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Preparation of cyclosporine A nanoparticles by evaporative precipitation into aqueous solution

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Abstract

Amorphous nanoparticle suspensions of a poorly water-soluble drug, cyclosporine A, are produced by a new process, evaporative precipitation into aqueous solution (EPAS). The rapid evaporation of a heated organic solution of the drug, which is atomized into an aqueous solution, results in fast nucleation leading to nanoparticles suspensions. Hydrophilic stabilizers, introduced in the organic or aqueous phases, limit particle growth and inhibit crystallization for drug concentrations as high as 35 mg/ml, and drug/surfactant ratios up to 1.0. The suspensions may be used in parenteral formulations to enhance bioavailability or may be dried to produce oral dosage forms with the potential for high dissolution rates due to the low crystallinity, small particle size and hydrophilic stabilizer that enhances wetting.

Keywords: Nanoparticle; Poorly water-soluble drug; Drug suspension; Precipitation

1. Introduction

The dissolution rate of many poorly water-soluble drugs limits their bioavailability via absorption into the gastrointestinal tract. Dissolution rates may be increased by reducing the particle size to increase the interfacial surface area and by inhibiting crystallization to form amorphous particles. Coating drug particles with polymeric and low molar mass hydrophilic stabilizers to enhance wetting and solvation by intestinal fluids may increase the rates further. The simultaneous application of all of these strategies would be desirable in attempting to achieve high dissolution rates; however, few existing micronization or particle formation techniques are capable of achieving such a goal.

Widely used mechanical techniques based on high shear or impaction, including microfluidization and milling can be limited by unfavorable yields due to solid losses, high polydispersity in particle size, shear-induced particle denaturation, long processing time, high energy requirements

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and the need for separating the product and processing agent (Rubinstein and Gould, 1987; Byers and Peck, 1990; Aiache and Beyssac, 1994; Illig et al., 1996). To overcome many of these limitations, particles may be formed from solution in semi-continuous spray processes, for example spray drying. In these solution-based processes, it is often not possible to formulate a solvent that can dissolve both the poorly water-soluble drug and hydrophilic stabilizer. Even if such a solution may be formed, the stabilizer may not coat the surface of the drug particle as it forms during solvent evaporation, since the vapor surrounding the particle is highly hydrophobic (Masters, 1979; Broadhead et al., 1992). In addition, it is difficult to form submicron particles in the above mechanical processes and spray drying.

Two important techniques have been developed to form small particles with compressed liquid and supercritical carbon dioxide, rapid expansion from supercritical solution (RESS) and precipitation with a compressed fluid antisolvent (PCA), which is also referred to as the SAS or SEDS process (Alessi et al., 1996; Subramaniam et al., 1997; Ghaderi et al., 1999; Reverchon, 1999; Lengsfeld et al., 2000; Thiering et al., 2000; Young et al., 2000; Chattopadhyay and Gupta, 2001; Young and Johnston, in preparation). The low solubility of water in carbon dioxide complicates the use of hydrophilic substances in both of these processes. RESS is often limited by the low solubility of drugs in supercritical fluids even when cosolvents are utilized. Since water is not very soluble in CO_2, ethanol has been used to aid water dissolution in the PCA process by mixing three streams in a tri-axial nozzle (Palakodaty and York, 1999). The complicated nature of the resulting phase separation and particle formation can make it challenging to control the particle morphology

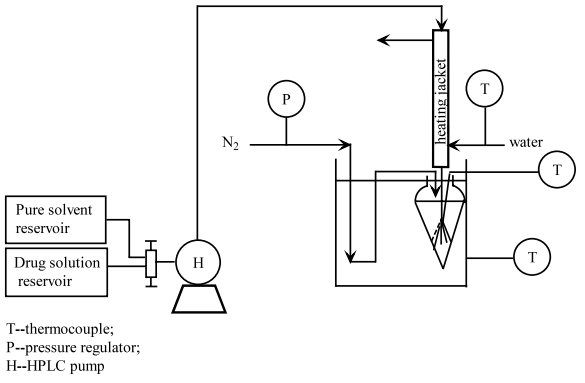


Fig. 1. Apparatus for EPAS.

The objective of this study is to develop a new process, evaporative precipitation into aqueous solution (EPAS), for forming submicron particles of a poorly water-soluble drug coated with hydrophilic stabilizers. As shown in Fig. 1, a drug dissolved in an organic solvent is sprayed through an atomizer into an aqueous solution containing a hydrophilic stabilizer to produce an aqueous dispersion. Intense atomization leads to rapid evaporation of the small organic droplets in the aqueous solution. The rapid evaporation produces large supersaturation of the drug. The resulting rapid nucleation of the drug has the potential to produce amorphous instead of crystalline particles. Hydrophilic stabilizers in water diffuse to the surface of the growing particles to inhibit particle growth and crystallization. The diffusion is facilitated by the intensity of the spray. The temperature may be chosen to optimize the evaporation rates of organic solvent and water, solubility of the organic solvent in water, while minimizing thermal decomposition of the drug. The EPAS suspensions may be dried by spray drying, lyophilization etc. The stabilization of the drug particles with water soluble stabilizers in the aqueous suspensions facilitates dissolution rates of the final powder after drying. Since the particle formation stage is distinct from the stage in which the aqueous solution is dried, EPAS has the potential to provide greater control over particle size and morphology than the above techniques. In this study, cyclosporine A, a water insoluble immunosuppresant for organ transplantation, was used as a model drug. The solvent, nature of the stabilizer(s), feed concentration of the drug, concentration of the stabilizer, temperature and drug-to-surfactant ratio were varied to control the particle size and morphology.

2. Materials and methods

2.1. Materials

Cyclosporine was obtained from North China Pharmaceutical Corporation and used without any further purification. L-α-phosphatidylcholine (Sigma), Myrj 52 (ICI Americas), Pluronic F127 (BASF), Polyvinylpyrrolidone (Sigma), Polyethylene glycol (Sigma), Brij 97 (Sigma) and Tween 80 (Aldrich) were used without further purification. The chemical structures of cyclosporine A and these surfactants are shown in Table 1. High performance liquid chromatography (HPLC) grade methanol was obtained from EM Science. Extra dry nitrogen was purchased from Matheson. Water was purified to Type I reagent grade by passing it through a Barnstead (NANOpure II) filtration system.

2.2. High performance liquid chromatography (HPLC) and gas chromatography (GC)

HPLC was used to measure the drug concentration in the aqueous suspension with a 250 mm long C-18 column (SGE ODS, 5 μm). The mobile phase was methanol and the detection wavelength was 210 nm. Gas chromatography (GC) was used to measure the residual concentration of the organic solvent in the aqueous suspension. It was equipped with a fused silica precolumn (i.d. = 0.53 mm, 0.5 m, methylated, Supelco) followed by a SPB-5 column (i.d. = 0.53 mm, 30 m, Supelco). To prepare the GC samples, 1 ml dimethylformamide was mixed with 1 ml EPAS suspension to dissolve the drug and then 1 μl of the internal standard 1-butanol was added into the solution. The temperature program was: 35 °C initial temperature for 1 min with a temperature ramp of 15 °C/min and final temperature 200 °C for 10 min. The injection volume was 1 μl, and FID detection was used. The retentions times were: dichloromethane (DCM), 0.68 min; 1-butanol, 1.36 min; DMF, 3.33 min.

2.3. Calculation of vapor–liquid–liquid equilibrium for the organic solvent-water system

For vapor–liquid–liquid equilibrium of an organic solvent and water binary system, there is only one degree of freedom. The equilibrium was calculated with the isothermal flash model at 1atm (ASPEN, Cambridge, MA), utilizing UNIQUAC to calculate the activity coefficients, in order to determine the solubility of the organic in the aqueous phase.

2.4. Particle size and particle size distribution

Particle size and particle size distribution were measured in aqueous suspensions by dynamic light scattering with a Brookhaven Zetaplus (Brookhaven Instruments Corporation, New York). To measure the particle size distribution, 0.5 ml suspension was diluted with distilled water to 8 ml before the measurement, and the results were based on the volume fraction distribution.

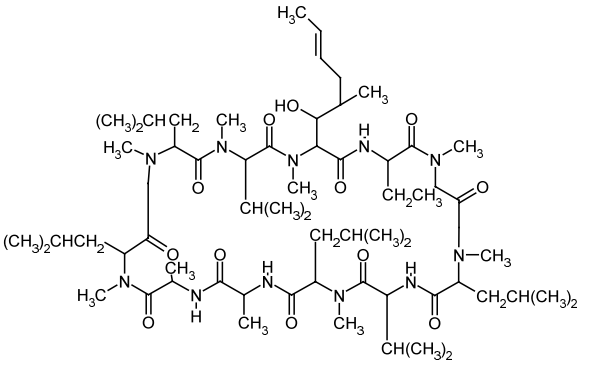
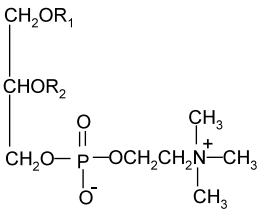
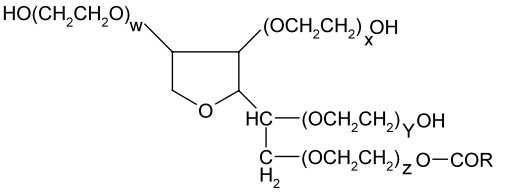
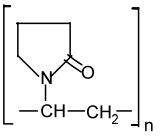
2.5. Particle crystallinity

The suspensions were flash frozen with liquid nitrogen and lyophilized to produce dry powder. The crystallinity of the dry powders was examined with a PW1720 X-ray generator (PHILIPS).

2.6. Preparation of phosphatidylcholine vesicles

Phosphatidylcholine was added into water to make a 10 wt.% solution. The solution was stirred and sonicated to break up any large clumps. Then it was passed 30 times through a high-pressure homogenizer (Avestin Emulsiflex C-5) at a shear pressure drop of 15000 psi to produce small unilamellar vesicles. The outlet of the homogenizer was submerged in an ice bath to keep the solution under 10 °C. At the end of the run, 0.1 M NaOH solution was added to adjust the pH between 7.5 and 8.0. Prior to the experiments, the phosphatidylcholine solution was filtered through a 0.2 μm filter.

Table 1
The chemical structures of drug and surfactants

Drug/surfactant	Chemical structure
Cyclosporine A	
L-α-phosphatidylcholine	 R_1, R_2 = Fatty Acid Residues
Tween 80	 W+X+Y+Z= 20 R=$CH_3(CH_2)_7CH{=}CH(CH_2)_7$
Polyvinylpyrrolidone	

2.7. Evaporative precipitation into aqueous solution (EPAS)

The EPAS apparatus is shown in Fig. 1. Either the drug solution or pure solvent could be fed to the HPLC pump. The organic cyclosporine A solution was fed through a 3 m long 1/16 in. o.d. × 0.030 in. i.d. stainless steel coiled tube con-

tained within a 1–1/2″ OD × 24″ long plastic water jacket (Alltech). Water was circulated through the jacket with a JULABO MP temperature controller. The organic solution was atomized through a crimped nozzle into hot water. The nozzle was made from a 10 in. long, 1/16 in. o.d. × 0.030 in. i.d. stainless steel tube. The tube was cut with a wire cutter to produce a crimped thin slit orifice. The tapered section of the tube was only 0.5 mm long. The tip was filed until the desired flow rate was achieved for a given pressure drop (Young et al., 2000; Young and Johnston, in preparation). The flow rate of the solution was checked by spraying drug solution in pure methanol, which would dissolve the drug completely, and the sample was analyzed by HPLC.

The aqueous stabilizing solution was contained in a 125 ml separatory funnel that was submerged in a temperature-controlled water bath. The nozzle was submerged approximately 2 cm under the surface of the aqueous solution. As shown in Fig. 2, the turbulence of the spray mixed the precipitated drug and the surfactant solution vigorously. To suppress and drain the foam produced by the organic vapor, nitrogen was blown downwards on top of the foam at 20 psi into the funnel through three 1/16 in. o.d. × 0.030 in. i.d. stainless steel tubes. A thermocouple was submerged next to the nozzle in the stabilizing aqueous solution to measure the actual temperature of the aqueous solution. About a 6 °C temperature drop resulted from the evaporation of organic solution at the thermocouple tip. Unless indicated otherwise, the stabilizer was added in the aqueous phase and was not present in the organic feed solution. After spraying for a required time to produce the desired drug/surfactant ratio, the suspension was recovered and analyzed within 1 h to determine the particle size by DLS. The suspensions were flash frozen in liquid nitrogen and lyophilized into dry powders for characterization by X-ray diffraction.

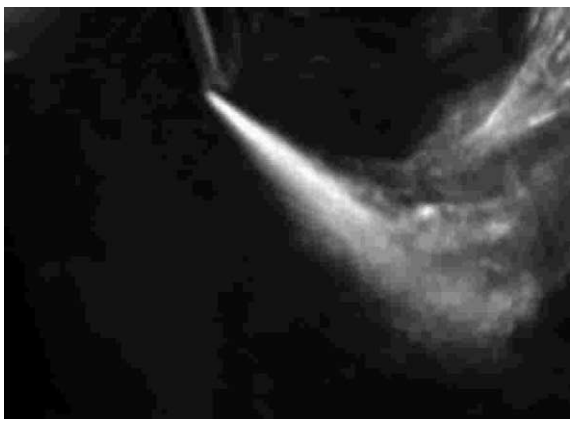


Fig. 2. Spray of 1% w/v cyclosporine A in DCM into aqueous solution in the EPAS process for a flow rate of 1 ml/min.

After the spray, the stabilizing solution was replaced by pure water and feed was switched to pure solvent to flush the remaining drug out of the tube. Without this pure solvent flush, the drug would precipitate and plug up the nozzle.

3. Results and discussion

3.1. Particle sizes with phosphatidylcholine in the aqueous solution

The organic solutions were sprayed into small unilamellar vesicles composed of phosphatidylcholine. All EPAS experiments in this section were performed with a surfactant concentration of 10% (w/w), a temperature of 75 °C for the aqueous solution and a preheater and a flow rate of 1 ml/min. The final concentration of drug in suspension was measured by HPLC and used to determine the drug/surfactant ratio. The yield was determined by comparing the measured drug concentration and the amount sprayed into the aqueous solution. The experiments with DCM were unsuccessful as this solvent modified the vesicles such that the phosphatidylcholine phase separated from the water during the spray.

The initial size of the SUVs produced during the homogenization process was only 48.7 nm. As shown in Table 2, when pure diethyl ether was sprayed into the surfactant solution without drug, the vesicle size increased modestly to 130 nm, with 65% by volume of the vesicles still smaller than 65 nm. This result indicates that the vesicles are still intact after exposure to the diethyl ether and would be available to stabilize a drug. Upon

Table 2
EPAS results for 10% (w/w) phosphatidylcholine in the aqueous solution

$C_{soln.}$ (% w/v)	D_{avg} (nm)	Particle size distribution (nm)	$C_{aq.}$ (mg/ml)	Yield (%)	Drug/surfactants ratio (w/w)
0	131	53–66 (65%); 225–323 (35%)	0	/	0
1	253	134–160 (48%); 324–386 (52%)	14.4	96	0.14
2	526	120–212 (13%); 375–663 (87%)	30.8	87	0.30
5	477	217–253 (20%); 507–615 (80%)	37.1	100	0.37
5	408	96–166 (19%); 287–496 (79%)	34.6	98	0.35
5	446	53–73 (44%); 188–414 (39%); 1252–2357 (17%)	31.8	90	0.32
36.0[a]	460	123–642 (97%)	35.2	83	0.35
36.0[a]	466	267–327 (61%); 653–801 (39%)	34.9	79	0.35

[a] Phosphatidylcholine in organic solution.

spraying drug solution into the vesicles, stable turbid suspensions were formed. The mean size of the drug particles increased with the concentration of drug in the aqueous suspension C_{aq}, which was a function of the drug concentration in the organic feed C_{soln}, and the spray time. As C_{aq} increased from 14 mg/ml to values over 30, a significant increase in particle size was observed. At a drug concentration of ~ 14 mg/ml, the dispersed particles had a mean size of 253 nm with a size range of 134–386 nm. At a concentration of ~ 35 mg/ml, the mean size increased to 526 nm with a very broad size distribution, including some particles larger than 1 μm. The particle size did not change significantly with a change in C_{soln} from 2 to 5%, as long as C_{aq} was constant. The three experiments with a drug concentration of 5% in diethyl ether showed that the experimental reproducibility in particle size was 7.5% and in yield was 4.2%. Several factors may contribute to this particle growth with an increase in C_{aq}. The collision frequency increases, which may increase aggregation. As the drug/surfactant ratio increases during the spray, surfactant is transferred from the empty vesicles to the surface of the drug particles. Fewer empty vesicles are available to stabilize the drug particles and prevent growth. Also, the coverage of the drug by surfactant is lower when the drug/surfactant ratio increases, leading to less effective stabilization against aggregation. Finally, the longer the spray, the longer the time for aggregation. Shear induced collisions during the spray and an increase in exposure time to organic solvent residues could lead to particle growth. However, this effect of shear was not observed as C_{soln} was increased from 2 to 5%, as this lowered the spray time from 18 to 7 min for a constant C_{aq} of 35 mg/ml.

To further explore the mechanism of surfactant stabilization, phosphatidylcholine was dissolved in diethyl ether with cyclosporine and sprayed into pure water, as shown in the last two entries in Table 2. Despite the lack of vesicles in the aqueous solution at the start of the process, the mean size and size distribution of the drug particles were similar to the case above where vesicles were already present in the water at the start of the spray. This comparison may be made in Table 2 at a nearly constant drug to surfactant ratio of 0.35. It is possible that the high shear in the spray produced vesicles. The particle sizes at the low end of the distribution were not as small as in the case where the vesicles were made in water prior to the EPAS spray. Thus, the vesicles formed during the EPAS spray appeared to be larger than those formed by homogenization. Regardless of the size of the vesicles, the surfactant was available to coat and stabilize the growing drug particles.

Heavy foam was formed in the aqueous solution when the organic drug-surfactant solution was sprayed into water. The shear from the evaporating organic jet created vapor bubbles that were stabilized by the phosphatidylcholine. In the earlier experiments where the phosphatidylcholine was present only in the aqueous solution, much less foam was produced. In this case the surfactant did not diffuse as quickly to the organic vapor–water interface to stabilize the foam. Despite the large degree of foam generation, the foam did not leave the top of the vessel containing the aqueous solution due to the downward flow of nitrogen.

3.2. Effect of solvents on particle size

Two solvents were compared in EPAS experiments, diethyl ether and DCM. They have similar boiling points, vapor pressures and heats of vaporization (Carl, 1999). At a temperature of 75 °C, the liquid phase concentration of DCM in water is 0.004 g/ml for the equilibrium flash calculation described above. The corresponding value for diethyl ether is 0.012 g/ml due to a lower value of the activity coefficient resulting from polar interactions and hydrogen bonding with water. In each spray, the solvent evaporated, and a layer of organic solvent was not observed in the precipitation vessel. As shown in Table 3, the size of drug particles was much smaller when the organic solvent was DCM versus diethyl ether for Pluronic F127 ($HO(CH_2CH_2O)_{98}(CH_2C(CH_3)HO)_{67}(CH_2CH_2O)_{98}H$, HLB = 18–23) as a stabilizer. Additional experiments were performed by spraying pure diethyl ether at 75 °C into a stable suspension formed by EPAS with DCM, which had an average particle size of 423 nm. After spraying diethyl ether for 10 min at 1 ml/min, large particles several microns in diameter formed and settled. This experiment indicates that the large particles formed with diethyl ether are influenced by the stability of the aqueous suspensions, and not just the particle formation stage. Given that the two solvents have similar volatilities and heats of vaporization, it is likely that the difference in size is related to the much different solubility of the solvents in water and the influence of the solvent on Ostwald ripening and/or steric interactions between surfactant coated drug particles.

Table 3
Solvent effect on the particle size for 1% w/v Pluronic F127 in the aqueous phase for a drug/surfactant ratio: 0.93–1.07

Solvent	D_{avg} (nm)	Size distribution (nm)	Yield (%)
Diethyl ether	1218	316–1000 (91%)	86
DCM	423	358–424 (96%); 909–990 (4%)	96
DCM	499	263–303 (38%); 576–689 (62%)	93

3.3. Effect of surfactant type and feeding drug concentration on particle size

In addition to phosphatidylcholine, a variety of other surfactants and polymers were used in the aqueous solution to stabilize the drug particles, as shown in Table 4. All of the experiments were done with preheater and aqueous solution temperatures of 75 °C, a flow rate of 1 ml/min, DCM as the solvent, a drug concentration of 1% (w/v), and a surfactant concentration of 1% (w/v). In these experiments, concentrations of cyclosporine were significantly higher than typical solubility levels in micelles composed of these surfactants (Young et al., 2000; Young and Johnston, in preparation). Unlike the case for the other surfactants, DCM/water emulsions were formed with Brij97 ($C_{18}H_{35}(OCH_2CH_2)_nOH$, $n = 1–10$, HLB = 12.4) due to its low HLB value. Much of the drug was lost to the emulsion droplets which settled, leaving an aqueous suspension with unusually low turbidity. For a feed concentration of 5%, the results were similar for the Tween 80 (HLB = 15.0) and Myrj 52 ($CH_3(CH_2)_{16}(OCH_2CH_2)_{40}OH$, HLB = 16.9) nonionic ethoxylated surfactants, which had similar HLB values. However, the particle sizes were much larger for the homopolymer stabilizers, which had much higher molecular weights. These particles may have had more time

Table 4
Effect of surfactant type on particle size for a drug/surfactant ratio: 0.3–0.5

Aqueous excipients	C_{soln} (% w/v)	D_{avg} (nm)	Size distribution (nm)	Yield (%)
1% Brij 97[a]	1	1177	664–1038 (90%); 3962–5663 (10%)	87
1% Myrj 52	1	625	512–738 (100%)	86
1% Myrj 52	5	339	324–349 (100%)	93
1% Tween 80	1	532	151–191 (19%); 529–713 (81%)	91
1% Tween 80	5	338	78–571 (100%)	96
1% PEG 8000	5	1365	1343–1387 (100%)	88
1% PEG 18 500	5	1018	77–437 (45%); 876–2091 (55%)	95
1% PVP 40 000	1	1076	909–1374 (100%)	95
1% PVP 40 000	5	595	577–741 (100%)	89
1% PVP K-15	5	1115	932–1222 (100%)	98

[a] Suspension concentration: 10 mg/ml water and drug/surfactant ratio = 1.0.

Table 5
Effect of surfactant concentration on the particle size for a drug/surfactant ratio: 0.3–0.4

Aqueous excipients	D_{avg} (nm)	Size distribution (nm)	Suspension conc. (mg/ml)	Yield (%)
1% PVP 40 000	1076	909–1374 (100%)	3.3	95
2% PVP 40 000	861	719–1136 (100%)	6.8	94
1% Tween 80	559	492–644 (100%)	3.7	101
2% Tween 80	361	226–435 (100%)	6.6	103
5% Tween 80	122	33–47 (23%); 124–177 (77%)	20.0	97

to grow due to slower diffusion of stabilizer to the particle surface. However, the steric stabilization provided by the high molecular weight of the adsorbed PVP molecules led to suspensions that were stable overnight. The suspensions for the other surfactants were only stable for 1–2 h.

As shown in Table 4, increasing the feed drug concentration to 5% (w/v) decreased the particle size even though the suspension concentration C_{aq} and drug/excipient ratios were approximately the same for Myrj 52, Tween 80, and PVP. With an increase in the feed drug concentration, C_{soln}, the supersaturation increases during evaporation, leading to smaller particles, if the steric stabilization is sufficient. Furthermore, the shorter spray time for the higher C_{soln} may produce less shear induced aggregation. For a given homopolymer, steric stabilization increased with molecular weight, as is evident in comparing particle sizes for PVP and PEG.

3.4. Effect of surfactant concentration in the aqueous solution

All of the experiments in Table 5 were conducted under the same conditions in Table 4 unless otherwise denoted. The drug to surfactant ratio was chosen to be approximately 0.35 at the end of each spray. As the surfactant concentration was doubled, the particle size decreased, even though the drug concentration in the suspension increased. Early in the sprays, the drug to surfactant ratio was higher in the case where the surfactant concentration was higher. This difference may be expected to lead to smaller particles, if the particles are well stabilized. It would not be expected to occur if the particles were not well stabilized, due to enhanced particle collisions with higher suspension concentrations. Thus surfactant concentration may have a large effect on the particle formation mechanism.

3.5. Effect of temperature on particle size

The temperature affects the equilibrium concentration of the organic solvent in water markedly. For example, the solubility of DCM in water without surfactant in vapor–liquid–liquid equilibrium is 0.0081 g/ml at a temperature of 55 °C but only 0.0023 g/ml at 85.0 °C. As shown in Table 6, the particle size increased with temperature for Tween 80 as a stabilizer, but decreased with temperature for PVP 40000. The other experimental conditions were the same as in Table 4. As temperature increases, more rapid evaporation of the organic solvent enhances nucleation favoring smaller particles. Higher temperature also increases the rate of diffusion of the PVP. These two factors may explain the decrease in particle size for the PVP data. For Tween 80 other factors were dominant. The hydrogen bonding between the EO group of Tween 80 and water is well known to become much weaker over this temperature range. In fact, this reduction of hydrogen bonding often leads to precipitation of EO based surfactants in water (Blankschtein et al., 1986). The steric stabilization from the EO tails in Tween 80 becomes weaker with this loss in hydration, and this may lead to particle growth, as observed experimentally.

3.6. Effect of drug loading on particle size

The drug loading, as reflected in the drug/surfactant ratio, increased linearly with the spray time. As shown in Table 7, with an increase in drug loading, and consequently, the concentration of the suspension, the mean particle size and

Table 6
Temperature effect on the particle size for a drug/surfactant ratio: 0.3–0.4

Surfactants	1% Tween 80		1% PVP 40 000	
T (°C)	D_{avg} (nm)	Size distribution (nm)	D_{avg} (nm)	Size distribution (nm)
55	308	37–68 (68%); 168–308 (6%); 765–1632 (26%)	1354	577–741 (38%); 1570–2017 (62%)
65	438	204–233 (3%); 400–473 (97%)	1144	503–563 (11%); 1149–1335 (89%)
75	559	492–644 (100%)	1076	909–1374 (100%)
85	774	622–925 (100%)	803	594–938 (100%)

Table 7
Effect of drug/excipient ratio (drug loading) on particle size for 1% Tween 80 in the aqueous solution

Feeding drug concentration (% w/v)	Drug/excipient ratio	Suspension concentration (mg/ml)	D_{avg} (nm)	Size distribution (nm)
1[a]	0.37	3.7	559	492–644 (100%)
1[a]	1.16	11.6	518	211–409 (76%); 695–1180 (24%)
5[a]	0.33	3.3	338	78–571 (100%)
5[a]	0.72	7.2	523	168–301 (89%); 2687–3598 (11%)
5[b]	2.50	25.0	921	563–691 (80%); 1740–2249 (20%)

[a] $Q = 1$ ml/min.
[b] $Q = 2.5$ ml/min.

Table 8
Concentration of residual organic solvent DCM in the aqueous suspension for a flow rate of 1 ml/min

Aqueous excipients	*t* (min)	Residual DCM concentration (g/ml)
1% PVP 40 000	10	0.0031
1% PVP 40 000	20	0.0029
1% PVP 40 000	30	0.0035
1% Pluronic F127	10	0.0041
1% Pluronic F127[a]	10	0.0044
1% Pluronic F127[b]	10	0.0021

[a] Pressure drop = 4000 psi.
[b] Temperature = 85 °C.

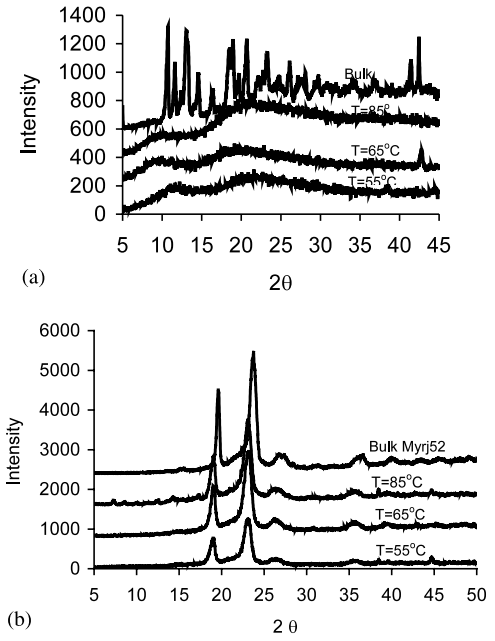


Fig. 3. (a) The X-ray diffraction pattern of cyclosporine A + PVP40, 000 powder (drug/excipient ratio = 0.4). (b) The X-ray diffraction pattern of cyclosporine A + Myrj52 powder (drug/excipient ratio = 0.4).

polydispersity increased. When the drug to surfactant was above 0.7, very large particles were formed, some as large as 3.6 μm. As the drug/surfactant ratio and the drug concentration in the suspension increased, less surfactant was available to stabilize the growing drug particles. Also, the coverage of surfactant on the drug particles decreased providing less stability to flocculation and agglomeration. Both of these reasons may contribute to the larger particle size at high drug loading.

3.7. Residual solvent concentration

The residual solvent concentration was measured by GC (HP 5890A). As shown in Table 8, the residual concentration of DCM did not change significantly with spray time, but did change slightly with surfactant type. In Pluronic F127 aqueous solution, some DCM will be dissolved in the micelles. This factor may contribute to higher residual concentration than for PVP, which does not form micelles. The residual concentrations of DCM in the suspension at 75 °C are slightly smaller than the equilibrium value for the binary system, which is 0.0039 g/ml. When the temperature increased from 75 to 85 °C, the residual solvent decreased from 0.0041 to 0.0021 g/ml, consistent with the decrease in solubility (binary system) from 0.0039 to 0.0023 g/ml. In a single experiment a new nozzle was formed that required a higher pressure drop of 4000 psi for the same flow rate to produce more intense atomization. However, the residual solvent was not changed. DCM is a class 2 solvent with a permitted daily exposure of 6 mg/day (Federal Register, 1997).

3.8. X-ray study

The drug particle suspensions were flash frozen with liquid nitrogen and lyophilized to dry powder. The X-ray diffraction patterns of several samples are shown in Fig. 3(a and b). Originally, cyclosporine A was crystalline with sharp peaks in the diffraction pattern. After the EPAS process, these sharp peaks disappeared indicating a large loss in crystallinity. The crystalline peaks of the samples with Myrj 52 come from the bulk Myrj 52 and not the drug. The large loss in crystallinity may be expected to enhance the bioavailability of this water-insoluble drug.

4. Conclusions

The rapid evaporation of the heated organic solution in EPAS results in fast nucleation leading to amorphous nanoparticle suspensions. A variety of hydrophilic stabilizers were found to diffuse to the surface of the growing particles rapidly enough to prevent growth of the nanoparticles. Nanoparticle suspensions with low drug crystallinity were formed for cyclosporine A with L-α-phosphatidylcholine vesicles, low molecular weight ethoxylated nonionic surfactants, and high molecular weight homopolymers. The drug concentrations were as high as 35 mg/ml, and drug/surfactant ratios reached unity. Both of these values are far in excess of the values obtained for solvation of this drug in micelles or vesicle bilayers (Young et al., 2000; Young and Johnston, in preparation). For the two solvents studied, diethyl ether and DCM, the difference in particle size is related to Ostwald ripening and/or steric interactions between surfactant coated drug particles, and in the case of L-α-phosphatidylcholine, to the effect of solvent on the vesicle stability. The particle sizes were larger for the high molecular weight homopolymer stabilizers versus the low molecular weight ethyoxylated surfactants. It is likely that the slower diffusion for the former allowed more time for particle growth. For a given drug/surfactant ratio, particle size decreased with increasing surfactant concentration, despite the increase in the concentration of the suspension. An increase in temperature speeds up evaporation and nucleation leading to smaller particles, except in the cases where it is detrimental to steric stabilization, as was found for ethoxylated surfactants.

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