Freeze-Drying above Room Temperature

MARC S. TESCONI, KIARESH SEPASSI, AND SAMUEL H. YALKOWSKY*

Contribution from *Department of Pharmaceutical Science, College of Pharmacy, University of Arizona, Tucson, Arizona 85721.*

Received December 10, 1998. Accepted for publication February 22, 1999.

Abstract □ This study investigates the use of solid, organic compounds to lyophilize drugs without conventional freeze-drying equipment. The aim of the investigation is to find a pharmaceutically acceptable solvent or solvent combination that is appropriate for freeze-drying on the basis of its ability to (1) solubilize hydrophobic drugs, (2) provide a stable environment for water-sensitive compounds, (3) be rapidly and completely removed from the product under vacuum, and (4) produce cakes that are readily reconstituted. A eutectic formed from 1,1,1-trichloro-2-methyl-2-propanol (chlorobutanol) hemihydrate and dimethyl sulfone ($DMSO_2$) is determined to be a suitable medium.

Introduction

The formulation of certain parenteral drugs as reconstitutable freeze-dried products offers the advantages of improved stability, dissolution rate, dosing accuracy, and sterility. However, freeze-drying is generally restricted to drugs that are water soluble and/or stable to hydrolysis for at least the time required for freezing. Compounds that hydrolyze rapidly are poor candidates because the amount of drug that remains in the dried product is likely to depend on the time required to freeze the sample. Hydrophobic nonelectrolytes are poor candidates because they require either large volumes of water or significant amounts of cosolvent to yield the desired quantity of drug in a reasonably sized container. Although cosolvents usually improve both stability and solubility, they can cause incomplete freezing and a drying stage that involves the simultaneous sublimation of ice and evaporation of liquid residue.[1] The resultant dried product is often very dense and difficult to reconstitute. Furthermore, since a surface skin is often produced when an incompletely frozen medium evaporates, drying times can be extended beyond the point of practicality.

It is also important to consider that if sublimation temperatures are significantly lower than normal to keep the medium frozen, then the condensing temperature required to recover the solvents may be lower than the −55 °C minimum of the lyophilizer.

The current study investigates the feasibility of using organic solvents that are solids at room temperature to lyophilize hydrophobic and water-sensitive compounds without conventional freeze-drying equipment. The aim of this investigation is to find a solvent or solvent combination that is suitable for freeze-drying on the basis of its ability to (1) solubilize hydrophobic drugs, (2) provide a stable environment for water-sensitive compounds, (3) be rapidly and completely removed from the product under vacuum, and (4) produce cakes that are easily reconstituted using pharmaceutically acceptable cosolvents.

* To whom correspondence should be addressed. Tel: (520) 626-1289. Fax: (520) 626-4063. E-mail: yalkowsky@pharmacy.arizona.edu.

Materials

Solvents were selected from the *Handbook of Pharmaceutical Excipients*[2] and *The Merck Index*[3] on the basis of their having low toxicity, melting points between 35 and 120 °C, and relatively low molecular weights. The compounds chosen for the study are listed in Table 1.

All compounds were >98% pure and were used as received from their respective suppliers except for anhydrous chlorobutanol, which was prepared by dehydration of a chlorobutanol hemihydrate melt.

Approach

The individual solvents in Table 1 were experimentally determined to be either too high melting to ensure solute stability or too nonpolar to solubilize semipolar compounds. Solvent combinations that formed eutectics were sought for their intermediate polarities and lower melting temperatures. The selection of a eutectic medium for freeze-drying involved seven steps: (1) screening solvent combinations for eutectic formation; (2) determination of the eutectic compositions; (3) measurement of sublimation rates; (4) assessment of solubilizing ability; (5) selection of an optimal medium; (6) freeze-drying sample compounds; and (7) analysis of freeze-dried products. Each of these steps is described below.

Experimental and Results

1. Eutectic Formation—The solvents in Table 1 were combined in a 1:1 mole ratio in quantities sufficient to yield approximately 2 g of material. The mixtures were melted, and those systems that exhibited solvent miscibility and stability were allowed to cool at room temperature for 2 h. These were refrigerated for 24 h at 4 °C to ensure complete solidification and then brought to room temperature. Samples that appeared "wet" were deleted from the study. Core samples of approximately 5 mg were taken from the remaining solids and analyzed with a DuPont Instruments model 910 differential scanning calorimeter using closed sample pans and a heating rate of 10 °C/min. Solvent combinations that exhibited a single, well-defined eutectic melt between 35 and 75 °C were considered further.

2. Eutectic Composition—The eutectic compositions were estimated using the van't Hoff equation

10.1021/js980478p CCC: $18.00
Published on Web 04/14/1999

Table 1—Compounds Used in This Study

compds used as solvents	T_m (°C)	compds used as solvents	T_m (°C)
coumarin	69	chlorobutanol (hemihydrate)	78
imidazole	90	chlorobutanol (anhydrous)[a]	97
2-amino-4-picolene	100	vanillin	82
2-aminopyridine	58	ethyl vanillin	77
salicylamide	140	ethyl paraben	116
tiglic acid	64	2-furoic acid	133
2-amino-5-nitrothiazole	187	other compounds	
menthol	35	anthracene	219
dimethyl sulfone	109	caffeine	238
camphene	51	fluasterone	175
thymol	52	progesterone	130
urea	133	coronene	438
salicylic acid	158	phenytoin	297
camphor	180	1,1-dicyano-3-nitrobenzyl-1-propene	161

[a] Prepared by dehydration of chlorobutanol hemihydrate.

$$\ln X_A = \frac{\Delta_{fus}H_A}{R}\left(\frac{1}{T_m} - \frac{1}{T_{eut}}\right) \quad (1)$$

with the molar heat of fusion, $\Delta_{fus}H_A$, determined by differential scanning calorimetry (DSC). In eq 1, X_A represents the mole fraction of component A, T_m is the melting point of pure component A, and T_{eut} is the eutectic melting temperature. Once the calculated mole fractions were determined (with $X_B = 1 - X_A$), solvent ratios slightly above and below the estimated value were prepared and a closer approximation of the eutectic composition was made experimentally using DSC. The eutectic composition was obtained as the mole ratio that produced a single melting endotherm. Hot stage microscopy was used to confirm the DSC results.

3. Sublimation Rates—Samples of the eutectics weighing ~50 mg were sublimed at 10 °C below their eutectic melting points at ~0.3 mmHg pressure. Evaporation rates were measured using a DuPont Instruments model 951 thermogravimetric analyzer. Two evaporation rates were observed in most cases. The greater first rate is due to the sublimation of both solvents. The second rate is due to the sublimation of the less volatile component after the more volatile solvent has been removed. The results for the most readily sublimed solvent combinations are given in Table 2.

4. Solubilizing Ability—To test the ability of the binary systems to solubilize both semipolar and nonpolar compounds, 20 mg of anthracene, caffeine, coronene, phenytoin, progesterone, and urea were added separately to 1 mL volumes of the eutectic melts at temperatures within 5 °C above their respective melting temperatures. All of the solvent systems were able to solubilize this quantity of the solutes. Solubility parameters were also calculated for the eutectic melts using the method of Adjel et al.[4] for combined solvent systems with group contribution values of cohesive energy density and molar volume from Fedors.[5] The solubility parameters shown in Table 3 are in the range of most drugs, which suggests that the melts are good solvents for drugs.

5. Premier Eutectic for Freeze-Drying—The chlorobutanol hemihydrate–dimethyl sulfone ($DMSO_2$) eutectic was found to be the most suitable media for freeze-drying on the basis of nontoxicity, solubilizing ability, and rate of solvent removal. As shown in Table 2, the eutectic is formed at a mole ratio of 60% chlorobutanol hemihydrate and 40% dimethyl sulfone, and it has a melting temperature of 50 °C. Figure 1 shows a phase diagram constructed from DSC analysis of several solvent ratios.

6. Freeze-Drying—The five compounds shown in Figure 2, progesterone, coronene, Fluasterone, phenytoin, and 1,1-dicyano-3-nitrobenzyl-1-propene, were lyophilized from the chlorobutanol hemihydrate–$DMSO_2$ eutectic. Samples were prepared in 10 mL freeze-drying vials with orifices of ~2 cm^2 by dissolving 30 mg of solute in 5 g of the eutectic melt at 60 °C. These were allowed to solidify for 2 h at 25 °C. The samples were then placed in a large Erlenmeyer flask that was connected to a vacuum pump. The base of the flask was submerged in a water bath that was heated by a hot plate. The solvents were sublimed at approximately 0.3 mmHg pressure using two drying stages:

stage 1: 40 °C, 6.5 h (−450 mg/cm^2-h: primarily chlorobutanol hemihydrate)

stage 2: 60 °C, 6.5 h (−150 mg/cm^2-h: $DMSO_2$)

The stage times were roughly equal in this study because both the mass and evaporation rate ratios are 3:1. However, the evaporation rate ratio is likely to vary with the temperatures and pressures used.

7. Freeze-Dried Product Analysis—*Residual Solvent*—Thermogravimetric (TG) analysis was used to measure the amount of residual solvent remaining in the cakes. Samples of ~10 mg were placed under vacuum and heated at a rate of 10 °C/min. The TG scans of the cakes and the solvents are shown in Figure 3. The analysis indicates that the cakes contain less than 1% residual solvent. The profiles for pure chlorobutanol–hemihydrate (-×-) and pure $DMSO_2$ (-+-) in Figure 3 indicate the temperatures at which weight loss due to residual solvent would be expected.

Solute Stability—The investigational compound 1,1-dicyano-3-nitrobenzyl-1-propene has a half-life in water of approximately 20 min, which precludes its freeze-drying by conventional methods. High-performance liquid chromatography was used to analyze the product immediately following lyophilization from the eutectic and after 15 months of storage at room temperature. Assays were performed using a Beckman System Gold HPLC with a Pinnacle ODS amine column (5 μm, 250 mm × 4.6 mm i.d., Restek, Bellefonte, PA) and a mobile phase consisting of 500 parts water, 500 parts acetonitrile, and 3 parts acetic acid at a pH of 3.45. A Beckman model 110A pump was used to maintain a flow rate of 1.0 mL/min. Ultraviolet detection was made using a Kratos Analytical Spectroflow model 757 detector at a wavelength of 300 nm. Quantification of drug in the effluent was made using a Hewlett-Packard model 3394 integrator. The relative retention times of 1,1-dicyano-3-nitrobenzyl-1-propene and its major degradation product are 3.4 and 2.8 min, respectively. No

Table 2—Compositions, Melting Temperatures, and Relative Evaporation Rates (at 10 °C below the Melting Temperatures) of the Eutectics

	solvents			sublimation rate[a] × 10^{-3}	
mol % A	A	B	T_{eut}	A +B	B
75	coumarin	salicylamide	58	0.6	0.0
65	2-amino-4-picolene	salicylamide	73	3.2	0.1
50	imidazole	coumarin	56	0.8	0.5
80	2-aminopyridine	salicylamide	46	1.3	0.0
24	2-amino-4-picolene	2-aminopyridine	44	1.3	1.3
60	chlorobutanol (hemihydrate)	dimethyl sulfone	50	7.9	0.8

[a] Expressed as a fraction of the estimated sublimation rate of ice at –10 °C and 0.3 mmHg (~10 mg/mm^2 min).

Table 3—Solubility Parameters for the Most Promising Solvent Combinations

solvents		solubility parameter of the eutectic melt
A	B	
coumarin	salicylamide	13.5
2-amino-4-picolene	salicylamide	11.8
imidazole	coumarin	12.5
2-aminopyridine	salicylamide	12.1
2-amino-4-picolene	2-aminopyridine	11.1
chlorobutanol (hemihydrate)	dimethyl sulfone	10.0

degradation was detected in the freeze-dried cake immediately after lyophilization or after 15 months of storage at room temperature.

Cake Structure and Particle Size—Figure 4a,b contains photomicrographs of the freeze-dried cakes of phenytoin and coronene, respectively, that were taken using a SPOT camera and a Leica DMLP polarizing microscope. The fiberlike structures in the phenytoin cake are loose aggregates of crystals that are approximately 1−2 μm long. The coronene cake is composed of bundles of roughly 5−10 crystals. The coronene crystals are the largest in the test set, having an average length of approximately 50 μm. The cakes of the other three compounds are similar in overall appearance and contain particles that range from 5 to 15 μm.

Reconstitution—The phenytoin cake was reconstituted using Millipore water adjusted to pH 12. Dissolution was complete with a single shake of the vial. With the exception of coronene, the other compounds were reconstituted using ethanolic solutions in which they were soluble. The progesterone cake was reconstituted using 8 mL of a 60% ethanol, 10% dimethylacetamide, and 30% water solution. The 1,1-dicyano-3-nitrobenzyl-1-propene and Fluasterone cakes were reconstituted using 8 mL of a nonaqueous 50% ethanol and 50% poly(ethylene glycol) 400 solution due to their low water solubilities, which are on the order of 1 μg/mL. For hydrophobic drugs such as these, the composition of the vehicles that are used for reconstitution, whether aqueous solutions or cosolvent concentrates, will depend on the solubilities of the drugs.

In all of the above cases, dissolution of the cakes was complete with 1−2 s of shaking. Note that coronene, which is not a therapeutic agent, was not soluble in pharmaceutical cosolvents.

Discussion

Solid organic solvents were successfully used to lyophilize hydrophobic and water-sensitive compounds. Because the solvents used for the freeze-drying medium were solids at room temperature, the process could be conducted without refrigeration and without conventional freeze-drying equipment. This also facilitated solvent collection, which was accomplished without using a cooled condenser.

A eutectic comprised of two solvents was used in order to achieve good solubilization of drugs in a medium with a moderate melting temperature. The latter property is required because drugs must be dissolved in a medium above its melting temperature and drug stability is compromised if that temperature is high. Since a eutectic is composed of two pure solid phases, these solvents are removed relatively independently of one another according to their vapor pressures. Consequently, two drying stages were used: one at 40 °C to remove (primarily) the more volatile chlorobutanol−hemihydrate and one at 60 °C to remove the $DMSO_2$. Note that these stages do not cor-

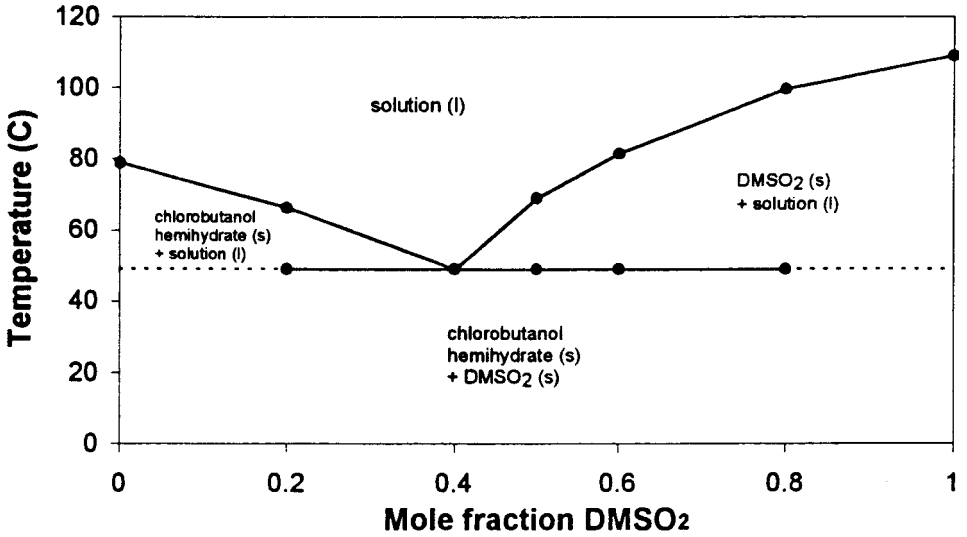

Figure 1—Chlorobutanol hemihydrate–$DMSO_2$ phase diagram.

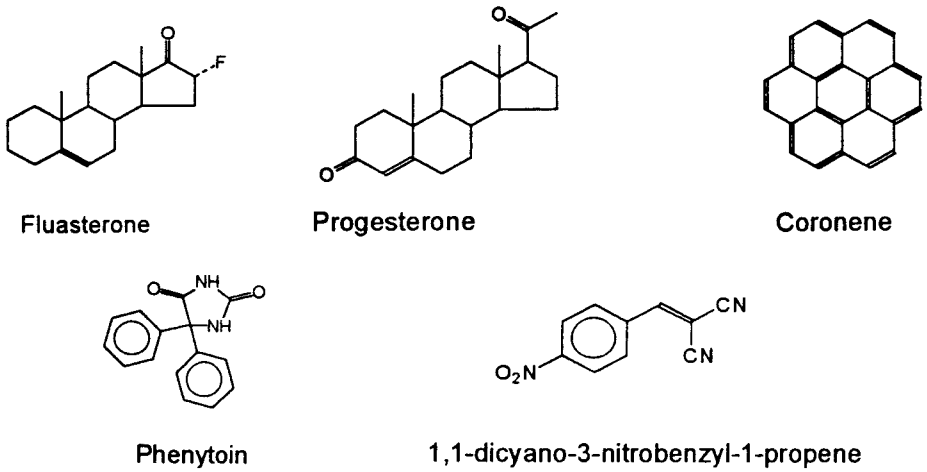

Figure 2—Compounds that were freeze-dried from the chlorobutanol hemihydrate–$DMSO_2$ eutectic.

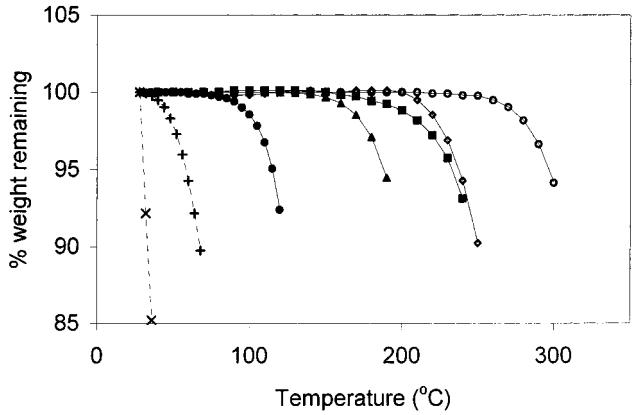

Figure 3—TG analysis of the pure solvents and the freeze-dried cakes: (×) chlorobutanol; (+) $DMSO_2$; (●) 1,1-dicyano-3-nitrobenzyl-1-propene; (▲) Fluasterone; (■) phenytoin; (◇) progesterone; (○) coronene.

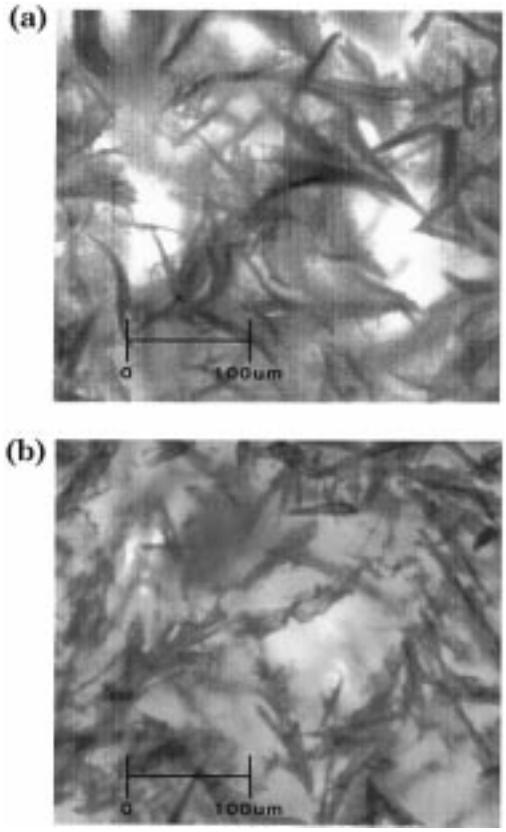

Figure 4—(a) Phenytoin cake. (b) Coronene cake.

respond to the drying stages used for ice in which the first removes frozen water and the second removes bound water.

Thermogravimetric analysis showed that the freeze-dried products did not contain significant amounts of residual solvent. This indicates that the 60 °C used to sublime the bulk $DMSO_2$ was sufficient to remove bound solvent. However, 60 °C is a relatively high temperature compared to what is normally used in freeze-drying, and care must be taken to avoid drug loss. It may be necessary to use lower temperatures and longer drying times when freeze-drying volatile compounds.

One of the major concerns in this study was that the structure of the eutectic should not be significantly altered by the presence of solute. The eutectic contains ~3.6% water that is believed to exist primarily in the form of chlorobutanol−hemihydrate crystals. If a dissolved drug caused the anhydrous form of chlorobutanol to crystallize on cooling, it could lead to incomplete freezing or variable solvent sublimation rates. Analysis of a eutectic containing 5% phenytoin indicated that the eutectic structure is largely unaffected by the presence of this solute. Figure 5a,b shows the DSC traces of the eutectic and the eutectic with 5% phenytoin, respectively. The similarity of the DSC traces suggests that the eutectic microstructures are the same.

The likeness of the photomicrographs of the eutectic and the eutectic with 5% phenytoin in Figure 6a,b, respectively, provides additional evidence for a stable eutectic structure. Hot-stage microscopy indicated that the apparent coarseness in the eutectic with phenytoin is due to presence of drug crystals in the solid. After fusion of the solvents at 50 °C, small, needlelike phenytoin crystals remain in the melt. These dissolved by 70 °C on heating the melt at a rate of 10 °C/min.

Evidence that a hydrated form of chlorobutanol exists in the eutectic is provided by the different thermal properties of the solid formed from the anhydrous chlorobutanol−$DMSO_2$ mixture at a 60:40 mole ratio. DSC and hot-stage microscopic analysis of the anhydrous chlorobutanol−$DMSO_2$ solid indicate a fusion event at 35 °C that is not exhibited by the solid formed from the hemihydrate. However, when 3.6% water is added to the anhydrous melt, the solid that is produced on cooling exhibits the same thermal properties as the solid prepared from the hemihydrate, i.e., a melting point of 50 °C without a 35 °C transition. Physically, the anhydrous solid is much softer than the hydrous form. The former also appears translucent, whereas the hydrous form (the eutectic) is a white

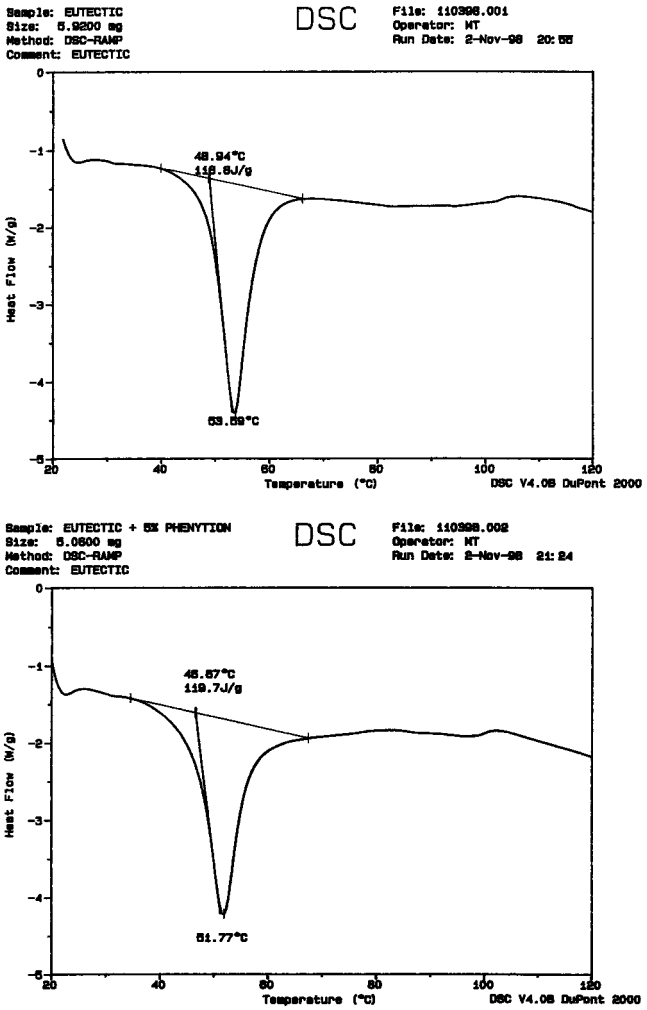

Figure 5—(a) DSC scan of the eutectic. (b) DSC scan of the eutectic + 5% phenytoin.

solid. Both properties may be due to the anhydrous form being incompletely frozen at room temperature. The physical properties of the hydrous eutectic also return with the addition of water to the anhydrous melt.

Thermogravimetric analysis indicated that the cakes produced in this study contained less than 1% residual solvent. Although human toxicity data are scarce, the available data suggest that trace amounts of the solvents which may remain in the freeze-dried products are well below harmful levels. Borody[6] found that plasma concentrations of chlorobutanol as high as 100 μg/mL produced sluggishness and slowed speech, but did not produce liver disease or abnormal electrolyte levels. Others have reported adverse reactions to high doses such as cardiovascular effects following the intravenous administration of heparin preserved with chlorobutanol, neurological effects following the administration of large doses of morphine with the preservative, and hypersensitivity reactions.[2] However, chlorobutanol is currently used in the U.S. as a parenteral preservative in concentrations of up to 0.5% in injectable (e.g., methadone, epinephrine, oxytocin, thiamine), opthalmic (e.g., pilocarpine, epinephrine, phospholine iodide), otic (e.g., Cresylate, Cerumenex), and cosmetic products. It is also the active ingredient in a nonprescription sleep aid (Seducaps: chlorobutanol 150 mg, salicylamide 300 mg) that is available in several countries outside of the United States.[2,7] The lethal dose of chlorobutanol is estimated to be 500 mg/kg[2].

Dimethyl sulfone is an oxidation product of dimethyl sulfoxide (DMSO) and is its major metabolite in all species

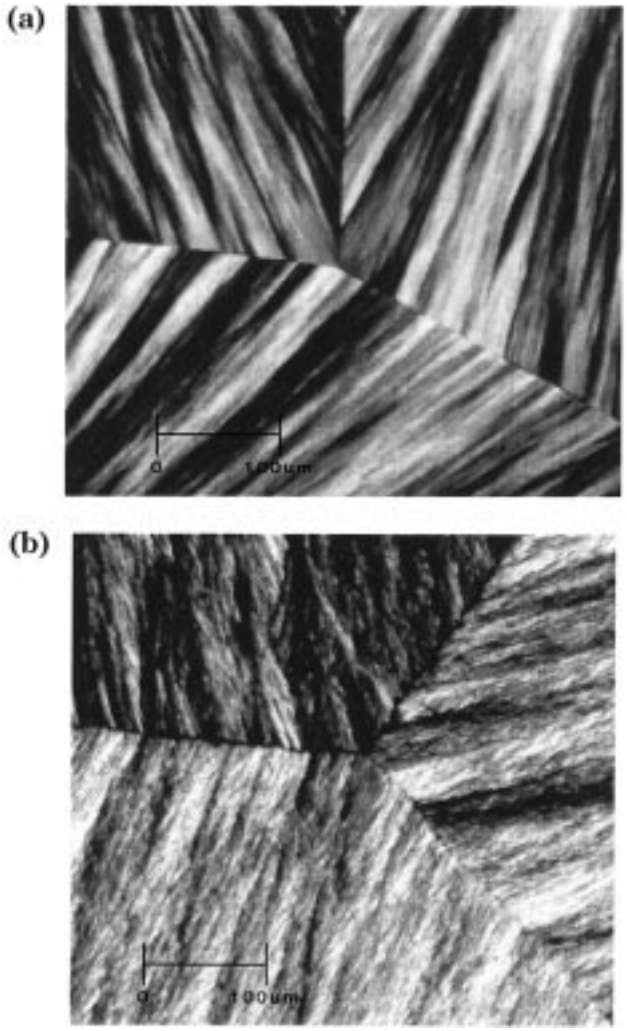

Figure 6—(a) Eutectic viewed with polarized light after crystallization between a glass slide and coverslip. (b) Eutectic with 5% phenytoin.

studied.[8] $DMSO_2$ was first identified as a natural constituent of biological systems in 1934 when Pfiffner and Vars isolated it from beef adrenals.[9] It was later isolated from beef blood and milk, and it has been indicated that human intake from these sources may be as high as a few milligrams daily.[8] The latter figure is consistent with Williams et al.'s finding of 4−11 mg of $DMSO_2$ in 24-h samples of human urine[10] and a reported circulating blood concentration of 0.2−0.5 ppm in the adult male.[11]

$DMSO_2$ has recently been tested for efficacy in the treatment of interstitial cystitis[11] without findings of any adverse effects. Bertken[8] also reported that a man who ingested approximately 4 g of $DMSO_2$ daily for 3 months suffered no ill effects. The only (related) report of toxicity that was found in the literature, other than the detrimental effects of $DMSO_2$ on nematode gamete production,[12] implicated dimethyl sulfate ($DMSO_4$) in a 1994 death.[13] However, as indicated by the authors of that paper, there is no established precedent for the direct conversion of $DMSO_2$ to $DMSO_4$.

Conclusion

The chlorobutanol hemihydrate−dimethyl sulfone ($DMSO_2$) eutectic was determined to be a suitable medium for lyophilizing drugs that are poorly water soluble and/or highly water sensitive. The eutectic is formed at a 60:40 mole ratio (75:25 weight ratio) and has a melting point of

50 °C. Lyophilization can be accomplished without refrigeration, and thus, without conventional freeze-drying equipment, because the melting point is above room temperature. The solvents are rapidly and thoroughly removed under vacuum with moderate heating. Two drying stages were used in the current study, both at a pressure of 0.3 mmHg. The first stage was at 40 °C to primarily remove the chlorobutanol hemihydrate, and the second was at 60 °C to remove the bulk of the $DMSO_2$. Each drying stage required approximately 6.5 h using standard 10 mL freeze-drying vials and solvent volumes of ∼5 mL. The cakes produced from the eutectic contain <1% residual solvent and are rapidly reconstituted. Trace amounts of residual solvent are below harmful levels.

References and Notes

1. Seager, H.; Taskis, C. B.; Syrop, M.; Lee, T. J. Structure of Products Prepared by Freeze-Drying Solutions Containing Organic Solvent. *J. Parenter. Sci. Technol.* **1985**, *39*, 161−181.
2. *The Handbook of Pharmaceutical Excipients*; Wade, A., Weller, P. J., Eds.; The Pharmaceutical Press: London, 1994.
3. *The Merck Index*, 12th ed.; Budavari, S., Ed.; Merck Research Laboratories: Whitehouse Station, NJ, 1996.
4. Adjei, A.; Newburger, J.; Martin, A. Extended Hildebrand Approach: Solubility of Caffeine in Dioxane−Water Mixtures. *J. Pharm. Sci.* **1980**, *69*, 659−661.
5. Fedors, R. F. A Method for Estimating Both the Solubility Parameters and Molar Volumes of Liquids. *Polym. Eng. Sci.* **1974**, *14*, 147−154.
6. Borody, T.; Chinwah, P. M.; Graham, G. G.; Wade, D. N.; Williams, K. N. Chlorobutanol Toxicity and Dependence. *Med. J. Australia.* **1979**, *7*, 288.
7. Nordt, S. P. Chlorobutanol Toxicity. *Ann. Pharmacother.* **1996**, *30*, 1179−1180.
8. Bertken, R. "Crystalline DMSO": $DMSO_2$. Arthritis Rheum. **1983**, *26*, 693−4.
9. Pfiffner, J. J.; Vars, H. M. Extraction Studies on Adrenal Cortical Hormone; Disrtibution Studies. *J. Biol. Chem.* **1934**, *106*, 645.
10. Williams, K. I. H.; Burstein, S. H.; Layne, D. S. Dimethyl Sulfone: Isolation forom Human Urine. *Arch. Biochem. Biophys.* **1966**, *113*, 251−252.
11. Childs, S. J. Dimethyl Sulfone ($DMSO_2$) in the Treatment of Interstitial Cystitis. *Urol. Clin. North Am.* **1994**, *21*, 85−88.
12. Goldstein, P.; Magnano, l.; Rojo, J. Effects of Dimethyl Sulfone ($DMSO_2$) on Early Gametogenesis in *Caenorhabditis Elegans*: Ultrastructural Aberrations and Loss of Synaptonemal Complexes from Pachytene Nuclei. *Reprod. Toxicol.* **1992**, *6*, 149−159.
13. Grant, P. M.; Haas, J. S.; Whipple, R. E.; Andrensen, B. D. A Possible Chemical Explanation for the Events Associated with the Death of Gloria Ramirez at Riverside General Hospital. *Forensic Sci. Int.* **1997**, *87*, 219−237.

JS980478P