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Phospholipid-Stabilized Nanoparticles of Cyclosporine A by Rapid Expansion from Supercritical to Aqueous Solution

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Timothy J. Young,[1,2] Keith P. Johnston,[1] Gary W. Pace,[3] and Awadhesh K. Mishra[3]

[1]Department of Chemical Engineering, University of Texas, Austin, TX
[2]Present address: Dow Chemical Company, Midland, MI
[3]RTP Pharma Inc, Quebec, Canada H3E 1A8

ABSTRACT

The purpose of this research was to form stable suspensions of submicron particles of cyclosporine A, a water-insoluble drug, by rapid expansion from supercritical to aqueous solution (RESAS). A solution of cyclosporine A in CO_2 was expanded into an aqueous solution containing phospholipid vesicles mixed with nonionic surfactants to provide stabilization against particle growth resulting from collisions in the expanding jet. The products were evaluated by measuring drug loading with high performance liquid chromatography (HPLC), particle sizing by dynamic light scattering (DLS), and particle morphology by transmission electron microscopy (TEM) and x-ray diffraction. The ability of the surfactant molecules to orient at the surface of the particles and provide steric stabilization could be manipulated by changing process variables including temperature and suspension concentration. Suspensions with high payloads (up to 54 mg/mL) could be achieved with a mean diameter of 500 nm and particle size distribution ranging from 40 to 920 nm. This size range is several hundred nanometers smaller than that produced by RESAS for particles stabilized by Tween 80 alone. The high drug payloads (~10 times greater than the equilibrium solubility), the small particle sizes, and the long-term stability make this process attractive for development.

KEYWORDS: supercritical fluid, carbon dioxide, rapid expansion, water-insoluble

Corresponding Author: Keith P. Johnston, Department of Chemical Engineering, University of Texas, Austin, TX 78712-1062; Tel: 512-445-4948; Fax: 512-471-7060; Email: kpj@che.utexas.edu

INTRODUCTION

The often low bioavailability of water-insoluble drugs leads to poor pharmacokinetic performance in the body. Techniques to improve bioavailability in oral or parenteral applications of water-insoluble drugs include powder micronization, the formation of micron- and submicron-size dispersions, solubility enhancement in aqueous solution by addition of appropriate surfactants, organic solvents or buffers, or drug-carrier systems such as liposomes. The payloads for drugs in liposomes are often limited because of the low volume fraction of hydrophobic regions.[1] The use of surfactants or organic solvents in parenteral administration can lead to phlebitis, anaphylaxis, hypotension, or even vasodilation.[1] Traditional micronization techniques such as spray-drying,[2,3] emulsion-solvent extraction,[4-6] and processes based on high shear (eg, microfluidization, high-pressure homogenization, ball milling, air jet milling)[7-11] can have certain drawbacks. With many of these techniques, particle size distributions tend to be broad, products can be denatured by exposure to high temperatures or organic solvents, residual solvent concentrations can be high without lengthy periods for additional extraction/evaporation, or undesirable processing agents need to be separated from the final products. Yields can be well below 100% due to losses during solids handling in milling and spray drying. Milling techniques also require cumbersome solids handling. Hence, processing techniques that do not rely on organic solvents or high temperatures and that can provide small particles with narrow distributions are highly desirable.

Dissolution rates of poorly-water soluble drugs may be increased by reducing the particle size to increase the surface area and by inhibiting crystallization to form amorphous particles. Both of these factors may be achieved by phase separation techniques that include rapid nucleation rates and prevention of particle growth. The process rapid expansion from supercritical solution (RESS) may be used to accomplish these goals according to theoretical models of nucleation. The expansion from the supercritical state to atmospheric pressure reduces the solvent density (or strength) and initiates intense nucleation.[12-22] The particle formation steps

include nucleation, condensation of solute molecules about the nucleii, and coagulation of particles in the free jet expansion. Recent work by Charoenchaitrakool et al has produced 2.5-µm particles by RESS with enhanced dissolution rates for the poorly water-soluble compound ibuprofen, likely due to the reduction in both particle size and crystallinity.[23] If coagulation can be minimized, it should be possible to produce 20- to 50-nm drug particles.[24] Similar findings have been noted by M. Weber and M. Thies (letter, March, 2001).

A novel process, rapid expansion from supercritical to aqueous solution (RESAS), has been developed to reduce the coagulation rate in the free jet expansion of RESS.[25] The supercritical solution is expanded through an orifice or tapered nozzle into an aqueous solution containing a stabilizer to minimize particle aggregation during free jet expansion. Previously, Young et al demonstrated the ability for Tween 80, a nonionic polysorbitan ester, to stabilize 400 to 700 nm cyclosporine particles produced by RESAS.[25] CO_2 was chosen as the supercritical fluid of interest as it is an environmentally benign solvent that is nonflammable, inexpensive, and essentially nontoxic. It also has relatively mild critical conditions, critical temperature (Tc) = 31°C, critical pressure (Pc) = 73.8 bar, and so allows processing at moderate temperatures to prevent thermal degradation. Sun et al have demonstrated a technique to form PbS nanoparticles by expanding a supercritical fluid containing one reactant, $Pb(NO_3)_2$, into a liquid solution containing a second reactant, Na_2S.[26]

In this study, the emphasis is on phospholipids to minimize coagulation in RESAS. Phospholipids are amphiphilic molecules, usually consisting of 2 lipophilic tails, which, when added to water, rapidly aggregate and form liposomes. Depending on the chemical compositions of the phospholipids, the concentration, and the method of formation, a variety of sizes and structures can be formed, such as 1- to 10-µm multilamellar vesicles (MLVs), 0.1- to 1.0-µm large unilamellar vesicles (LUVs), and <0.1-µm small unilamellar vesicles (SUVs).[27,28] In this study, the focus is on SUVs. High drug:lipid ratios with particle sizes <1.0 µm have been achieved by micronizing drugs while in the presence of phospholipid stabilizers.[1] It is suggested that in these systems the drug partitions into the bilayers of the SUVs employed, and colloidal drug particles are stabilized by a monolayer of phospholipid and either successive bilayers or small loosely associated vesicles.

The goal of this study was to use phospholipid vesicle solutions to stabilize nanoparticle aqueous dispersions of cyclosporine, a water-insoluble immunosuppressant, with substantial payloads (eg, above 20 mg/mL) by RESAS. To place these experiments in perspective, new RESAS results are presented for stabilization by a series of micelle-forming surfactants (without phospholipids). The phospholipids were mixed with small amounts of micelle-forming surfactants to enhance fluidity of the surfactant bilayers. The results were compared for aqueous solutions stabilized with vesicles with only micelle-forming surfactants to provide mechanistic insight into the stabilization processes. The effects of several variables, such as drug concentration in the suspension, stabilizing solution temperature, preheater temperature, and flow rate, were examined.

MATERIALS AND METHODS

Cyclosporine was obtained from North China Pharmaceutical Corporation (Shijiazhuang, China) and used without any further purification. Lipoid E80 (Vernon Walden, Madison, NJ), Phospholipon 100H (American Lecithin, Oxford, CT), Myrj 52 (ICI Americas, Wilmington, DE), Pluronic F127 (BASF, Ludwigshafen, Germany), methanol (high performance liquid chromatography [HPLC] grade, EM Science, Gibbstown, NJ), and Tween 80 (Aldrich, Milwaukee, WI) were used without further purification. Mannitol, sodium lauryl sulfate, and sodium deoxycholate were purchased from Sigma (St Louis, MO) and used without further purification. Water was purified to Type I reagent grade by passing it through a Barnstead (NANOpure II) filtration system (Barnstead International, Dubuque, IA). Instrument grade CO_2 (Matheson, Albuquerque, NM) was used for all of the experiments. The structures of the various surfactants used in this study are shown in Figure 1.

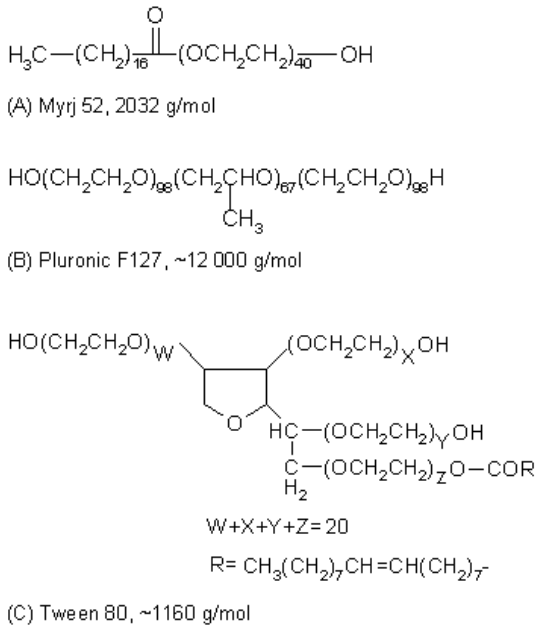

Figure 1. Structures of the surfactants used in this study: (A) Myrj 52, 2032 g/mol, (B) Pluronic F127, ~12 000 g/mol, and (C) Tween 80, ~1160 g/mol.

HPLC combined with UV detection was used to quantify the drug concentration from the aqueous suspensions. The column used for HPLC was a 250-mm long C-18 column (SGE ODS, 5 μm). Samples were prepared by withdrawing 0.5 mL of the suspension and adding to 5 mL of methanol, of which 9.6 μL was actually injected onto the column. The mobile phase consisted of pure methanol, and the detection wavelength was 210 nm.

The solubility of cyclosporine in the surfactant solutions was determined from a saturated solution at 25°C. Excess drug was added to 10 mL of each surfactant solution and allowed to equilibrate with stirring for 1 week at 25°C. The dissolved drug content was determined by the above-mentioned HPLC method by analyzing a filtrate of each saturated solution.

The intensity-weighted particle size distribution was determined by Dynamic Light Scattering (DLS) via a Brookhaven Zetaplus (Brookhaven Instruments, Holtsville, NY). Particle size measurements were made within 24 hours of preparation and at 1-month time intervals. Multimodal size distributions were determined by a nonnegative least squares method, and the mean diameters and size ranges were reported.

X-ray diffraction data was taken with a PW1720 x-ray generator (Philips Electronic Instruments, Inc, Mahwah, NJ). Suspensions examined were frozen and lyophilized, and the dry powder remaining was examined. Samples containing liquid surfactants, such as Tween 80, could not be examined by this technique, only those forming a dry powder.

Differential scanning calorimetry (DSC) was used to determine the phase transitions of the phospholipids. A TA Instruments (New Castle, DE) DSC 2920 calorimeter was used. Typically, 8 to 15 mg of sample was loaded into the pan. A heating rate of 1°C/min was used to scan over the temperature range desired.

Confirmation of vesicle and particle size and structure was determined by transmission electron microscopy using a Philips EM208 microscope (Philips). Diluted samples were dropped onto copper grids. The grid was allowed to stand for 5 minutes before the negative stain was dropped onto the grid. The stain used was a 1.0% (wt/wt) solution of uranyl acetate, which had been brought to a neutral pH to prevent damage to vesicle structure.[29] After staining for 5 minutes, the grid was washed lightly (3 drops) with pure water and then the excess water was removed by touching the edge of the grid to an absorbent cloth.

Preparation of Phospholipid Vesicles

Phospholipid solution "A" was typically made with an overall aqueous batch size of 200 g. First, Tween 80 and mannitol were added to the requisite amount of water and stirred until completely dissolved. The solution was chilled in an ice water bath, and then the phospholipid was added. The solution was stirred and sonicated (Branson Sonifier 250, Branson Ultrasonics Corporation, Danbury, CT) while still chilled to break up any large clumps. At this time, the pH was adjusted to 7.0 by adding 0.1M NaOH. This solution was then passed 30 times through a high-pressure homogenizer (Avestin Emulsiflex C-5, Avestin Inc, Ottawa, Ontario, Canada) at a shear pressure drop of 15 000 psig to produce small unilamellar vesicles. The outlet flow from the pump was passed through a chilling tube, which was submerged in an ice bath, before returning to the pump feed supply to keep the temperature of the phospholipid solution entering the pump at 10°C. At the end of the run, the pH was again checked and, if necessary, 0.1M NaOH was added to bring the final pH to between 7.5 and 8.0. This solution was then refrigerated at 4°C until ready for use.

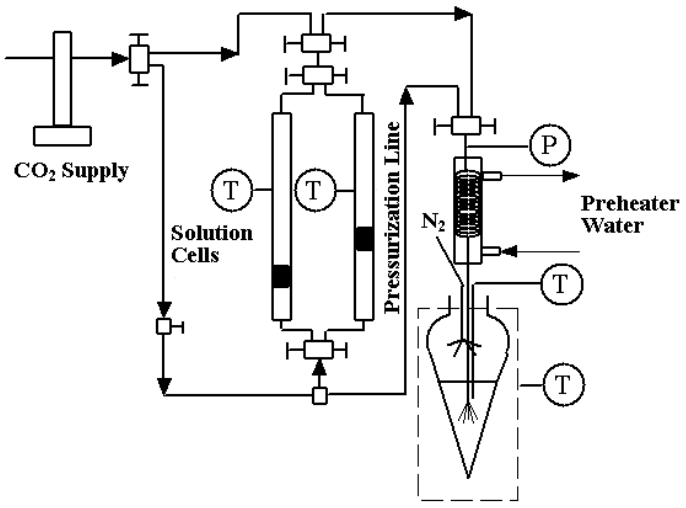

Figure 2. Schematic of apparatus used for rapid expansion from supercritical to aqueous solution (RESAS). T: temperature indicator, P: pressure indicator.

Rapid Expansion from Supercritical to Aqueous Solution

The RESAS apparatus is shown in Figure 2. The CO_2 was supplied to the system by means of a high-pressure computer-controlled syringe pump (ISCO). The 2 solution cells were composed of 4-foot-long, 11/16-inch inner diameter (id) × 1-inch outer diameter (od), stainless steel tubes set up in parallel and equipped with pistons. Each cell was loaded with a predetermined mass of drug above the piston, sealed, and then loaded with a known volume of CO_2. The back side (or bottom) of the piston was subsequently pressurized by CO_2 to the desired pressure. Each cell was heated by four

2-foot-long strips of heating tape connected to heating controllers to maintain the temperature to within ±0.3°C. For initial solution equilibration and mixing, only 2 of the heating tape strips were heated (the second and fourth from the top) to produce density fluctuations within the fluid to aid mixing. At the same time, the pressure within the cell was cycled via the syringe pump to create further mixing by moving the piston up and down. Typically, the solution would come to the equilibrium concentration within 24 hours. The solution concentration was checked by spraying the solution into pure methanol to collect dissolved drug, and the sample was analyzed by HPLC. If the aforementioned mixing technique was not utilized it could take the solution up to a week to reach equilibrium. The preheater consisted of a 30-foot-long piece of 1/16-inch od × 0.03-inch id stainless steel tubing coiled within a 1½-inch diameter × 20-foot-long tube (column heater, Alltech Associates, Deerfield, IL) with heated water circulating through it at a rate of 2.4 L/min. This heat exchanger allowed very uniform heating of the preheater coil, preventing hot spots, which could otherwise produce crystallization of the drug within the coil.

For most of the experiments, the nozzle was made from a 10-inch-long, 1/16-inch od × 0.03-inch id stainless steel tube, in a similar manner to that previously described.[25] Two sizes of nozzle were made by filing the end of the nozzle, allowing flow rates of 0.88 mL/min or 2.5 mL/min at a constant pressure drop of 345 bar. Figure 3 provides an example of what the spray pattern looks like exiting the 2.5-mL/min nozzle operated at a pressure drop of 345 bar. For comparison purposes, a 10-inch-long piece of 50-µm id fused silica capillary tubing (Polymicro Technologies, Phoenix, AZ) was used as the nozzle for a few experiments.

The aqueous stabilizing solution was held in a 125-mL separatory funnel. The expanding diameter of the funnel helps to destabilize foam formation created by CO_2 bubbling through the solution, while providing sufficient depth for small volumes of liquid to allow the nozzle to be submerged. The nozzle was submerged approximately 1 cm below the surface of the aqueous solution. In this way, the particles being formed are brought into close contact with the stabilizing solution. The separatory funnel was submerged within a temperature-controlled water bath. A thermocouple was submerged next to the nozzle in the stabilizing solution to measure the local temperature of the solution. To suppress and drain any foam produced during RESAS, N_2 gas was blown down on top of the foam at 7.8 L/min and 2 bar into the funnel approximately 4 cm above the foam through four 1/16-inch od × 0.03-inch id stainless steel tubes. Prior to each experiment, 10 mL of the stabilizing solution was filtered with a 0.2 µm sterile disposable syringe filter (Whatman, Kent, UK) and then added to the funnel. Immediately prior to collecting the particles, the stabilizing solution in the funnel was submerged in the heated water bath to bring it to the desired collection temperature.

Figure 3. Spray profile for a CO_2 solution expanding through a tapered elliptical nozzle with a flow rate of 2.5 mL/min at 345 bar.

To produce the suspensions, first the preheater and nozzle assemblies were prepressurized with CO_2. The prepressurization prevented plugging of the nozzle. Once the flow of CO_2 had equilibrated, the flow was switched so as to push the solution out of the cell and through the nozzle. Initially, the spray was conducted by spraying into a separatory funnel containing pure water. Once drug particles began to accumulate in the water, the separatory funnel was switched to one of the prepared and preheated stabilizing solutions. A measured volume (as measured by the syringe pump) of solution was sprayed to produce a suspension of a desired concentration. Upon completing the spray, the stabilizing solution was replaced by pure water again, and the flow switched back to pure CO_2. Sufficient CO_2 was allowed to spray through the nozzle to ensure that all drug was swept out of the system to prevent nozzle plugging upon depressurization. For all of the samples using phospholipid-based surfactants, the pH of the suspension was adjusted again, after the spray was completed, to 7.0 by adding sufficient 0.1M NaOH. Typically the pH of the phospholipid solution went from 7.5 to 3.5 during the course of the spray. pH neutralization was required to ensure long-term stability of the phospholipids. The samples were stored with a N_2 headspace by purging the air from the sample vial through a sep-

Table 1. Solubility of Cyclosporine in Various Surfactant Systems at 25°C*

Surfactant Name	Surf Concentration in H_2O % (g/g)	Drug Solubility (mg/mL)	Drug/Surf ratio (g/g)
Tween 80[†]	1	0.56	0.056
	2	1.2	0.060
Myrj 52	2	1.7	0.085
SLS	2	27.0	1.4
Pluronic F127	2	0.01	0.0005
Lipoid E80/Tween 80/mannitol	10/2/5.5	4.75	0.04
	1/0.2/0.55	0.32	0.03

*SLS indicates sodium lauryl sulfate; and surf, surfactant.
[†]Reference 26.

tum in the vial cap to prevent oxidation of the phospholipid components. The samples were refrigerated at 4°C.

RESULTS AND DISCUSSION

Cyclosporine Solubility

The phase behavior of cyclosporine in CO_2 has previously been reported.[25] At 30°C and 345 bar, 1.0% (wt/wt) of cyclosporine is readily soluble in CO_2. All of the RESAS experiments in this work were performed with an initial solution concentration of 1.0% (wt/wt). It is important to note that the solubility of cyclosporine in CO_2 decreases with temperature at constant pressure (cloud point increases). The solubilities of cyclosporine in the surfactant solutions used for stabilization were measured to serve as a control, as shown in Table 1 at 25°C. The goal of RESAS was to produce suspensions with a drug/surfactant ratio much higher, and at least twice as high, as is obtained from the equilibrium solubility in the surfactant solution. As a result, sodium lauryl sulfate (SLS) was not chosen as one of the surfactants to examine since the solubility of cyclosporine in this solution is quite high.

Effect of Surfactant Type on Particle Size

While the bulk of the RESAS experiments will focus on phospholipid-based systems, micelle-forming surfactants including Tween 80, Pluronic F127, and Myrj 52 were studied for comparison, as shown in Table 2. All of the experiments shown in Table 2 had the following initial conditions: a drug concentration of 1.0% (wt/wt) in CO_2, a solution temperature of 30°C, a preheater temperature of 60°C, a pressure drop of 345 bar with a nozzle that produced a flow rate of 2.5 mL/min, and a stabilizing solution bath temperature of 25°C. The table shows the final concentration of drug in the suspension as measured by HPLC, the mean particle size, the particle size distribution (with the relative percentage of particles found in each peak for multipeak distributions), and the drug to surfactant ratio for the equilibrium solubility as well as for the actual suspension.

As seen in Table 2, Tween 80 stabilized particles with mean diameters from 660 to 970 nm with fairly broad distributions at relatively high drug/surfactant ratios of nearly 0.6, which was 10 times the equilibrium solubility. These results agree reasonably well with previous work.[25] The new experiments utilized smaller aqueous stabilizer volumes and higher flow rates that did not seem to modify the particle size. To minimize aggregation resulting from particle collisions, the surfactant must reach the surface of the primary particle rapidly and orient such that it can provide steric stabilization. Previously,[25] the particle size increased markedly for a drug:surfactant ratio >0.6 due to insufficient surface coverage. In contrast, using Pluronic F127 led to substantially larger particles despite the lower drug/surfactant ratio of approximately 0.25. It appears that this surfactant has a lower affinity for the particle surface, which is consistent with the low equilibrium solubility. The lipophilic propylene oxide group makes up only 30% (wt/wt) of the surfactant molecule, which might not be enough for sufficient adsorption at the particle surface. The use of Myrj 52 also yielded larger cyclosporine particles than Tween 80. While this surfactant has a modestly different lipophilic group than Tween 80 (stearate compared with oleate), it also has a more linear, or less bulky, hydrophilic group. Tween 80 has several ethylene oxide side chains providing greater steric repulsion in the continuous aqueous phase.

The compositions of several phospholipid-based surfactant mixtures are provided in Table 3. The 2 phospholipids included commercially available compounds Lipoid E80 (from chicken egg white) and Phospholipon 100H (hydrogenated soybean), which both consist primarily of dipalmitoylphosphatidylcholine (DPPC) and include small amounts of impurities such as phosphatidylethanolamine, sphingomyelin, and lyso-phosphatidylcholine. Each of these systems was prepared so as to have initial structures consisting of small unilamellar vesicles <1.0 μm in water. Preparations

Table 2. Effect of Surfactant Type on Cyclosporine Microparticles Prepared by RESAS of a 1.0% (wt/wt) Solution into 10.0 mL of 2.0% (wt/wt) Aqueous Surfactant Solution*

Surfactant Type	Drug Conc (mg/mL)	Yield % (wt/wt)	Particle Mean (nm)	Particle Size Distribution (nm)	Drug/Surf Ratio @ max sol	Drug/Surf Ratio (g/g)
Tween 80†	6.1	68	970	210-320 (21%), 500-760 (59%), 2200-3400 (20%)	0.056	0.61
Tween 80†	5.4	64	660	220-290 (25%), 700-940 (75%)		0.54
F127	4.0	46	1010	140-250 (12%), 400-860 (75%), 3400-5400 (13%)	0.0005	0.20
F127	4.7	48	1200	180-310 (7%), 520-870 (66%), 2100-4100 (27%)		0.24
Myrj 52	4.6	49	840	90-120 (2%), 310-590 (58%), 1100-2100 (40%)	0.085	0.23
Myrj 52	3.2	38	1240	150-270 (9%), 410-850 (79%), 4800-7500 (13%)		0.16
SLS	------	------	-----	-------	1.4	

* Initial solution conditions were temperature (T_{soln}), 30°C; preheater temperature ($T_{preheater}$), 60°C; pressure drop (ΔP), 345 bar; flow rate, 2.5 mL/min; and stabilizing bath temperature (T_{bath}), 25°C. Conc indicates concentration; max sol, maximum solubility; RESAS, rapid expansion from supercritical to aqueous solution; and surf, surfactant.
†1.0% (wt/wt)

Table 3. Compositions of the Various Phospholipid Surfactant Systems Used in This Study

Designation	Components	Concentrations % (wt/wt)	Vesicle Size Mean (nm)	Vesicle Size Range (nm)
Phospholipid A	Lipoid E80/ Tween 80/ Mannitol	10/2/5.5 1/0.2/0.55	39.6 35.1	20-50 10-50
Phospholipid B	Phospholipon 100H/ Tween 80/ Mannitol	2/2/5.5	90.2	70-280
Phospholipid C	Phospholipon 100H/ Myrj52/ Sodium Deoxycholate/ Mannitol	2/1/0.25/5.5	131.2	60-360
Phospholipid D	Phospholipon 100H/ Myrj52/ Mannitol	2/2/5.5	140.5	70-440

made with Lipoid E80 had starting vesicle sizes of 10 to 50 nm, while those made with Phospholipon 100H were slightly larger, as confirmed by DLS. The nonionic surfactants can act to make the vesicle bilayer more fluid,[30] facilitating transport across the bilayer. All of these systems included mannitol to enhance vesicle stability, and also to act as a cryoprotectant to prevent loss of structure during lyophilization.[31-33] Sodium deoxycholate was utilized in system "C" to supplement the steric stabilization with electrostatic stabilization.

Results are shown in Table 4 for the various phospholipid-based surfactant systems. The table shows the final concentration of drug in the suspension as measured by HPLC, the mean particle size, the particle size distribution (with the relative percentage of particles found in each peak for multipeak distributions), and the drug/surfactant ratio for the equilibrium solubility as well as at the final suspension concentration. In order to be able to distinguish between vesicles and particles in the final solutions, a few control experiments were performed. First, in the range of 10°C to 50°C, it was found that the size of the SUVs does not

Table 4. Effect of Surfactant Type on Cyclosporine Microparticles Prepared by RESAS for a Stabilizing Solution Bath Temperature of 25°C*

Phospholipid Surfactant Composition	Drug Conc (mg/mL)	Yield % (wt/wt)	Particle Mean (nm)	Particle Size Distribution (nm)	Drug/Surf Ratio (g/g)
A†	6.5	93	220	30-50 (4%), 100-160 (66%), 330-560 (30%)	0.54
A†	6.9	100	220	80-120 (32%), 250-300 (68%)	0.58
B	4.6	54	660	80-420 (91%), 3300-6300 (9%)	0.12
B	5.1	62	640	80-420 (93%), 5100-7100 (7%)	0.13
C	4.1	26	2740	140-300 (16%), 720-1740 (60%), 7500-10 000 (34%)	0.13
C	4.2	34	4110	280-470 (56%), 7700-10 000 (44%)	0.13
D	7.7	63	390	150-200 (54%), 560-750 (46%)	0.19
D	6.9	59	460	110-180 (54%), 320-560 (27%), 1180-2060 (19%)	0.17

* Initial solution conditions were temperature (T_{soln}), 30°C; preheater temperature ($T_{preheater}$), 60°C; pressure drop (□P), 345 bar; flow rate, 2.5 mL/min; and stabilizing bath temperature (T_{bath}), 25°C. Conc indicates concentration; RESAS, rapid expansion from supercritical to aqueous solution; and surf, surfactant.

† 1.0% Lipoid E80/ 0.2% Tween 80/ 0.55% Mannitol (wt/wt); T_{bath} = 45.0°C

change when measured by DLS. Second, if pure CO_2 is sprayed into the solution for a time similar to that in the rest of the experiments (up to an hour), there is also no change in the vesicle size even with the resulting change in pH from 7 to 3. pH is known to affect vesicles by increasing DPPC hydrolysis rates, and pH gradients have been utilized in the formation of SUVs.[34] Therefore, it is reasonable to believe that any change in particle size distribution as measured by DLS after RESAS with a drug would correspond to the presence of stabilized drug particles.

The low-concentration solution A was able to stabilize cyclosporine particles at concentrations of ~7 mg/mL (~0.6 drug:surfactant ratio) with mean diameters of 220 to 230 nm and relatively narrow size distributions as seen in Table 4. The distributions are bimodal. The smaller peak is somewhat larger than for the initial drug-free vesicles, suggesting that the drug induces modest aggregation of these vesicles. The larger peak is 5 to 10 times the size of the initial vesicles. It is likely that most of the drug is contained in these larger aggregates. The drug/surfactant ratios are over 20 times the equilibrium solubility in the vesicles. While the examples discussed here were made with the lower concentration of formulation A, these suspensions tended to be less stable (discussed in more detail later) and were incapable of stabilizing drug payloads up to 50 mg/mL. Therefore, the majority of the rest of the work detailed herein was conducted using the higher concentration of formulation A.

Figure 4 shows transmission electron micrographs (TEMs) taken of several of the suspensions formed in this study. Figure 4A shows the initial empty SUVs of phospholipid formulation A before RESAS, with particle sizes that are between 10 and 80 nm. Upon exposure of the aqueous vesicles to CO_2, there was no noticeable change in the TEM micrographs. Note that in some regions, the vesicles associate loosely to form aggregates. Also, the vesicle shape appears distorted from a spherical shape. Some distortion can occur due to collapse or close packing of the vesicles upon water evaporation.[27,34] The negative staining technique can be used to observe multilamellar structures (MLVs), if present. MLVs could be seen (not shown) before homogenization to form SUVs. Figure 4B shows the particles at a drug:surfactant ratio of 0.15, showing both the large stabi-

lized drug particles (~200 nm) as well as small vesicles. The DLS results showed particles as large as 400 nm, so there may be some aggregation occurring within the suspension between the larger drug particles and the remaining SUVs.

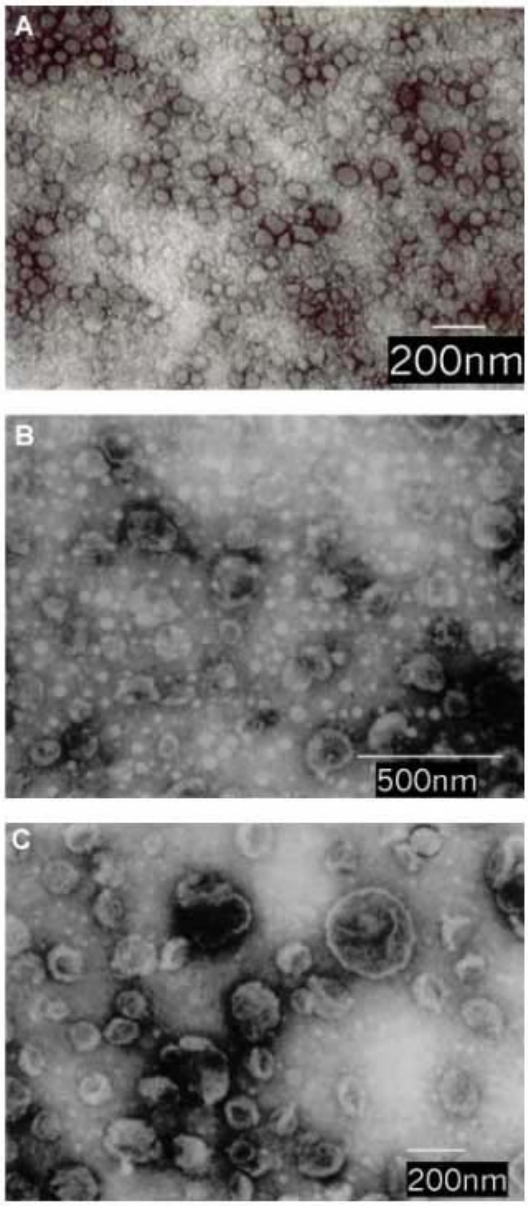

Figure 4. TEM micrographs of (A) initial SUVs made from phospholipid formulation A before RESAS, (B) drug-loaded vesicles of phospholipid formulation A at a concentration of 17.6 mg/mL and stabilizing solution temperature of 31.1°C; operating conditions: T_{soln} = 30°C, T_{pre} = 60°C, ΔP = 345 bar, T_{bath} = 45°C, solution flow rate = 2.5 mL/min, (C) drug-loaded vesicles of phospholipid formulation A at a concentration of 54.0 mg/mL and stabilizing solution temperature of 50.3°C; operating conditions: T_{soln} = 30°C, T_{pre} = 60°C, ΔP = 345 bar, T_{bath} = 80°C, solution flow rate = 2.5 mL/min.

Unfortunately, with this staining technique, we were unable to visualize the monolayer of surfactant on the particles, as the stain simply deposits around the outside of the particle. Also, we saw no evidence of successive bilayers surrounding the particles. While it was somewhat difficult to distinguish between drug particles and vesicles, the larger objects seen in this micrograph never appeared during inspection of phospholipid vesicles with no drug. Also, the entities we believe to be the drug particles have highly irregular surfaces, which is another characteristic not observed with unloaded vesicles. Figure 4C shows the particles formed at a high drug:surfactant ratio of 0.45, depicting very few small vesicles remaining with numerous large stabilized drug particles, approaching 300 nm. The DLS results in this case indicated 3 size ranges (40 to 60, 100 to 200, and 500 to 920) with the bulk of the material present in the last peak. This distribution again suggests that some of the particles in the TEM associate or aggregate.

The particle diameters of the phospholipid-stabilized suspensions were markedly lower than those produced with micellar-forming surfactants for similar surfactant concentrations and drug/surfactant ratios. To stabilize such small particles, the surfactant must be able to rapidly adsorb onto the surfaces of the precipitating particles in order to hinder particle growth in the jet. Since the bulk of the surfactant is now in the structure of a vesicle, the stabilization mechanism may be expected to be different than for the micelle-forming surfactants in Table 2. In the case of vesicles, the aggregation number of surfactant is much larger than for micelles. Thus, in a single vesicle the local concentration of surfactant that can coat a growing drug particle is higher than for a single micelle. The preferred curvature of the surfactant is more favorable for vesicles than micelles. For vesicles, the interface with water is less curved than for the much smaller micelles and will more closely match that of the drug particles. The better match in curvature may be expected to favor particle stability. The growing drug nuclei may collide with a bilayer of the vesicle and dissolve. The presence of the nonionic surfactant Tween 80 can aid the transport through the bilayer. Since vesicles tend to be relatively stable, growth of drug particles by collision/coagulation may be expected to be minimized. Thus, the particle may be expected to grow mostly due to a condensation mechanism within the bilayer as additional drug nuclei enter, although detailed studies would be needed to quantify this mechanism. When the drug particle size becomes large enough, it can disrupt the vesicle structure and essentially cause an "unzipping" of the bilayer as the vesicle breaks. The rearrangement of the surfactant may be expected, leaving a monolayer of the phospholipid on the particle surface with the polar heads solvated by water. SUVs may then become loosely associated with the hydrophilic groups on the outside surface of this monolayer. Previous

work has demonstrated the ability of phospholipid surfactants to adsorb on a hydrophobic surface as a monolayer.[1]

Unfortunately, in all the cases where Phospholipon 100H was used, the solutions foamed extensively and quickly became very viscous or, as in the case of solution C, even gelled during the spray. Changing the temperature of these solutions from 25°C to 75°C had no observable or measurable effect on the particles collected or the nature of the solution during RESAS. When the solution becomes too thick, any further spraying yields large particles, as the surfactant can no longer diffuse to the particle surfaces as they precipitate and grow. In the case of phospholipid solution B, particles were stabilized with mean diameters from 370 to 660 nm with broad distributions from 30 nm to 7 μm. Phospholipid solution C quickly gelled during the spray, yielding the largest particles of any of the phospholipid combinations with approximate mean particle sizes of 2 to 4 μm and broad size distributions. Phospholipid solution D stabilized particles moderately well, with mean sizes of 290 to 460 nm and particles ranging from 70 nm to 3 μm. Solutions B, C, and D also each had low trapping yields as the solutions became too viscous. It is likely that the difference between the performance of Lipoid E80 and Phospholipon 100H lies in the source (egg vs soybean), and resulting differences in impurities. Since phospholipid solution A produced the smallest particles, it will be the focus of the experiments in later sections concerning the effects of temperature, drug concentration, etc.

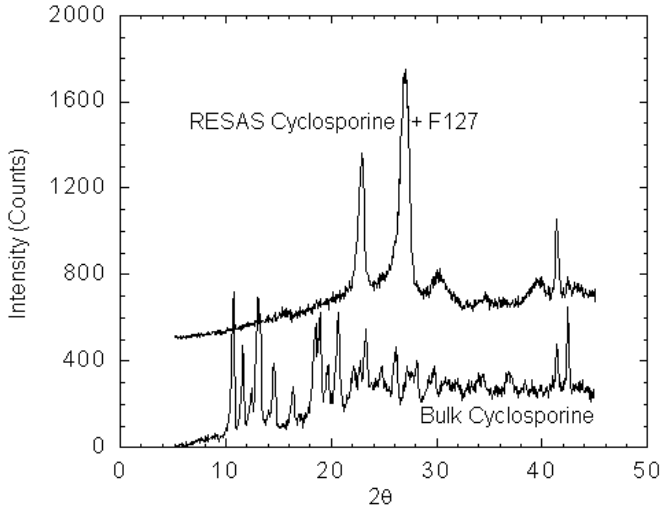

Figure 5. X-ray diffraction patterns for bulk cyclosporine (bottom) and cyclosporine processed by RESAS and stabilized by Pluronic F127 (drug/surfactant ratio = 0.2).

In Figure 5, we show the x-ray diffraction patterns for cyclosporine before RESAS (lower curve), and for cyclosporine stabilized by Pluronic F127 (upper curve). Prior to processing, the cyclosporine is crystalline, showing multiple sharp peaks. After processing by RESAS and stabilization by F127, the cyclosporine crystal peaks have disappeared, suggesting the drug is now trapped in an amorphous state. The 2 large peaks seen are due to the surfactant, Pluronic F127. Since the entire sample produced with F127 was dried and analyzed (including the large particles), and the entire sample was amorphous, it is not unreasonable to assume that the smaller drug particles trapped with the other surfactant systems could also be amorphous.

Effect of Suspension Concentration

Tables 5-7 illustrate the effect of drug concentration, which is a function of spray time, on particle size for bath temperatures of 25°C, 45°C, and 80°C, respectively. In all of the experiments, the stabilizing solution was phospholipid formulation A at the higher concentration levels, as shown in Table 3. The starting vesicle size in the solution, as listed in Table 3, was 10 to 50 nm, and they are likely SUVs. Upon examination with TEM, the vesicles appeared similar to those shown in Figure 4A. Tables 5-7 show the actual stabilizing solution temperature measured within the separatory funnel in addition to the related entries found in Table 4.

Table 5 shows the particle size for a bath temperature of 25°C. At a drug concentration of ~20 mg/mL, particles collected had a mean diameter of 500 to 650 nm with sizes ranging from 70 nm to 2 μm. At ~40 mg/mL, the mean particle size increased to 730 nm with very broad distributions. The average particle size grew by as much as 50% when doubling the drug concentration. A further increase in drug concentration to 46 mg/mL produced a substantial increase in particles larger than 1 μm, indicating loss of stabilization against aggregation.

In Table 5 versus Table 6 the only difference in experimental conditions was the stabilizing solution bath temperature, which was 45°C. Initially, at a drug:surfactant ratio of ~0.1 (roughly 2 times the equilibrium solubility), the particle mean diameters were only 160 to 180 nm with narrow size distributions. At a drug surfactant ratio of ~0.15, the particle mean increased to 260 to 290 nm, and at 0.25, it reached 310 to 390 nm with a slightly broader size distribution. In all cases, the particles were much smaller than those produced with Tween 80.

In Table 7, the stabilizing solution bath temperature was 80°C. For a given drug loading, each of the properties of the particles was similar to that for a bath temperature of 45°C. In all 3 cases, the particle size increased substantially with drug concentration, which increases with spray time. Figure 6 demonstrates the trends in particle growth more clearly. For the 2 higher stabilizing solution temperatures, the particle size increases approximately linearly with drug concentration, with only a small increase in size with temperature.

Table 5. Effect of Suspension Concentration on Cyclosporine Microparticles Prepared by RESAS for a Stabilizing Solution Bath Temperature of 25°C*

Stabilizing Solution Temp °C	Drug Conc (mg/mL)	Yield % (wt/wt)	Particle Mean (nm)	Particle Size Distribution (nm)	Drug/Surf Ratio (g/g)
14	18.4	89	650	70-140 (28%), 240-450 (36%), 1000-1900 (36%)	0.15
13.3	19.4	96	500	90-190 (45%), 530-1260 (55%)	0.16
14.8	24.2	110	630	120-210 (47%), 760-1440 (53%)	0.20
15.8	39.0	110	730	50-80 (4%), 160-290 (33%), 750-1500 (63%)	0.33
13.7	39.8	80	960	80-160 (14%), 480-910 (74%), 2700-5200 (12%)	0.33
13.6	45.9	94	1700	80-160 (12%), 500-1150 (74%), 5000-10000 (14%)	0.38

* Initial solution conditions were temperature (T_{soln}), 30°C; preheater temperature ($T_{preheater}$), 60°C; pressure drop (ΔP), 345 bar; flow rate, 2.5 mL/min; and stabilizing bath temperature (T_{bath}), 25°C. Conc indicates concentration; RESAS, rapid expansion from supercritical to aqueous solution; surf, surfactant; and temp, temperature.

Table 6. Effect of Suspension Concentration on Cyclosporine Microparticles Prepared by RESAS for a Stabilizing Solution Bath Temperature of 45°C*

Stabilizing Solution Temp °C	Drug Conc (mg/mL)	Yield % (wt/wt)	Particle Mean (nm)	Particle Size Distribution (nm)	Drug/Surf Ratio (g/g)
32	10.3	70	160	80-110 (67%), 260-350 (33%)	0.09
33	13.0	87	180	80-110 (47%), 220-290 (53%)	0.11
31.2	15.8	89	280	40-70 (6%), 140-220 (67%), 450-760 (27%)	0.13
29.8	17.5	95	290	110-160 (44%), 370-470 (56%)	0.15
31.1	17.6	93	260	90-120 (33%), 290-400 (67%)	0.15
30.3	26.2	72	380	40-80 (10 %), 200-370 (71%), 690-1100 (19%)	0.22
31.2	24.4	74	310	40-100 (13%), 200-520 (87%)	0.20
30.5	31.7	83	390	30-50 (2%), 140-230 (44%), 430-820 (54%)	0.26

*Initial solution conditions were temperature (T_{soln}), 30°C; preheater temperature ($T_{preheater}$), 60°C; pressure drop (ΔP), 345 bar; flow rate, 2.5 mL/min; and stabilizing bath temperature (T_{bath}), 45°C. Conc indicates concentration; RESAS, rapid expansion from supercritical to aqueous solution; surf, surfactant; and temp, temperature.

Table 7. Effect of Suspension Concentration on Cyclosporine Microparticles Prepared by RESAS for a Stabilizing Solution Bath Temperature of 80°C*

Stabilizing Solution Temp °C	Drug Conc (mg/mL)	Yield % (wt/wt)	Particle Mean (nm)	Particle Size Distribution (nm)	Drug/Surf Ratio (g/g)
56.8	12.6	88	220	60-90 (45%), 270-380 (55%)	0.11
54.3	14.8	88	230	60-90 (40%), 290-390 (60%)	0.12
57.2	17.6	72	320	60-100 (28%), 310-600 (72%)	0.15
53.7	23.8	62	390	80-120 (34%), 440-650 (66%)	0.20
53.8	27.0	72	460	100-140 (31%), 500-750 (69%)	0.23
52.7	39.8	99	480	50-100 (48%), 170-360 (18%), 860-1770 (34%)	0.33
50.3	54.0	139	500	40-60 (26%), 100-200 (11%), 500-920 (63%)	0.45

*Initial solution conditions were temperature (T_{soln}), 30°C; preheater temperature ($T_{preheater}$), 60°C; pressure drop (ΔP), 345 bar; flow rate, 2.5 mL/min; and stabilizing bath temperature (T_{bath}), 80°C. Conc indicates concentration; RESAS, rapid expansion from supercritical to aqueous solution; surf, surfactant; and temp, temperature.

For a temperature of 14°C, the size is larger by a factor of about 2, and the scatter about the linear correlation with drug concentration is much larger.

Several factors may be expected to cause the increase in particle size with drug concentration. The first factor is surfactant depletion in the aqueous solution as the SUVs coat the particles and form drug-surfactant aggregates. As the spray time and drug concentration increase, fewer of the initial SUVs are available for stabilizing incoming particles. Another factor is that the particle collision rate increases approximately with the square of the particle concentration. In addition, an increase in the drug concentration raises the drug/surfactant ratio in the aqueous solution. The resulting increase in total drug surface area and decrease in surfactant coverage of this drug surface area may lead to greater aggregation of the surfactant-coated drug particles. Another factor is simply the greater time for particle growth due to shear-induced aggregation caused by the flowing CO_2. Once removed from the process, however, the drug suspensions were extremely stable with little change in particle size measurable by DLS even after weeks of storage, as discussed in greater detail below.

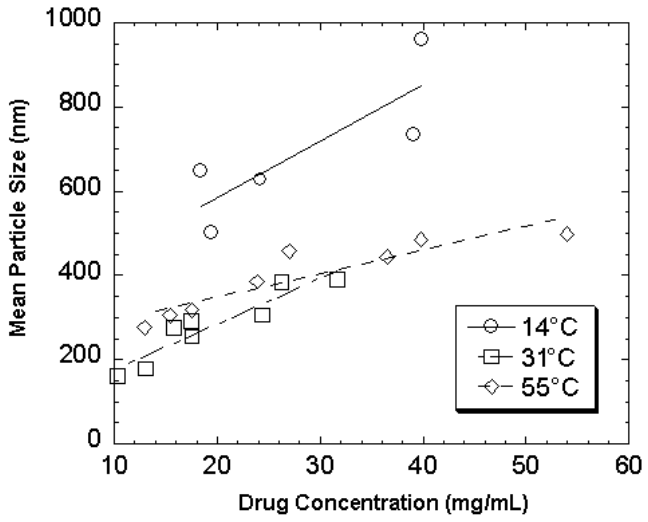

Figure 6. Effect of drug concentration and stabilizing solution temperature on cyclosporine particles produced by RESAS with phospholipid solution A as the stabilizer. T_{soln} = 30°C; T_{pre} = 60°C; ΔP = 345 bar; T_{bath} = 25°C, 45°C, 80°C; solution flow rate = 2.5 mL/min.

Effect of Stabilizing Solution Temperature

The ability for the phospholipid-based surfactants to stabilize the particles may be expected to depend upon the temperature of the medium within the receiving vessel. Most phospholipid vesicles exhibit a transition temperature from a rigid gel-like state to a fluid liquid-crystalline state. As temperature increases, the phospholipid chains within the vesicles go from a very rigid, ordered state, to one that is more flexible and can allow diffusion across the bilayer. The rigidity of the phospholipid tails, and hence the vesicles, could affect the surfactant's ability to rearrange in order to stabilize the drug particles as they precipitate. The effect of lipid chain melting behavior, and hence the lipid's ability to orient at the surface of emulsion droplets has been studied.[27] Dry Lipoid E80 powder was analyzed by DSC to determine the chain melting point, as shown in Figure 7. The melting transition appears slightly above room temperature, at 24°C to 29°C. While the location of the transition agrees with previous studies,[27] the transition enthalpy is lower here. This difference is likely due to adsorbed water. When 10% (wt/wt) of Lipoid E80 was added to water, however, the DSC thermogram was dominated by the water melting peak, and since the transition enthalpy was already low, it could no longer be detected. This effect has been seen previously.[27] Also, the fact that it is hard to detect a clear transition in solution is not unexpected for lipid samples that are not made of pure components or that have additives such as cholesterol.[35] While the presence of Tween 80, mannitol, and even the drug in the vesicles would be expected to further broaden/shift the transition point,[27,35-40] this effect could unfortunately not be demonstrated with this system.

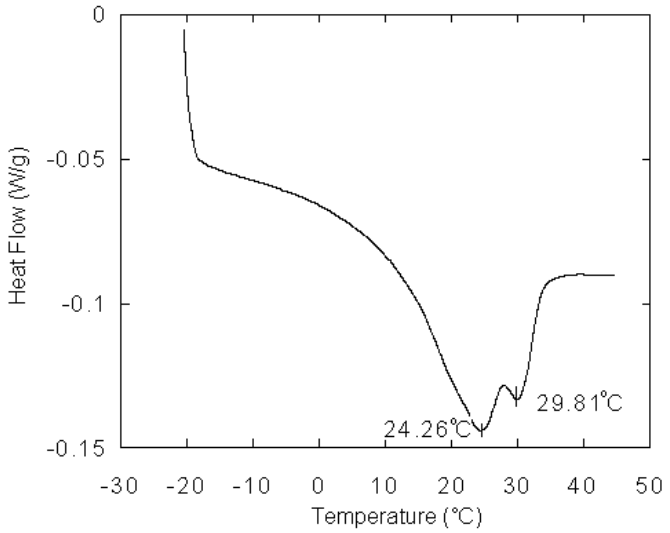

Figure 7. DSC thermogram of bulk Lipoid E80.

Note that in each of the tables, the actual stabilizing solution temperature near the nozzle is significantly lower than that of the water bath used to heat the medium. This cooling is due to the expanding CO_2 gas. As seen in Figure 4, the particle sizes measured when the stabilizing solution temperature was above 30°C were nearly the same at similar drug loadings, regardless of the stabilizing solution bath temperature. However, a large increase in particle size was noted for the cases in which the stabilizing solution was only 14°C. It is likely that at this temperature the phospholipid chains are too rigid to rearrange and stabilize the growing particles as rapidly as at the higher temperatures. The increase in the viscosity of water at locally low temperatures could further inhibit diffusion and rearrangement of surfactant. There could also be an effect of locally colder temperatures in the vicinity of the nozzle tip decreasing supersaturation based on the phase diagram,[25] slowing particle nucleation and producing larger particles. This supersaturation effect is discussed in greater detail in the next section on the effect of preheater temperature for a constant stabilizing solution temperature.

Effect of Preheater Temperature

Cyclosporine becomes less soluble in CO_2 as temperature increases.[25] Therefore, it is expected that at higher preheater temperatures, the depressurization in the nozzle will cause the solution to pass through the phase boundary more quickly than at lower temperatures, leading to higher levels of supersaturation.[15,25,41] The greater supersaturation, in turn, leads to higher rates of nucleation, which would lead to the formation of smaller particles, if aggregation is controlled.

Table 8 illustrates the effects of the preheater temperature on the cyclosporine particle size. The first 2 entries show the results through a nozzle at a flow rate of 0.88 mL/min at a change in pressure (ΔP) of 345 bar. At a low preheater temperature of 30°C, the particles produced at this flow rate were larger than 10 μm and quickly settled out, leaving only drug solubilized in the SUVs. With a bath temperature of only 30°C, the stabilizing solution temperature became too cold for adequate stabilization as discussed above. At a higher preheater temperature of 60°C and at a drug:surfactant ratio of 0.24, the particles had a mean size of 530 nm. The higher preheater temperature resulted in less local cooling for this nozzle flow rate and allowed the stabilization of particles.

In the remainder of the results in Table 8, the solution flow rate was 2.5 mL/min at the same ΔP of 345 bar. As the preheater temperature was changed from 30°C to 80°C, the mean particle size decreased. The drug/surfactant ratio was fairly constant, especially for the 2 higher preheater temperatures. Notice that the preheater temperature had essentially no effect on the stabilizing solution temperature because of the efficient heat transfer in the nozzle and the jet,

Table 8. Effect of Preheater Temperature on Cyclosporine Microparticles Prepared by RESAS of a 1.0% (wt/wt) solution into 10.0 mL of phospholipid mixture A*

T_{pre} °C	Stabilizing Solution Temp °C	Drug Conc (mg/mL)	Yield % (wt/wt)	Particle Mean (nm)	Particle Size Distribution (nm)	Drug/Surf Ratio (g/g)
30†	22.0	2.5	30		30-130, >10 μm	0.02
60†	21.2	24.0	80	530	190-290 (82%), 1500-2500 (18%)	0.24
30	32.1	27.8	100	400	50-240 (40%), 440-720 (60%)	0.23
30	31.7	19.3	82	380	90-170 (15%), 270-590 (85%)	0.16
60	32.2	14.3	98	280	40-180 (68%), 420-770 (32%)	0.12
60	31.8	11.9	84	320	110-150 (41%), 400-540 (59%)	0.10
80	31.6	11.7	79	110	70-100 (90%), 280-380 (10%)	0.10
80	32.0	13.1	78	190	20-140 (70%), 280-380 (30%)	0.11

*Initial solution conditions were temperature (T_{soln}), 30°C; pressure drop (ΔP), 345 bar; flow rate, 2.5 mL/min; and stabilizing bath temperature (T_{bath}), 45°C. Conc indicates concentration; RESAS, rapid expansion from supercritical to aqueous solution; surf, surfactant; and temp, temperature; and T_{pre}, preheater temperature.
†T_{soln} = 30°C; T_{bath} = 40°C; ΔP = 345 bar; solution flow rate = 0.88 mL/min.

and the large change in latent heat for compressed CO_2 relative to the sensible heat. Consequently, the rigidity of the phospholipids in the aqueous solutions was essentially constant. Thus, the decrease in particle size is most likely due to the faster nucleation rate produced by the higher supersaturation at the higher preheater temperatures.

Effect of Nozzle Size

Table 8 also illustrates the effect of nozzle size on the particle size. Two crimped nozzles were compared with similar operating pressures, ΔP = 345 bar, but with flow rates of 0.88 mL/min and 2.5 mL/min. The greater restriction for the slower flow rate nozzle indicates that the crimping and filing process produced a smaller elliptical orifice. The smaller, low flow rate nozzle appears to produce larger particles than the high flow rate nozzle. While smaller droplet sizes would be expected with the smaller nozzle due to more intense atomization, the dominating factor on particle stability in this system appears to be the conditions of the stabilizing solution. At the lower flow rate, the jet is less forceful and creates less mixing/turbulence within the stabilizing solution. The weaker mixing is evident in the lower local temperature within the stabilizing solution. At the higher flow rates, the solution is agitated more intensely, and heat is transferred throughout the fluid within the separatory funnel more efficiently, producing a higher local temperature. Since the lower flow rate nozzle creates less turbulence, the particles and surfactant are not mixed as well, leading to less-effective stabilization. Also, the particles are not carried away from the jet as quickly, allowing for potentially more collisions prior to complete stabilization. In the future, this limitation could be overcome by stirring the solution.

The use of a much larger 50-μm id × 10-cm long straight capillary nozzle in RESAS led to low yields (<35%) and the formation of >10 μm particles. Also, this nozzle tended to plug easily as compared with the crimped nozzle design, which never plugged during a spray. In the case of the capillary nozzle, the depressurization profile occurs over the entire length of the nozzle, resulting in some particle nucleation and growth within the nozzle where it cannot be stabilized.

Long-term Stability

For all of the samples produced, initial particle size measurements were made within 24 hours of the RESAS spray. Also, the stability of the particle size after 1 month of stor-

Table 9. Suspension Stability After 1 Month of Storage at 4°C for Phospholipid Mixture A 10%/ 2%/ 5.5% (wt/wt) Lipoid E80/Tween 80/mannitol

Original Mean (nm)	Mean After 1 Month (nm)	Distribution After 1 Month (nm)	% Change
260	320	30-50 (25%), 120-190 (16%), 410-710 (59%)	20
220	750*	50-230 (17%), 660-1200 (83%)	240
1700	720	100-160 (10%), 650-1000 (90%)	-40
390	700	80-140 (29%), 200-300 (6%), 820-1230 (65%)	80
460	560	50-240 (47%), 560-1780 (53%)	20
400	740	60-90 (41%), 170-350 (16%), 1200-2000 (43%)	85
320	420	50-150 (46%), 220-410 (15%), 680-1120 (39%)	30

*1.0%/0.2%/0.55% (wt/wt) Lipoid E80/Tween 80/mannitol.

age at 4°C and under nitrogen was examined as shown in Table 9. Each sample was gently inverted 5 to 10 times and then allowed to sit for 1 hour prior to analysis. For each of the nonphospholipid surfactants, the particles settled completely and caked at the bottom of the vial. These samples were not redispersible, even by sonication. The samples produced by RESAS with phospholipid solution A had good stability, with no settling observed for most of the suspensions without shaking. Even in cases where there was some settling present, the samples were easily redispersed by the gentle inversions. Most of the samples experienced some growth, or small broadening in the size distribution. In one case, the particle size grew considerably during the 1-month period (likely because of the very high drug:surfactant ratio (>0.5) in this system due to the low amount of surfactant) and also showed instability in the form of settling. In the case in which the particle size appears to decrease, it is likely that some of the larger particles have settled out (not visible to eye) and thus were simply not captured in the sample analyzed by DLS.

In summary, many of the size distributions were bimodal, according to DLS measurements and TEM, consisting of vesicles with very low drug concentrations and drug particle aggregates stabilized with vesicles. As the local temperature of the aqueous stabilizing solution is increased above 25°C, the vesicles become less rigid and stabilize the drug particles more effectively, leading to smaller particles. For a given stabilizing solution temperature, the particle size also decreases with an increase in preheater temperature due to greater supersaturation in the nozzle and more rapid nucleation. High drug loadings in the aqueous suspensions, as high as 54 mg/mL, could be achieved with particle sizes of ~500 nm. This concentration is ~10 times the equilibrium solubility in the aqueous surfactant solution. For Pluronic F127 as a stabilizer, the particles are stabilized rapidly enough to trap the drug in an amorphous state. Long-term stability studies of the suspensions stored at 4°C indicate only modest particle growth over 1 month. The particle sizes in RESAS are much smaller than those produced by RESS as a result of the stabilization provided by the surfactant. The sizes are comparable with those produced by homogenization.

CONCLUSION

Phospholipid vesicles mixed with nonionic surfactants stabilize cyclosporine particles produced by RESAS with mean diameters as small as 200 to 300 nm. This size range is several hundred nanometers smaller than produced by RESAS for particles stabilized by Tween 80. The high drug loadings in the aqueous suspensions, reaching 50 mg/mL (~10 times greater than the equilibrium solubility); the small particle

sizes; and the long-term stability make this process attractive for development.

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