Drug Development and Industrial Pharmacy, 23(11), 1087-1093 (1997)

RESEARCH PAPER

Sterile Filtration of NanoCrystal™ Drug Formulations

Jane Y. Zheng and H. William Bosch

NanoSystems L.L.C., 1250 S. Collegeville Road, Collegeville, Pennsylvania 19426

ABSTRACT

A NanoCrystal™ dispersion of the iodinated x-ray contrast agent iodipamide was prepared by wet milling the drug substance in the presence of Pluronic® F127 stabilizer. The mean particle size of the formulation was 98 nm and all drug particles in the formulation were smaller than 220 nm as determined by dynamic light scattering. Approximately 1 liter of dispersion was filtered through a sterile 0.2-μm disposable capsule filter (Supor® 200 DCF™, 0.1 m^2 effective filtration area [EFA], Gelman Sciences) to condition the capsule. No drug concentration or size distribution changes were detected after the filtration process. The microbiological validation tests were performed using Pseudomonas diminuta organisms to challenge the capsule under simulated worst-case process conditions. The results showed that the Supor 200 DCF was able to retain 100% Pseudomonas diminuta organisms ($\geq 10^7$ organisms per cm^2 of effective filtration area). Thus, this study demonstrated that terminal filtration is a feasible process to sterilize NanoCrystal™ drug dispersions that may be chemically or physically unstable at elevated temperatures and thus not amenable to terminal heat sterilization.

INTRODUCTION

Drug substances that possess low aqueous solubilities often present significant challenges to the formulator, especially when the intended dosage form is an injectable product (1). In these instances, solubilizing excipients such as ethanol, propylene glycol, Polysorbate 80, or Cremaphor EL are usually added to the formulation. While these additives may adequately solubilize the drug substance, they are generally an unattractive option due to toxicity concerns. Furthermore, injectable drug products that contain insoluble drug substances may have to be delivered very gradually in order to avoid precipitation of the drug substance in vivo. A novel and attractive alternative to the use of solubilizing agents in injectable product development is the application of NanoCrystal™ 1 technology (2), whereby aqueous dispersions of drug substance are processed via wet milling techniques to yield formulations with mean particle sizes of approximately 250 nm or less. In these systems,

the drug substance particle size is approximately 50 times smaller than the diameter of a human capillary, making the dispersions safe for intravenous injection. Furthermore, the concentration of drug substance in solution does not exceed its thermodynamic solubility, which eliminates the risk of drug precipitation in vivo. An added benefit is that formulations can be prepared at very high (up to 40%) solids content, allowing low-volume injections to deliver substantial quantities of drug. To date, a variety of different NanoCrystal drug compounds have been safely administered to laboratory animals via the intravenous route. The terminal sterilization of parenteral NanoCrystal formulations presents a unique challenge in this arena because of the colloidal nature of the products. NanoCrystal dispersions, which are physically stable at room temperature, may be prone to degradation at elevated temperatures (e.g., 121°C) due to aggregation, Ostwald ripening, or other mechanisms, making terminal heat sterilization a difficult approach. An alternative method of sterilization that is especially attractive for heat-sensitive drug compounds in solution is filtration through a sterile 0.2-μm membrane filter. The application of sterile filtration to NanoCrystal dispersions is clearly a viable approach as long as essentially all of the drug crystals are less than 0.2 μm in diameter. This study reports the results of a filtration process and a microbiological validation study in which a Supor® 200 DCF™ (Gelman Sciences, Ann Arbor, MI) was used to sterile filter a NanoCrystal iodipamide formulation.

EXPERIMENTAL

Materials and Methods

Iodipamide (3,3′[adipoyldiimino]bis[2,4,6-triiodobenzoic acid]) was obtained from the Sigma Chemical Company and Pluronic® F127 NF Prill was supplied by BASF. Both reagents were used as received. Ceramic grinding media (zirconia-silica composite, 0.8–1.00 mm in diameter) were obtained from SEPR. Particle size measurements were performed on a Leeds & Northrup Microtrac Ultrafine Particle Analyzer. UV-visible spectroscopy was performed using either an ATI model UV 4 or a Hewlett-Packard 8452A. Polyethersulfone membrane filters were obtained from Gelman Sciences. The microbiological validation study was performed by Gelman Sciences, Ann Arbor, MI.

Preparation of NanoCrystal™ Iodipamide

The meglumine salt form of iodipamide (3,3′[adipoyldiimino]bis[2,4,6-triiodobenzoic acid]) is highly water soluble and has previously been used for hepatobiliary x-ray image enhancement (3). However, the dicarboxylic acid form of iodipamide has low aqueous solubility (0.45 mg/ml) and is a suitable candidate for NanoCrystal formulation development.

The NanoCrystal drug dispersions used in this study were prepared using conventional wet milling techniques. The block copolymer Pluronic F127 was selected as the stabilizer for the formulation after a screening study. During the course of this study, several batches of dispersions were prepared with drug concentrations of 5%, 7.5%, 15%, 20%, and 30%. The drug to stabilizer (F127) ratio in each dispersion was 5 to 1. In a typical large-scale milling experiment, approximately 1 liter of 15% (w/v) iodipamide dispersion in 3% Pluronic F127 stabilizer solution was prepared by combining 150 g of iodipamide drug substance with 30 g of Pluronic F127 and diluting with distilled water to a total volume of 1 liter. The formulation was then added to a suitable quantity of ceramic grinding media in several Pyrex media bottles. The formulations were processed via ball milling until particle size analysis indicated that the desired drug particle size distribution had been achieved. At that time the formulations were isolated from the grinding media by vacuum filtration.

Filtration Studies

The sterile filtration feasibility study was initiated with the qualitative evaluation of different membranes. Eight different types of membranes (polyethersulfone, cellulose acetate, PVDF, cellulose esters, PTFE, nylon, nonorganic, and hollow fiber) were tested by filtering a NanoCrystal iodipamide dispersion. The first seven membranes were used as syringe filters and the eighth one (hollow fiber) was in a tangential flow device. The iodipamide dispersion was pushed through each membrane and the filtering capacity among different membranes was compared. In addition to the filterability test, drug concentration yield after filtration was also evaluated. Aliquots of the drug dispersion taken before and after filtration were dissolved in known quantities of aqueous sodium hydroxide and analyzed by UV-visible spectroscopy (λ = 236 nm) in order to determine if significant quantities of drug substance had been retained

by the membrane. Particle size distribution before and after the filtration was examined by dynamic light scattering (Microtrac UPA).

Based on the results of these initial studies, the membrane with the highest filtering capacity and drug yields was selected for larger scale tests. A 1-liter sample of NanoCrystal iodipamide (15%) in F127 solution (3%) was placed in a 4-liter stainless steel pressure vessel, the outlet of which was connected to a polyethersulfone disposable capsule filter (Supor 200 DCF, 0.1 m^2 of EFA). The capsule consisted of a 0.2-μm membrane with a 0.8-μm prefilter. The flow rate decay at 30 psi was monitored. Particle size distribution and drug concentration before and after the filtration were examined. Upon completion of the process, a bubble point test was performed to verify the integrity of the membrane filter.

MICROBIOLOGICAL VALIDATION STUDY

The microbiological validation study of NanoCrystal iodipamide (7.5%) in a Supor 200 DCF was performed by Gelman Sciences (Ann Arbor, MI) (4). The purpose of this study was to validate that Supor 200 membrane filtration can be used to sterilize NanoCrystal dispersions and to evaluate the effect of a NanoCrystal dispersion on a sterile membrane filter.

Bactericidal Determination of NanoCrystal Iodipamide Dispersion on *Pseudomonas diminuta*

A tube of trypticase soy broth (TSB) was inoculated with frozen stock *Ps. diminuta* seed culture 48 hr prior to commencement of the bactericidal testing and incubated for 24 hr at 35°C. A sample of saline lactose broth (SLB) was then inoculated with 2 ml of *Ps. diminuta* seed culture per liter of SLB and incubated for 24 hr at 35°C. The concentration of organisms in the broth was determined by turbidity. Two aliquots containing approximately 5×10^7 organisms were transferred into each of two sterile centrifuge bottles, which were then centrifuged at 2500 rpm for 20 min at 4°C. After centrifugation the supernatant liquid was removed from both bottles, leaving only a thin layer of SLB covering the pellets. To one bottle, 100 ml of sterile 0.9% saline solution was added; to the other bottle, 100 ml of NanoCrystal iodipamide dispersion was added which had been filtered through a 0.2-μm VacuCap® apparatus to remove any organisms that may have already been in the formulation. Thus, each bottle contained 100 ml of liquid with approximately 5×10^7 organisms per ml. The plates were then incubated for 48 hr at 35°C. Aliquots (1 ml) were taken at 0, 0.5, 1, 2, 4, 7, and 24 hr intervals and the number of colony forming units (CFU) evaluated.

Preparation of Challenge Suspension

A tube of TSB was inoculated with frozen *Ps. diminuta* stock seed culture and incubated for 24 hr at 35°C. An appropriate amount was then added to SLB and incubated for an additional 24 hr at 35°C. Based on the density of the sample (determined via turbidity), a suspension of $>10^7$ organisms per cm^2 of effective filtration was prepared.

Negative Control and Preconditioning Integrity Test

A sterile pressure vessel connected to a manifold was fitted with a Supor 200 DCF at one port and a 47-mm negative control filter holder at the second port. The 47-mm filter holder contained a 47-mm GN-6 membrane that was used as a negative control (this verifies sterility of the system). The downstream end of the capsule filter was then fitted with a sterile stainless steel hose, which was placed in a beaker containing 60/40 isopropyl alcohol. The pressure vessel was charged with 5 liters of deionized water and pressurized to 15 psi. The valve to the Supor DCF was opened and 4 liters of water was allowed to flow through in order to wet the capsule. The valve to the negative-control membrane was then opened and 100 ml of water was collected. After the negative-control membrane had been wetted, all valves were closed and the system depressurized to 10 psi. The valve to the Supor DCF was then reopened and the pressure gradually increased until the bubble point of the capsule was reached. In this instance the bubble point of the DCF was determined to be 56 psi (minimum bubble point of this capsule in water is ≥45 psi). After preconditioning, the negative-control filter was aseptically transferred to a trypticase soy agar (TSA) plate and incubated for 48 hr at 35°C.

Product Conditioning

The pressure vessel was charged with approximately 2.5 liters of 7.5% NanoCrystal iodipamide dispersion in aqueous F127 and the system was primed. The vessel was then pressurized to 30 psi and 1.2 liters of iodi-

pamide dispersion was filtered through the Supor 200 DCF. A 142-mm filter holder containing a Supor 450 (0.45 μm) membrane filter (positive control) was then connected aseptically to the third port on the manifold and conditioned with 400 ml of iodipamide formulation. The post-conditioning bubble point of the Supor 200 DCF was then determined as described above and found to be 44 psi.

Challenge Procedure

To remove the iodipamide from the capsule because of its bactericidal properties, the pressure vessel was charged with 15 liters of sterile deionized water and then pressurized to 30 psi. The valves leading to the Supor 200 DCF were opened and the capsule flushed with 10 liters of water. The 142-mm positive-control membrane was flushed with 3 liters of water. The last 100 ml of the capsule flush was set aside for water toxicity testing.

The challenge dispersion was added to the pressure vessel and the system pressurized to 60 psi. The valve to the Supor DCF was then opened; a total of 2.0 liters of challenge dispersion was collected at a flow rate of 5.26 LPM per 0.1 m^2 of membrane surface area. The valve connecting the Supor DCF to the manifold was then closed and the system pressurized to 30 psi. The valve leading to the 142-mm positive-control filter was opened and 250 ml of challenge dispersion was collected in a sterile bottle. The system was depressurized and the downsteam hose from the collection vessel disconnected. Next, the collection vessel port was plugged and the discharge hose placed in a beaker of 60/40 isopropyl alcohol. The system was pressurized to 5 psi and the valve to the recovery filter opened. The effluent was collected in an Erlenmeyer flask. When collection was completed, the Supor 200 recovery filter holder was detached and set aside for plating. In addition, 250 ml of the challenge suspension was filtered through a Supor 450 (0.45 μm) positive-control filter. Upon completion of bacterial challenge, the test capsule was evaluated for membrane integrity. The bubble point of the Supor DCF capsule was found to be 45 psi and that of the positive control (Supor 450) was 36 psi. The Supor 200 recovery filter was aseptically plated onto a 150-mm TSA plate and incubated for 7 days at 35°C. Samples collected from the Supor 450 positive-control plate were diluted in saline and incubated in triplicate on TSA.

Toxicity Testing of Rinse Water

Three aliquots of rinse water (9 ml) and one aliquot of sterile water used as a control were aseptically transferred to sterile test tubes and spiked with 1 ml of diluted culture density medium. TSA spread plates were prepared and incubated for 48 hr at 35°C.

RESULTS AND DISCUSSION

Preparation of NanoCrystal Iodipamide

NanoCrystal iodipamide dispersion was successfully formulated at solids contents up to 30% after 5–10 days of ball milling. The mean particle size of a typical iodipamide formulation was around 100 nm. Furthermore, all drug particles in the formulations were smaller than 0.2 μm as measured by dynamic light scattering. The success in formulating drug dispersions with this kind of particle size distribution using the NanoCrystal technology makes terminal filtration a possible process for sterilization of aqueous drug dispersions.

Filtration Studies

In the initial feasibility study, significant differences in filterability were observed among different membranes. In some cases the filters plugged right away and no filtrate could be collected; in other cases the filters plugged quickly and little filtrate could be collected; only with two (polyethersulfone and cellulose acetate) out of eight membranes could the iodipamide dispersion pass through without difficulty. Among eight membranes, polyethersulfone (Gelman Sciences Supor) provided the highest filtering capacity. The results of drug yield in concentration after the filtration also favored polyethersulfone. With this type of membrane, no drug loss was detected during the filtration at 15% solids content. The comparison of filtration results with eight different membranes is presented in Table 1. These experimental results show that polyethersulfone (Supor) is superior to the other membranes for filtering iodipamide dispersion in terms of high flow rate, high filtering capacity, and low drug binding.

In a larger scale filtration experiment, the iodipamide dispersion passed easily through the Supor 200 DCF under less than 30 psi with no significant flow rate decay. Approximately 850 ml of filtrate was collected in 5 min and the flow rate was about 170 ml/min. No change in particle size distribution or drug substance concentration was observed after the filtration. The re-

Table 1

Concentration Yields of Iodipamide Formulations After Filtration Through Different Membranes

Membrane (0.2 μm)	Vendor	Drug Yield (at 5%)	Drug Yield (at 15%)
Polyethersulfone	Gelman	100%	100%
Cellulose acetate	Sartorius	95%	80%
PVDF	Millipore	95%	Plugged
Cellulose esters	Millipore	85%	Plugged
Nylon	Gelman	75%	Plugged
PTFE	Whatman	Plugged	–
Nonorganic	Whatman	Plugged	–
TFF (hollow fiber)	Microgon	Plugged	–

sults of particle size analysis before and after the filtration and UV assay are shown in Table 2. These results demonstrate that terminal filtration is a feasible process for sterilizing NanoCrystal drug dispersions that have the appropriate particle size distributions.

After the filtration, the bubble point of the Supor 200 DCF was tested and found to be 40 psi. The results indicated that no detectable deterioration of the membrane filter had occurred.

Microbiological Validation Study

Bactericidal Evaluation

In the bactericidal evaluation experiment, a sample of iodipamide dispersion that had been inoculated with the bacterium (*Ps. diminuta*) was found to have no viable organisms remaining after 7 hr (Table 3). As a result of this test, the iodipamide dispersion was indicated to be bactericidal to *Ps. diminuta*. Therefore, 0.9% saline was

Table 2

Particle Size Distributions of Iodipamide Suspensions Measured by Microtrac UPA Before and After 0.2 μm Filtration

Filter Type	Solids Content	Volume (ml)	Yield	Size (nm) Before		Size (nm) After	
				Mean	90%	Mean	90%
4.9 cm^2 Syringe	15%	2	100%	102	155	96	157
0.1 m^2 Capsule	15%	850	>98%	105	182	111	172

Table 3

Bactericidal Test Results of Iodipamide to Pseudomonas diminuta

Time (hr)	No. of Organisms/ml in Iodipamide Suspension	No. of Organisms/ml in 0.9% Saline
0	2.10×10^7	2.11×10^7
0.5	2.01×10^7	2.59×10^7
1.0	1.69×10^7	2.06×10^7
2.0	1.75×10^7	2.21×10^7
4.0	1.30×10^6	1.77×10^7
7.0	0	1.51×10^7
24.0	0	8.57×10^6

used as the challenge base for the microbiological validation study.

Negative Control and Preconditioning Integrity Test

Initially the Supor DCF test capsule was evaluated for membrane integrity and the bubble point was found to be 56 psi. A sample of sterile water was filtered through the GN-6 filter (negative control), collected, and incubated to ensure that the system was not contaminated prior to the challenge procedure. The negative-control sample was incubated in TSA for 48 hr at 35°C and found not to contain viable microorganisms.

Product Conditioning Step and Post-Conditioning Integrity Test

Since the iodipamide suspension was found to be bactericidal to *Ps. diminuta*, the test capsule was conditioned with the suspension by filtering 1.2 liters of formulation through it at 30 psi. In addition, 400 ml of suspension was filtered through a Supor 450 (0.45 μm) positive-control filter. Upon completion of product conditioning, the test capsule was evaluated for membrane integrity and the bubble point found to be 44 psi. The bubble point of the membrane after the formulation was filtered through was expected to be lower, since F127 surfactant used in the formulation has significantly lower surface tension than that of water.

Challenge Procedure

Prior to the microbiological challenge, the test capsule and positive control were rinsed with 10 liters and 3 liters of sterile DI water, respectively. The last 100 ml of rinse water from the Supor 200 DCF was retained for toxicity testing. A suspension of *Ps. diminuta* in 0.9% saline (4.2×10^7 cfu/cm^2) was then filtered through the test capsule; similarly, an amount equal to 1.7×10^7 cfu/cm^2 was passed through the positive control. Samples of test article from both the Supor 200 DCF and positive control were collected and retained for incubation. The challenge flow rate was 5.26 LPM/0.1m^2 of EFA at 60 psi.

Challenge Verification

After 48 hr of incubation, the number of *Ps. diminuta* organisms was evaluated for the Supor 200 effluent, positive-control effluent, negative-control effluent, and rinse water. In each case the Log Reduction Value (LRV) was calculated according to the equation

$$\text{LRV} = \text{Log}_{10} (\text{total challenge/no. cfu on filter})$$

the results of which are presented in Table 4.

The sterile effluent from the Supor 200 test capsule was found to have zero colony forming units, hence the LRV for this sample is >10.6. The positive-control effluent yielded 1164 cfu, which corresponds to an LRV of 6.3. Toxicity testing of the rinse water showed a colony forming unit level equal to 81% of that found in the control sample, indicating that the test capsule was adequately rinsed prior to the bacterial challenge. The results are presented in Table 5. Also, the negative-control sample was found to have zero colony forming units, indicating that the apparatus was adequately sterilized prior to the bacterial challenge. The effluent colonies were confirmed to be *Ps. diminuta* by Gram stain and biochemical tests specific for this organism.

Table 4

Results of Pseudomonas diminuta Challenge and Filter Integrity Tests

Filter ID	0.2 μm DCF	0.45 μm Positive control
Volume (ml)	2000	250
Flow rate (LPM/0.1m^2)	5.26	N/A
Pressure (psi)	60	30
Challenge/cm^2 of EFA	4.2×10^7	1.7×10^7
No. of colonies	0	1164
LRV	>10.6	6.3
Pass or fail	Pass	Fail[a]
Bubble point (post)	45 psi	36 psi

[a]The positive control is expected to fail when the test organism is grown according to HIMA guidelines.

Table 5

Toxicity Testing of Rinse Water Results

Sample ID	CFU/ml in Toxicity Test	% of Control	Actual Challenge/cm^2	"Corrected" Challenge/cm^2
Control	2.6×10^6	-	-	-
Capsule	2.1×10^6	81%	4.2×10^7	3.4×10^7

[a]"Corrected" challenge/cm^2 = actual challenge/cm^2 multiplied by percent of control.

Criteria for Acceptance

According to Health Industry Manufacturers Association (HIMA) guidelines (5), a 0.2-μm filter is considered to be sterilizing grade when the effluent is sterile after the filter has been challenged with $>10^7$ *Ps. diminuta* per cm^2 of effective filtration area at a minimum flow rate of 3.86 LPM/0.1 m^2; e.g., the filter must be 100% retentive of the test organism under these conditions. The positive control must permit passage of organisms, and the negative control must indicate the absence of microbiological contamination in the system prior to the challenge. Furthermore, the rinse water must show <50% decrease in organism viability as an assurance that the bactericidal formulation was adequately rinsed from the test capsule and thus did not have a detrimental effect on the growth of the test organism.

CONCLUSIONS

This study demonstrated that terminal filtration is a feasible process for sterilizing NanoCrystal drug dispersions. Using NanoCrystal technology, water-insoluble drugs can be formulated into aqueous NanoCrystal dispersions in which all of the drug particles ar smaller than 200 nm. This study evaluated eight different types of membranes and identified that the polyethersulfone (Supor) membrane can be used to sterile filter NanoCrystal drug dispersions with a desirable flow rate and filtering capacity. The results of microbiological challenge tests showed that the Supor 200 polyethersulfone disposable capsule filter has the capacity to remove microorganisms from a NanoCrystal dispersion of iodipamide and thus provides a method of sterilizing these systems. A microbiological validation study performed under HIMA guidelines demonstrated that the capsule was 100% retentive of the test organism *Ps. diminuta* while the formulation could be filtered through the capsule without measurable drug loss. This study demonstrates the feasibility of using membrane (0.2 μm) filtration as a method of sterilizing NanoCrystal drug formulations that have the prerequisite particle size distributions and are compatible with the membrane composition.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the contributions of Michelle L. Berner, Larry Scheer, Valerie McInroe, and Linda Belkowski (Gelman Sciences, Ann Arbor, MI).

REFERENCES

1. P. P. DeLuca, J. C. Boylan, Formulation of Small Volume Parenterals, in *Pharmaceutical Dosage Forms: Parenteral Medications* (vol. 1) (K. E. Avis, L. Lachman, and H. A. Lieberman, eds.), Marcel Dekker, Inc., New York, 1984.
2. G. L. Liversidge, K. C. Cundy, J. F. Bishop, and D. A. Czekai, Surface Modified Drug Nanoparticles, U.S. Patent No. 5,145,684, Sept. 8, 1992.
3. R. N. Berk, J. T. Ferrucci, Jr., G. R. Leopold, *Radiology of the Gallbladder and Bile Ducts*, W. B. Saunders Co., Philadelphia, PA, 1983.
4. *Gelman Sciences Validation Studies of a Supor DCF Capsule with Iodipamide Suspension for NanoSystems*, Gelman Sciences, Ann Arbor, MI, 1995.
5. *Microbiological Evaluation of Filters for Sterilizing Liquids*, Health Industry Manufacturers Association, Doc. No. 3, Vol. 4, April, 1982.