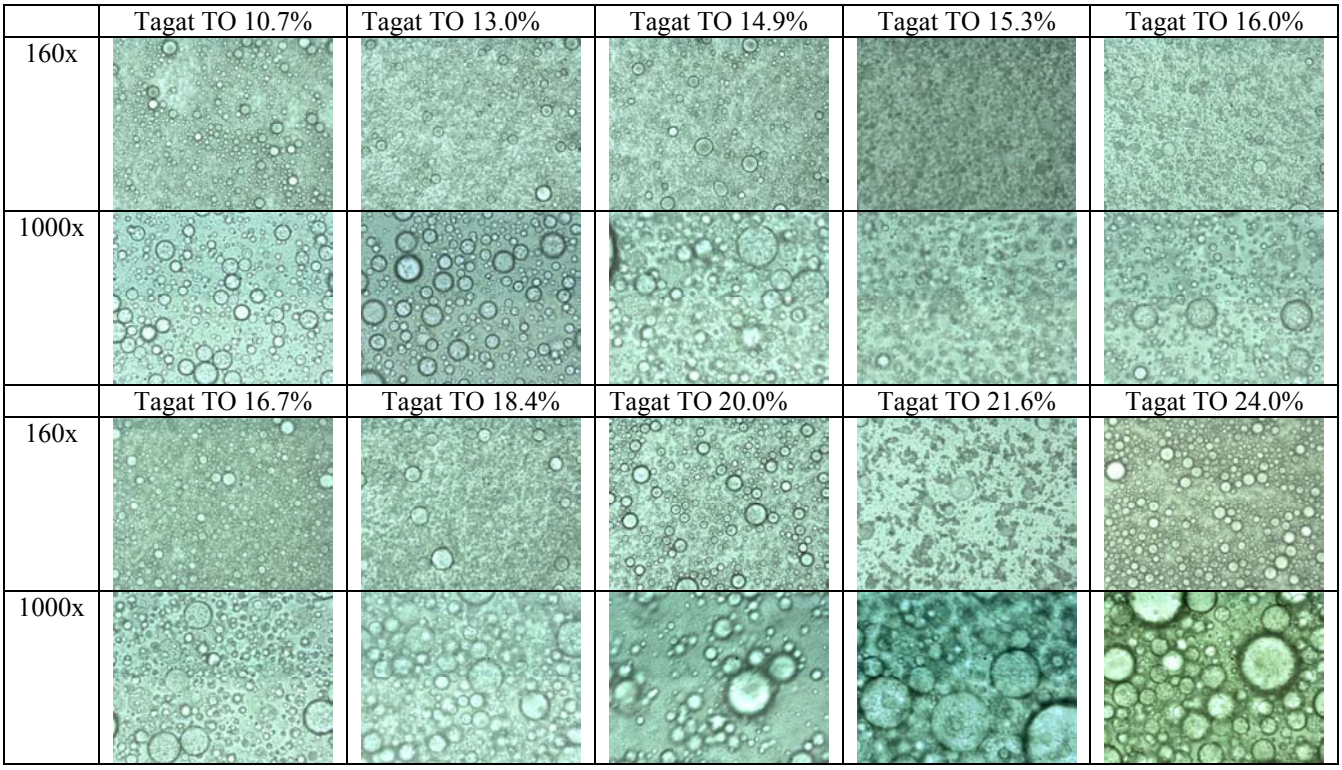


Figure 8-19: Microscopic images of SEDDS containing cyclosporine and Tagat TO with varying but low concentrations

As expected, the reduction of Tagat TO within the cyclosporine containing SEDDS increased the quality of the SEDDS obtained here. Though the theoretically expectation, arrived from surface measurements, could be proven. The calculated value of 15.43% Tagat was well in agreement with the results found here; the best SEDDS were obtained for concentrations of Tagat TO between 15.5-15.7 % (Figure 8-19).

8.2.7 Conclusion

Cyclosporine dissolves not only in peppermint oil but also in Tagat TO. However storage of dissolved cyclosporine in Tagat TO is not possible as re-crystallisation occurs within only 24 hours. Therefore the upper limit of cyclosporine is 33% in ratio to the peppermint oil content of the formulation. The resulting particle size and size distribution of SEDDS is dependent on the production parameters applied to the system. The more energy input is applied, the smaller is the size and the narrower size distribution of the SEDDS produced. The development of SEDDS containing peppermint oil and Tagat TO was successful. The best drug-free SEDDS was obtained with a concentration of Tagat TO of 38.7% (w/w). For those systems droplet sizes of only 157nm were analysed. SEDDS containing cyclosporine could also be produced. The best SEDDS containing cyclosporine 33% (w/w) in ratio to peppermint oil were obtained with a concentration of Tagat TO being only 15.5% (w/w). The large difference in concentrations of Tagat TO needed for SEDDS without and for SEDDS with cyclosporine can be explained by surface tension measurements. The surface tension of pure peppermint oil increases in a concentration dependent manner when Tagat or cyclosporine is added to it. Systems containing Tagat TO and cyclosporine were analysed to have even a higher surface tension, which can be reduced by reducing either the concentration of cyclosporine or the concentration of Tagat TO. Therefore only the reduction of Tagat TO in the formulation containing cyclosporine led to suitable results. However, sizes of SEDDS containing cyclosporine were much larger than SEDDS without drug.

In conclusion, SEDDS on the base of peppermint oil and Tagat TO containing cyclosporine are an interesting formulation principle for the oral delivery of cyclosporine. In this study it was shown that the production is possible. However, further studies (e.g. screening for co-surfactants, viscosity measurements, and influence on emulsification temperature, etc.) are required to further optimise the formulations found here.

8.3 Development of an emulsifier free delivery system containing cyclosporine

8.3.1 Introduction

As mentioned before the formulation of cyclosporine in systems containing emulsifiers is discussed controversely. The absorption mechanism of cyclosporine is passive diffusion. There is evidence that passive diffusion can be decreased in the presence of emulsifiers above the critical micelle concentration. It is thought that the drug is trapped into the micelles formed, from which it can not be released (Kasim et al. 2004). All formulations investigated in the previous chapters contain emulsifiers well above the critical micelle concentration. Also the often as "standard" referred to microemulsion pre-concentrate Sandimmun® Optoral contains high concentrations of an emulsifier. The older formulation Sandimmun does not contain an emulsifier; it only consists of dissolved cyclosporine in corn oil - filled into gelatine capsules. Nevertheless, the bioavailability could be greatly enhanced when cyclosporine was formulated as a microemulsion pre-concentrate (Mainzer et al. 1998; Rote Liste 2003). This effect observed is explained as the particle size of the microemulsion Sandimmun® Optoral is small, where no further diminution or emulsification is required (30-70nm). In contrast the older formulation Sandimmun. The oily solution is dependent on the availability of digestive emulsifiers, which vary intra individually. Droplet sizes reported are around 2µm for this formulation in the gut (Mainzer et al. 1998). Sandimmun entered the market 1983. The improved formulation Sandimmun® Optoral was launched 11 years later in 1994. At those times the importance of p-glycoprotein was not fully recognised. Literature research therefore gave no results about publications comparing systems containing cyclosporine and inhibitors of p-glycoprotein with and without emulsifiers, supporting the assumption at this time that only the particle size is the influencing parameter for the increased bioavailability of cyclosporine. However from the literature and the recent controversy, such a study could clarify doubts and assumptions. The basic formulation; peppermint oil containing dissolved cyclosporine at a maximal concentration of 33% (w/w) fulfils the requirement of p-gp inhibition and no content of emulsifier. Though, this simple system is thought to be of high interest for in vivo studies. However, a small sized system without emulsifier but with inhibitory properties for p-glycoprotein would be the formulation of choice. Therefore the aim of this study was the development of such a small sized system on the base of peppermint oil and dissolved cyclosporine. Intense literature research put the interest to Aeroperls® 300 pharma. Aeroperls® 300 are 100% pure, highly disperse, hydrophilic and amorphous silicon dioxides of pharmaceutical grade. It is a granulate consisting of single particles of an approximate particle size of 30µm. Each particle is highly porous as a sponge.

Therefore the special property is its high absorption capacity for liquids. Up to 300% (w/w) of a liquid can be absorbed from Aeroperls®, where it still remains a flowing powder (Degussa AG 2005).

The aim of this study was to formulate cyclosporine as an emulsifier free but small sized dosage form. For that the pre-formulation of dissolved cyclosporine in peppermint oil was absorbed to Aeroperls® 300. The final product should be still a flowing powder. Therefore first the maximal absorption weight of the pre-formulation (peppermint oil with cyclosporine) was investigated. The quantity of the formulation absorbed to Aeroperls® was increased subsequently until the obtained formulation turned from a flowing powder into an agglomerated system. The highest weight of the formulation at which the Aeroperls® remained flowing, was set to be the maximal weight. Microscopic imaging was performed to characterise the systems obtained. The systems produced were observed dry and after water was added, in order to study the behaviour after administration. LD measurements were performed in parallel. Last, DSC measurements were performed to observe possible re-crystallisation of cyclosporine during storage. For comparison also the previously developed SEDDS containing cyclosporine was absorbed to Aeroperls® and was investigated in the same manner. The formulations absorbed to the Aeroperls® are listed in Table 8-9.

Table 8-9: Fromulations investigated

formulation	peppermint oil in % (w/w)	cyclosporine in % (w/w)	Tagat TO in % (w/w)
pre-formulation - cyclosporine solution	67	33	0.0
SEDDS pre-concentrate	56.72	27.93	15.35

8.3.1.1 Screening for maximal absorption volume

In this experiment the maximal load of cyclosporine dissolved in peppermint oil in the Aeroperls® was investigated. The cyclosporine solution was added to the Aeroperls® subsequently (2.00g Aeroperls, placed in a 30ml vial, droplets from a disposable pipette were added one by one, approx 0.02g per droplet). After appropriate times the vial was shaken to absorb the solution into the Aeroperls® and to control the flow of the Aeroperls®. The last quantity which could be added to the system before the Aeroperls® did not remain flowing, was set to be the maximal load. The maximal load was also investigated for peppermint oil alone and for the SEDDS formulation.

The maximal load was found to be 1.0g for the cyclosporine peppermint solution per 1.0g Aeroperls®. At this concentration the Aeroperls® remained flowing, but the loaded Aeroperls® tended to stick to the wall of the vial. The concentration where no adhesion was observed was found to be 0.5g solution per 1.0g Aeroperls®. For further investigations the maximal loaded (1.0g solution/1.0g Aeroperls®) Aeroperls® were used. The maximal load for peppermint oil alone was 1.5g per 1.0g Aeroperls® and 1.2g for the SEDDS formulation. Further investigations were performed at concentrations similar to the cyclosporine peppermint oil solution (1.0g peppermint oil or SEDDS/1.0g Aeroperls®)

8.3.1.2 Comparison of non loaded Aeroperls® and Aeroperls® loaded with cyclosporine dissolved in peppermint oil

The cyclosporine loaded Aeroperls® (Cycloperls) were compared to non loaded Aeroperls® by electron microscopy. Figure 8-20 shows the images obtained. The Aeroperls® without load show a smooth surface. Beside this a large amount of disintegrated Aeroperls® was observed (left column). The Cycloperls are shown on the right side. They seem to be larger than the original perls and do not contain as much fragments as seen in the unloaded Aeroperls®. From this it is assumed that Aeroperls® might swell when liquid is added to them and that splinters of dry Aeroperls® can adhere to the intact perls. The main interest of the study was to gain information about the peppermint oil with dissolved cyclosporine. The images clearly show that it is not adsorbed but absorbed, as no droplets can be seen on the surface of the pearls. From the images it is concluded that cyclosporine dissolved in peppermint oil can be successfully formulated as a dry dosage form by a simple absorption into Aeroperls® 300.

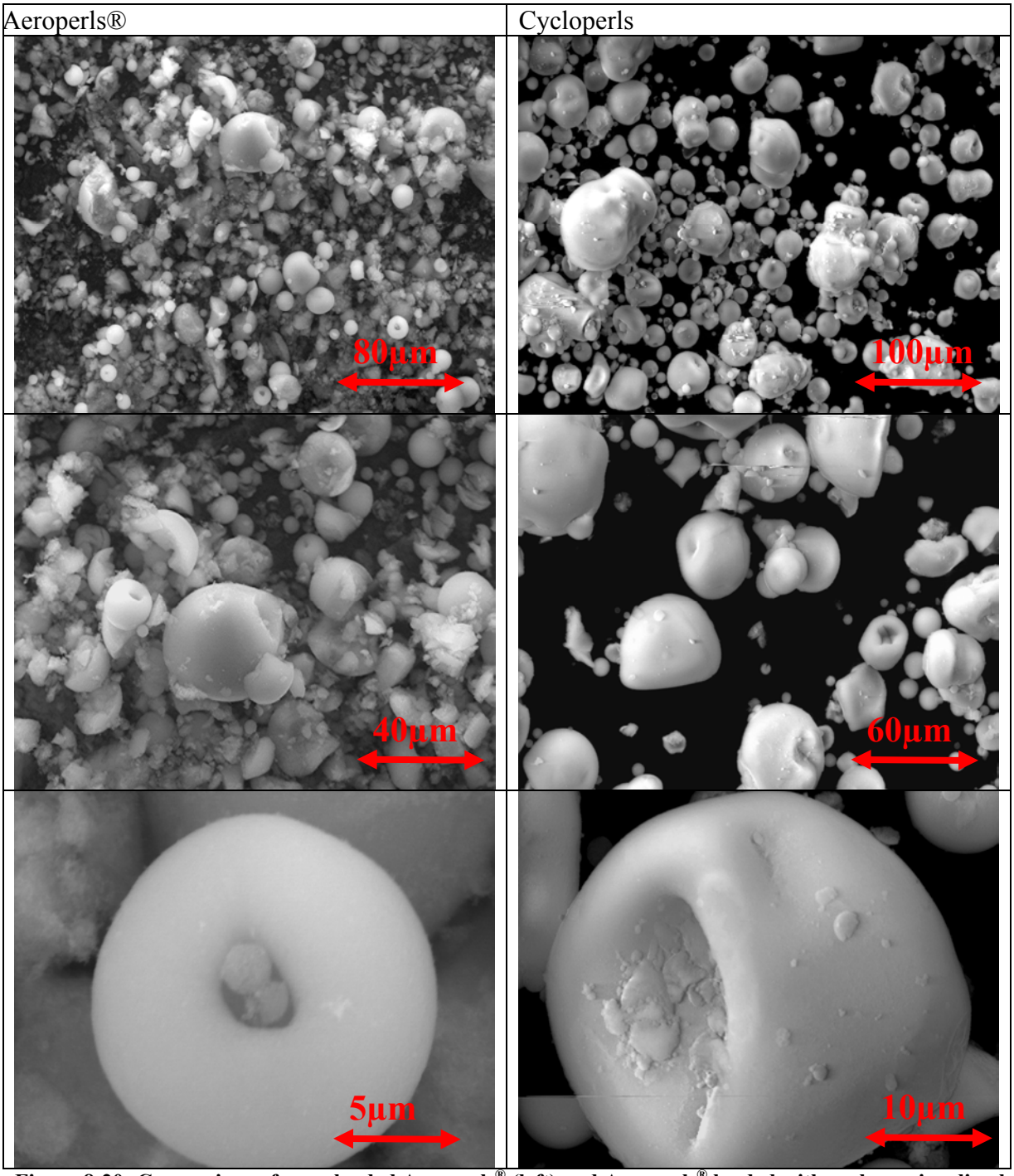
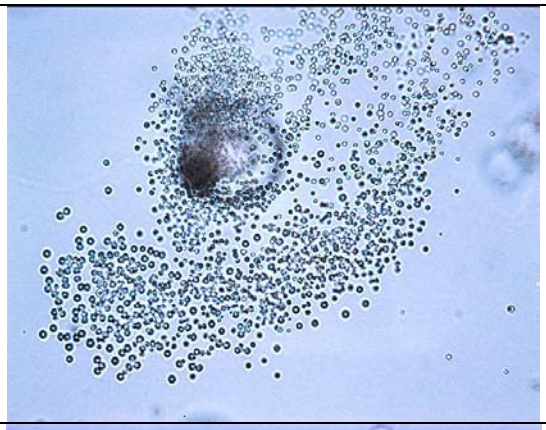
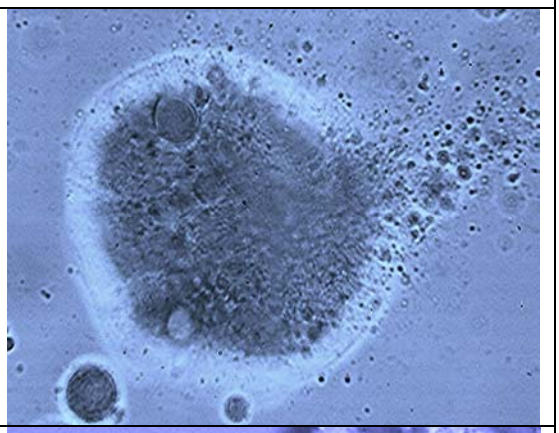
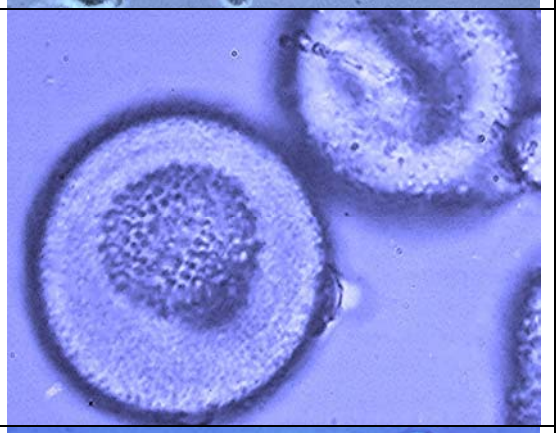
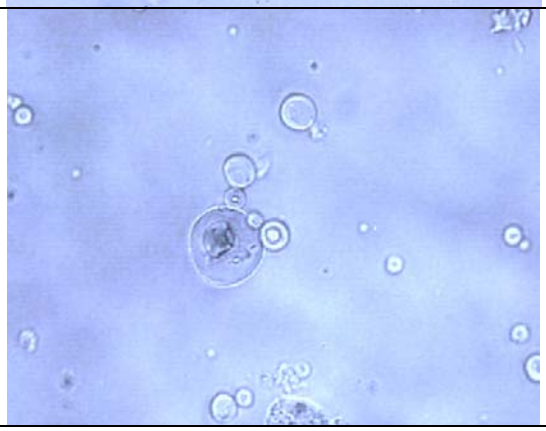
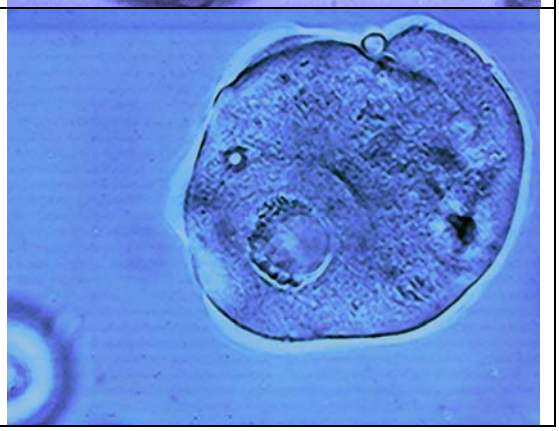


Figure 8-20: Comparison of none loaded Aeroperls® (left) and Aeroperls® loaded with cyclosporine dissolved in peppermint oil (load 1.0g solution / 1.0g Aeroperls®), = Cycloperls, (right)

8.3.1.3 Comparison of Aeroperls® loaded with cyclosporine / peppermint oil and SEDDS

The aim was to study the release of the formulations when liquid was added to them. Aeroperls® loaded with cyclosporine dissolved in peppermint oil (Cycloperls) were compared to the Aeroperls® loaded with the SEDDS (SEDDSperls).

Table 8-10: Images obtained from light microscopy for Cycloperls (first row), SEDDSperls (second row) and unloaded Aeroperls (third row) in water; Cycloperls show a rapid release after water is added, in SEDDSperls no rapid release can be obtained, only some droplets occur on the surface of the SEDDSperls without dispersion of the droplets released. No changes or effects are seen for unloaded Aeroperls.

	enlargement 630x	enlargement 1250x
Aeroperls® loaded with cyclosporine/ peppermint solution in water (Cycloperls)		
Aeroperls® loaded with SEDDS pre-concentrate in water (SEDDSperls)		
Aeroperls® unloaded in water		

An appropriate amount of the respective formulation and a droplet of water were placed on a microscope slide. The systems were observed by light microscopy immediately after the preparation. Aeroperls® without loading were also investigated with this method. The images obtained from this study are shown in Table 8-10. Cycloperls showed a rapid release of droplets (first row). It was observed, that the released droplets underwent coalescence shortly after their release, caused by the absence of emulsifiers. Therefore it was hoped that the Aeroperls® loaded with the SEDDS would release small droplets without coalescence as a large amount of emulsifiers is present in this system. However no fast release of droplets could be observed for the SEDDSperls (second row). The observation done was the appearance of a few large oil droplets around the SEDDSperls after about 5min. The release of very small droplets (e.g. < 200nm) can not be excluded, because such small particles are not accessible by light microscopy. For Aeroperls® without load no effect or changes could be observed (third row). In order to prove the observation form light microscopy, the Cycloperls and SEDDSperls were analysed by laser diffractometry. The refractive index was measured for cyclosporine (33 w%) in peppermint oil and for the SEDDS. The peppermint solution had a real refractive index of 1.467. The real refractive index for the SEDDS formulation was 1.470. For the imaginary part 0.1 was used, as obtained for both systems by UV/VIS measurements (0.102 (λ= 750nm) for the solution and 0.097(λ= 750nm) for the SEDDS).

The main interest of the LD measurements was the detection of small particles in the presence of a main large sized population. Therefore PIDS was included into the measurements. In the LD chapter it was investigated that the analysis as numeric distribution might be useful if small particles are expected within a larger bulk population. As this was the case for these measurements, the measured data were calculated as numeric and volumetric distribution. The data for the volume distribution are shown in Figure 8-21. Figure 8-22 shows the results as number distribution. The results of the volume distribution are very interesting. In principle they show that the Cycloperls are smaller in size than the SEDDSperls as well as that both systems change over the time of the measurement. But taking the results from the LD chapter into account, the results indicate the existence of smaller particles in the Cycloperls suspension, even though they are not detected.

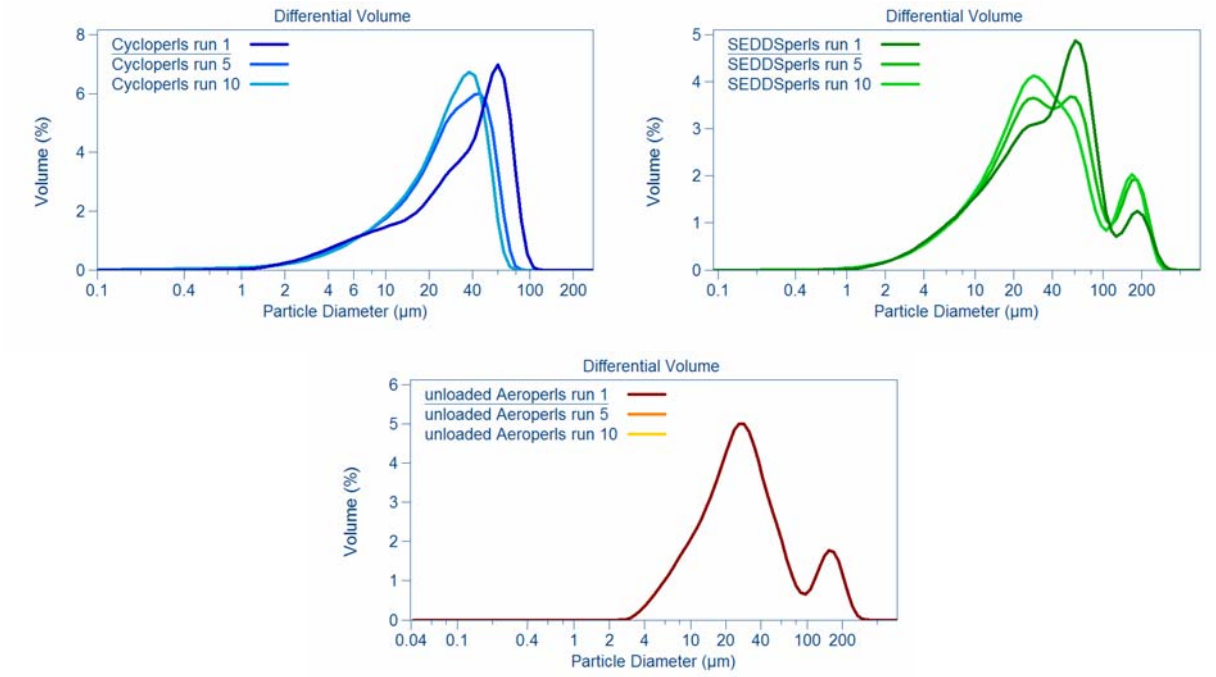


Figure 8-21: Comparison of LD-measurements (run 1, 5 and 10) of Cycloperls (upper, left, blue); SEDDSperls (upper, right, green) and unloaded Aeroperls (lower, middle, dark red), analysed as volume distribution; the results change over the time of the measurement, indicating the release of droplets, Cycloperls are incorrectly analysed to be smaller than the SEDDSperls and the unloaded Aeroperls: clearly this result is false and is due to the existence of smaller particles (not visible within this analysis mode)

Clearly there is a second peak at approximately 185µm found for the SEDDSperls (middle) but not for the Cycloperls (upper). To avoid misinterpretations, the Cycloperls and the SEDDSperls were produced again and the measurements (each consistent of 10 single runs) were repeated two more times yielding the same result each time. Non loaded Aeroperls® were measured, yielding a second population at 190µm similar to the peak detected for the SEDDS perls. Because the results are reproducible they can be judged as follows:

It was shown that larger particles might be overseen by LD analysis if smaller particles are present in the sample and if PIDS is included into the measurement. The larger particles correspond to the Aeroperls® in both of the systems. As the same batch was used for the production of both formulations, the particle size measured by LD should be equal for the Cycloperls and the SEDDSperls in theory. However in practice the Cycloperls are smaller than the SEDDSperls. The only reason can be the existence of smaller particles within the Cycloperls suspension, which overwhelm the impact and the detection of the larger particles. This expectation can be proven if the result is viewed as number distribution (Figure 8-22). It gratefully shows that the droplets released from the Cycloperls are much smaller than those released from the SEDDSperls and that the unloaded Aeroperls do not release small droplets.

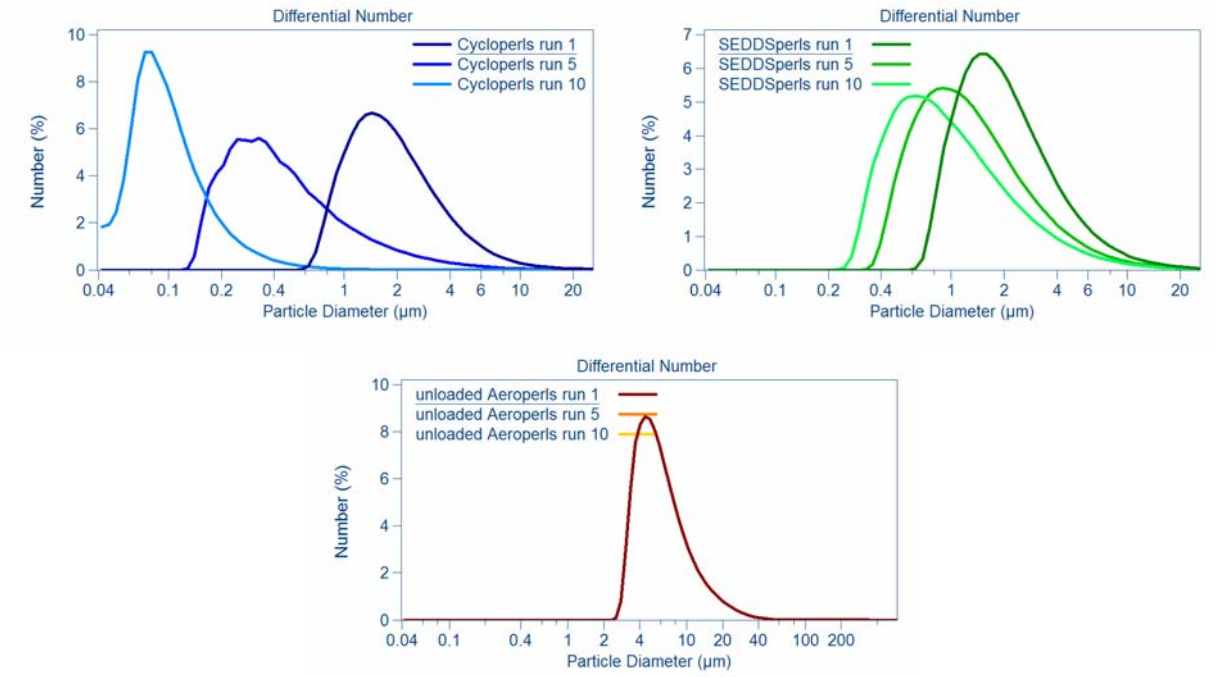


Figure 8-22: Comparison of LD-measurements (run 1, 5 and 10) of Cycloperls (upper, left, blue); SEDDSperls (upper, right, green) and unloaded Aeroperls (lower, middle, dark red), analysed as number distribution;, in this mode the much smaller droplets released from the Cycloperls can be detected

In conclusion the emulsifier free formulation containing only cyclosporine dissolved in peppermint oil seems to be suitable for a rapid release. The formulation containing a SEDDS formulation seems to get trapped in the Aeroperls®. No rapid release could be observed whether neither from microscopy nor from LD measurements.

8.3.1.4 DSC measurements

DCS measurements were performed in order to investigate if re-crystallisation of cyclosporine occurs over the time of storage. In Figure 8-23 the DSC measurements are shown. The diagram left shows the data for the raw materials. Only for cyclosporine powder a melting peak can be obtained. In the middle the DSC data for combined systems are shown (i.e. peppermint oil in Aeroperls®, cyclosporine dissolved in peppermint oil and the final formulation containing cyclosporine dissolved in peppermint oil absorbed to Aeroperls® (Cycloperls)). The diagram on the right side shows the Cycloperls and the Aeroperls® with absorbed peppermint oil after ten month of storage (stored at room temperature). Only for cyclosporine powder a melting peak can be obtained (left diagram, red curve). None of the other DSC curves shows a melting peak. From the data it can be concluded that no cyclosporine re-crystallised over the time of observation. Therefore the formulation can be judged as physically stable.

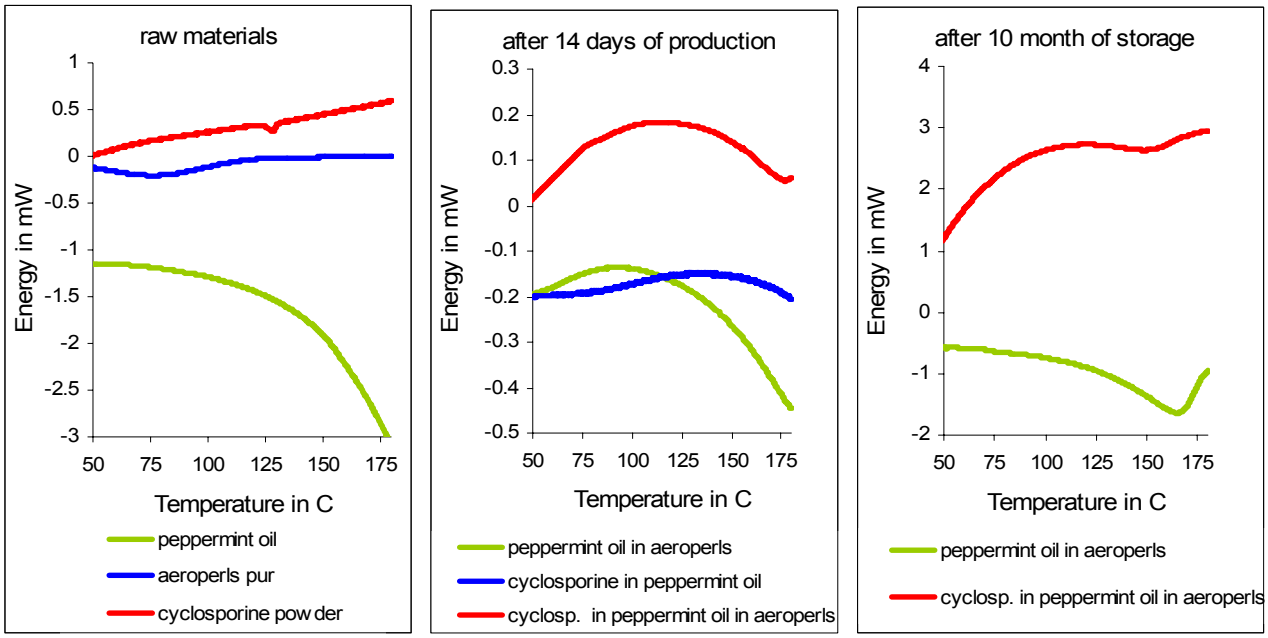


Figure 8-23: DSC analysis left: for the raw materials (cyclosporine powder, unloaded Aeroperls and peppermint oil) middle: for cyclosporine dissolved in peppermint oil, peppermint oil absorbed to Aeroperls and Cycloperls at day 14 and right: for Cycloperls and peppermint oil absorbed to Aeroperls after 10 months of storage

In addition also light microscopy was performed after 10 month of storage. Also from this analysis no crystals of cyclosporine could be detected (Figure 8-24)

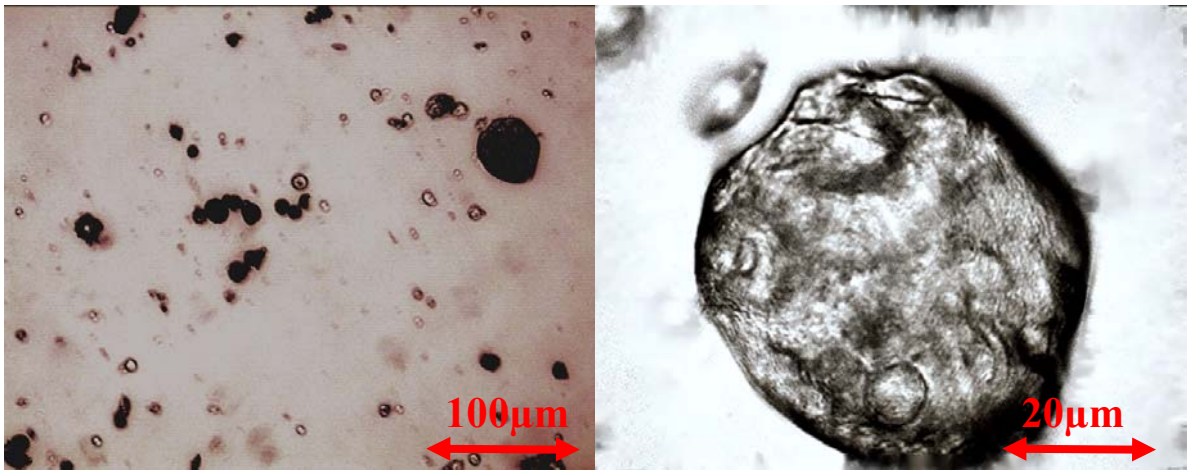


Figure 8-24: Microscopic images of Cycloperls after 10 months of storage, no crystals of cyclosporine are detected (magnification 160x left magnification 1250x right)

8.3.2 Conclusion

The formulation of cyclosporine dissolved in peppermint oil and absorbed onto Aeroperls® 300 yielded a physically stable solid dosage form over a time of at least ten months. It was proven that dissolved cyclosporine is completely absorbed from the Aeroperls and that the Aeroperls release the solution rapidly if liquid medium is added to them. The concentration of cyclosporine in the final formulation is 16.5w% (=165mg cyclosporine per gram). In comparison the microemulsion pre concentrate contains 100mg cyclosporine per ml pre-concentrate. Sandimmun Optoral® is also available in capsules with concentrations of 10, 25, 50 and 100mg cyclosporine. Therefore the concentration of cyclosporine within the Cycloperls represents a suitable concentration for the practical application. In conclusion, Cycloperls represent a solid, emulsifier free delivery system for cyclosporine with inhibitory effects for p-glycoprotein. The developed system is thought to be from interest for further investigations (e.g. in vitro and in vivo studies).
Additionally it could be shown again, that meaningful LD analysis can only be performed by a sound knowledge of the technology and the application of the optimised standards developed within this thesis.

8.4 Characterisation of Sandimmun Optoral® (neoral)

The most sold commercial product on the market containing cyclosporine as active is Sandimmun® Optoral. In countries different to Germany it is also sold under the trade name Sandimmun® Neoral. The aim of this thesis was to formulate the drug cyclosporine as an alternative dosage to the existing formulation. In order to judge the results the commercial product was analysed similar to the alternative systems developed here.

Sandimmun® Optoral is a microemulsion pre-concentrate. The final formulation is obtained upon the dilution of the system with water. For the characterisation by light microscopy the pre-concentrate was diluted with water (1:100) as suggested from the manufacturer. Light microscopy was started immediately after the preparation. The formulation was observed for a period of 1h. The images obtained are shown in Figure 8-26.

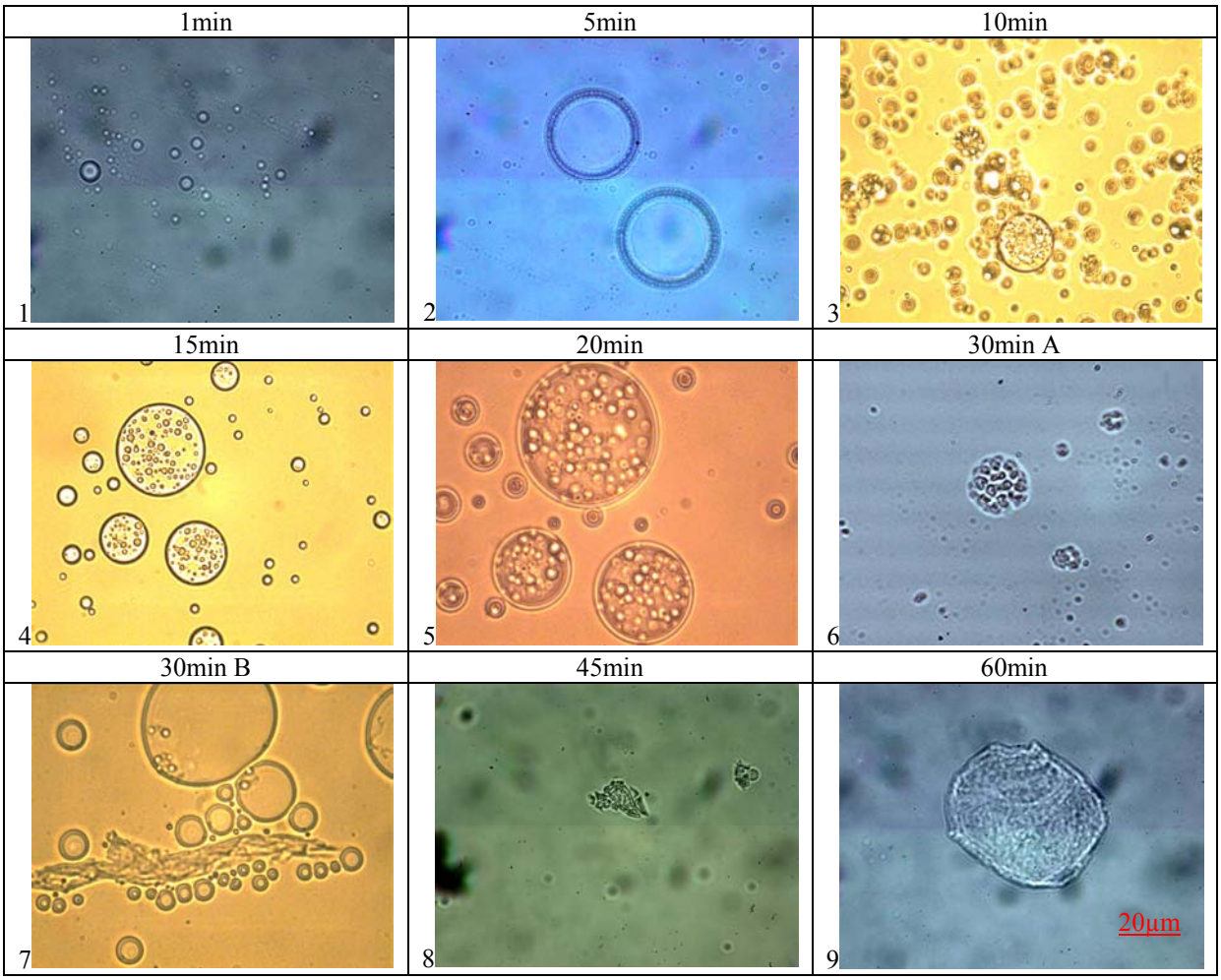


Figure 8-26: Microscopic images (magnification 1000x) of Sandimmun Optoral diluted with water (1:100) observed for 1 hour, pictures shown were taken after 5, 10, 15, 20, 30, 45 and 60 minutes.
Already in the freshly prepared formulation droplets larger 5µm can be detected (1min), the droplets increase and smaller droplets adhere to them (5min-20min). After 30min the large droplets seem to collapse (30min A) crystals of cyclosporine were observed first after 30min (30min B), the size and the amount of particles was further increased at 45 and 60min, the largest particle observed was about 45µm in size (60min)

The manufacturer (Novartis, Basel, Switzerland) suggests a particle size of the droplets being in the range of 70nm after dilution. Therefore it was expected that no particles would be observed from microscopy. In contrast already the freshly prepared formulation contained droplets larger than 5µm. The size of the droplets further increased with ongoing observation to a maximal size of 100µm (maximal size in the images shown is 50µm (image 5, 20min). It was observed that smaller droplets adhere to the larger droplets. After 30min the amount of large droplets with adhered small droplets decreased and smaller objects shown in image 6 (30min) occurred. It is thought that the large droplets collapsed and the smaller adhered droplets remained forming the object observed. At the same time crystals were detected. The amount of crystals and their size increased over the time of observation. The largest crystals observed had a particle size of 50µm and were detected after 60min. The crystals are thought to be cyclosporine precipitates. All other compounds* in this formulation are liquids and cannot precipitate. (*ethanol, corn oil, propylenglycole, macrogol glycerol hydroxyl stearat, α-tocopherol)

Laser diffractometry was used to analyse the particle size distribution. The measurements were performed with and without PIDS. The results were analysed with Mie theory (real refractive index: 1.465; imaginary refractive index: 0.01) and were calculated as volume and number distribution (Figure 8-27).

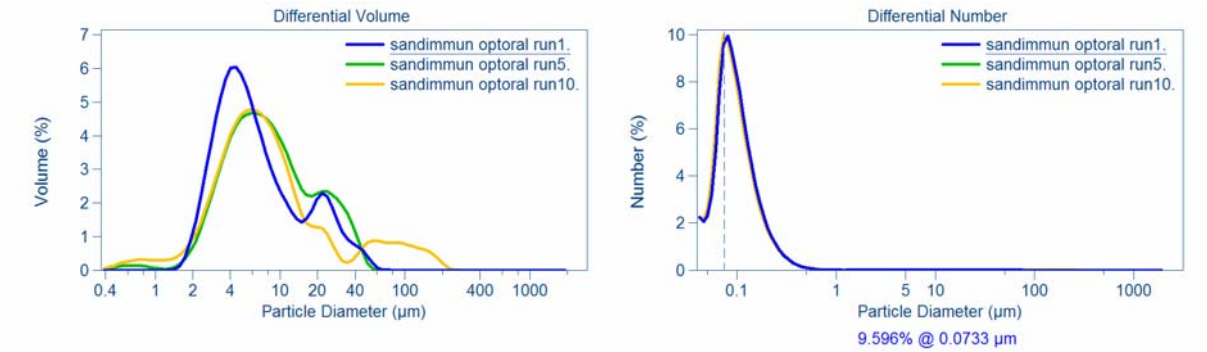


Figure 8-27: LD measurements of Sandimmun Optoral; left without PIDS as volume distribution; right with PIDS as number distribution

The volume distribution (left diagram) clearly shows that the droplet size increases over the time of the measurement. The sizes obtained are well in agreement with sizes obtained from light microscopy. The number distribution detects particles with a size of 75nm, which is well in agreement with the suggestions from the manufacturerer and with measurements by photon correlation spectroscopy (PCS). The z-average was 76.2nm for the formulation investigated, the polydispersity index was 0.247)

8.4.1 Comparison of Sandimmun with cyclosporine SEDDS

When Sanimmun Optoral was characterised it was shown that it does not only consists of nanodroplets with a droplet of 70nm. Because the results were worse than expected, Sandimmun Optoral was compared with the best SEDDS developed within this thesis. For microscopic analyses also the SEDDS was diluted with 100ml water, similar to the Sandimmun formulation. The images of the SEDDS (left) and from the Sandimmun Optoral formulation (right) obtained 10min after the preparation are shown in Figure 8-28.

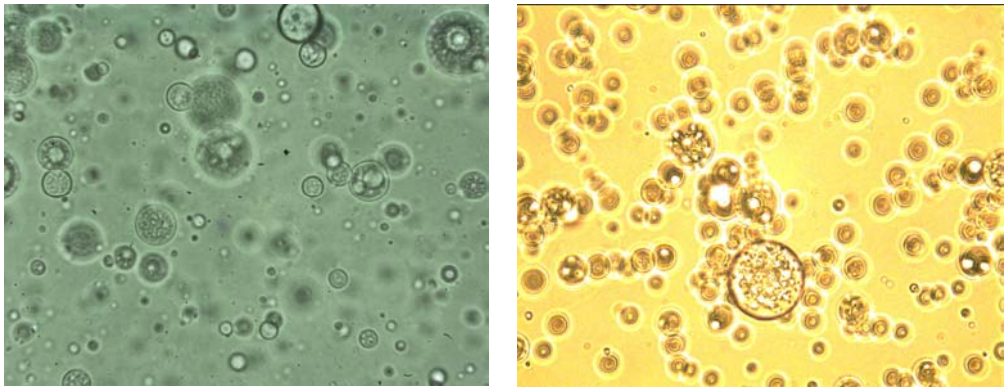


Figure 8-28: Microscopic images of SEDDS containing cyclosporine, peppermintoil and Tagat TO (left) and Sandimmun Optoral (right) taken 10 min after production (magnification 1000x)

The comparison of the images suggests that the SEDDS is more ore less similar to the Sandimmun Optoral formulation. The comparison of the LD data (Figure 8-29) suggest that the SEDDS consists of more large particles than the Sandimmun Optoral formulation, but in principle the distribution was the same (left diagram, volume distribution) From the number distribution (right diagram) there is evidence that also the SEDDS contains particles as small as 75nm.

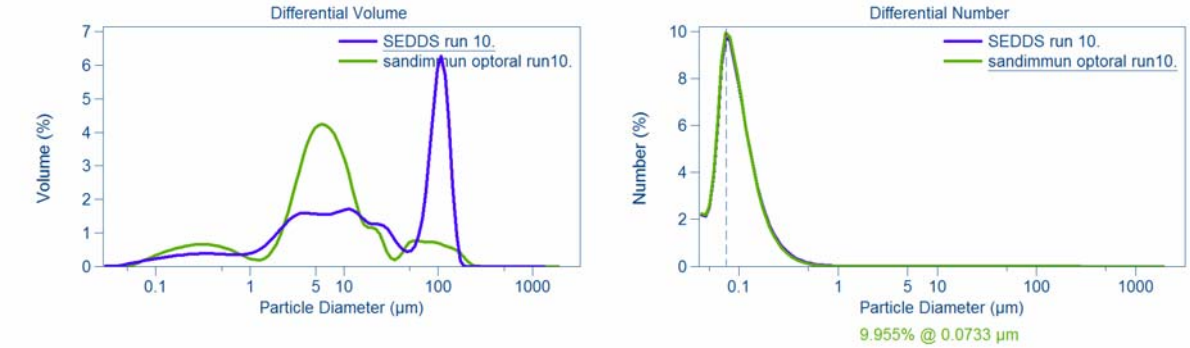


Figure 8-29: LD measurements of Sandimmun Optoral and SEDDS 5a; left without PIDS as volume distribution; right with PIDS as number distribution

PCS analysis did not show a similarity between the systems. The z-average of the SEDDS was calculated with 234.1nm whereas the z-average of the Sandimmun formulation was only 76.2nm.

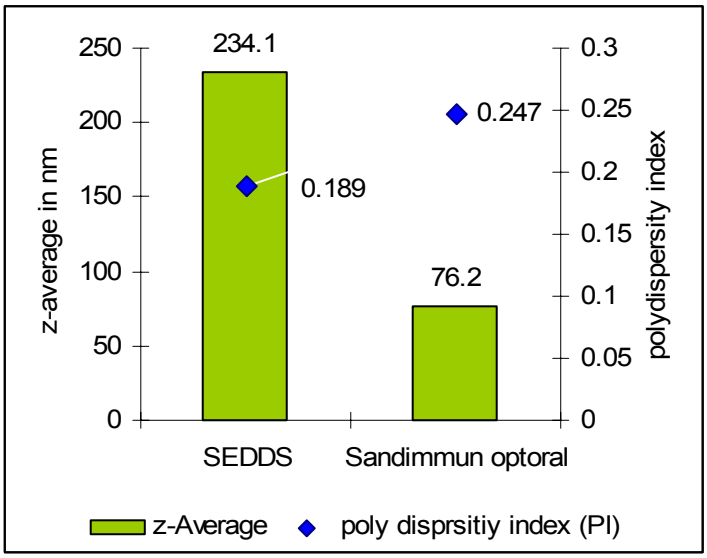


Figure 8-30: Comparison of PCS data from SEDDS and Sandimmun Optoral

8.4.2 Conclusion

The characterisation of the commercial product Sandimmun Optoral showed that it also contains larger droplets beside small droplets of only 75nm when it is diluted with water. It was shown that the formulation changes over time. The droplets increase in size and re-crystallisation of cyclosporine was observed. The variability of the bioavailability of the microemulsion is reduced when compared to the first Sandimmun, but still some variation is left. From the data obtained it can be assumed that the variations in bioavailability for Sandimmun Optoral can also be caused by the changes in particle size and due to precipitation of cyclosporine in the gastrointestinal tract. The changes of the formulation were time dependent. Therefore also the time between diluting the pre-concentrate with water and the administration can be considered to be important for the bioavailability.

The SEDDS developed in this thesis was compared with the commercial product. The PCS diameters showed that the commercial product is much smaller in comparison to the SEDDS. However LD analysis and light microscopy showed that the formulations are comparable in respect to larger particles. From this the developed SEDDS is judged to be worth for further investigations.