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Use of pure *t*-butanol as a solvent for freeze-drying: a case study

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Abstract

1-(2-Chloroethyl)-3-sarcosinamide-1-nitrosourea, (SarCNU) (NSC-364432) is a new antitumor drug that is of interest to the National Cancer Institute. It is intended for use as an intravenous injection. Although SarCNU is sufficiently soluble in water to obtain the desired dosage, it is highly unstable. Its T_{90} in aqueous solution at room temperature is less than 6 h. Neat tertiary butyl alcohol (TBA), a low toxicity, high vapor pressure and low melting solvent, was determined to be an excellent freeze-drying medium. Lyophilization of SarCNU from pure TBA produces a uniform cake composed of needle-shaped crystals. Thermal analysis and gas chromatography indicate that the cake contains less than 0.001% residual solvent. The SarCNU cake can be readily reconstituted with either water or an aqueous solution of 40% propylene glycol and 10% ethanol. The reconstituted solutions are stable for 4 and 13 h, respectively.

Keywords: 1-(2-Chloroethyl)-3-sarcosinamide-1-nitrosourea; Lyophilization; Tertiary butyl alcohol

1. Introduction

1-(2-Chloroethyl)-3-sarcosinamide-1-nitrosourea (SarCNU) (Fig. 1) was selected for formulation by the National Cancer Institute because of its therapeutic advantage in the treatment of malignant glioma. Most nitrosoureas are stable in acidic media at pH 3–5, and in nonaqueous solvents such as methanol, ethanol (EtOH), and tertiary butyl alcohol (TBA), but they are very labile in neutral aqueous media (Bosanquet, 1985). SarCNU is a chloroethylnitrosourea that is methylated in the *N*-3 position to make it more stable. In spite of its being more stable than other nitrosoureas, we have found that it still degrades rapidly in aqueous media, especially in the presence of light and at high temperatures. Although it is soluble in aqueous media, the instability of SarCNU prevents the formulation of the drug in an aqueous or a semi-aqueous vehicle.

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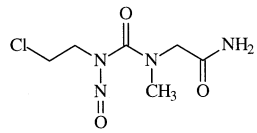

Fig. 1. Structure of SarCNU.

Generally a product is freeze-dried, if it is not stable in aqueous media. Although inherently expensive in terms of manufacturing costs, freeze-drying is often the processing method of choice for the production of an unstable parenteral product. Generally a reconstitutable freeze-dried product has improved stability, dosing accuracy, and rapid reconstitution. However, freeze-drying is commonly restricted to drugs that are soluble and stable in an aqueous medium for at least the time required for dissolution, filtration, filling, and freezing of the product.

The ideal freeze-drying medium has a high vapor pressure, a melting point either below or slightly above room temperature, a high viscosity, and a low toxicity. It must provide a stable environment for freeze-drying and be rapidly and completely removed to produce a readily reconstitutable cake. In this report, we will show that neat TBA is an excellent nonaqueous solvent for freeze-drying highly water unstable drugs, such as SarCNU.

2. Experimental

2.1. Materials

SarCNU (NSC-364432) was provided by the Pharmaceutical Resources Branch, Developmen-

Table 2
Vapor pressure of TBA and water

Temperature (°C)	TBA[a]	Water[b]
	Vapor pressure (mmHg)	Vapor pressure (mmHg)
−20.5	1	0.74
−3.0	5	3.57
5.5	10	6.77
14.5	20	12.38
24.4	40	22.92
31.0	60	33.69
39.8	100	54.74
52.5	200	104.65
68.0	400	214.17
83.0	760	400.60

[a] All data were obtained from reference (Chemical Engineer's Handbook, 1973).

[b] All data were obtained from reference (Lange's Handbook of Chemistry, 1992).

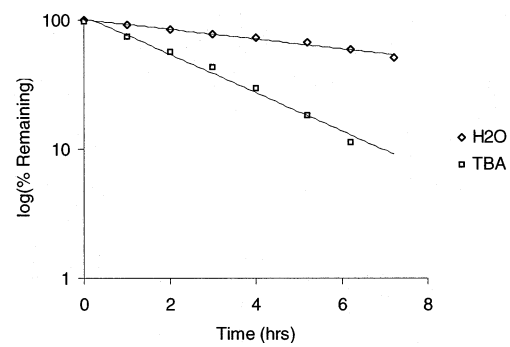

Fig. 2. Sublimation of TBA and H_2O during the primary drying (− 20 °C and 60 mTorr).

Table 1
The physical properties of the solvents

Name	Molecular weight	Melting point (°C)	Boiling point (°C)	Density (g/ml) 25 °C	Vapor pressure (mmHg) 25 °C	Viscosity (mPa·s) 20 °C
H_2O	18.02	0	100	0.9970	23.78	1.00
HAc	60.05	17	118	1.0429	15.75	1.31
TBA	74.12	25	82	0.7812	41.25	3.62
DMSO	78.14	19	189	1.0955	0.60	2.20

All data were obtained from reference (Handbook of Chemistry and Physics, 1991–1992), except for the viscosity of TBA that was experimentally determined.

tal Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). 'Baker Analyzed' *tert*-butyl alcohol was at least 99.9% pure. All other chemicals were analytical or high pressure liquid chromatographic assay (HPLC) grade.

Table 3
Degradation of SarCNU in different solvents at 25 °C

Solvent	Degradation rate constants (per day)	T_{90} (days)
HAc	1.8324	0.06
DMSO	0.0912	1.16
MeOH	0.0216	4.88
H_2O	0.4128	0.26
20% TBA/H_2O	0.2880	0.36
50% TBA/H_2O	0.1032	1.02
80% TBA/H_2O	0.0336	3.14
TBA	0.0072	14.93

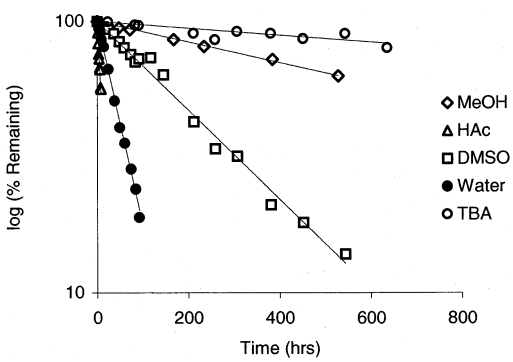

Fig. 3. Degradation of SarCNU in different solvents at 25 °C.

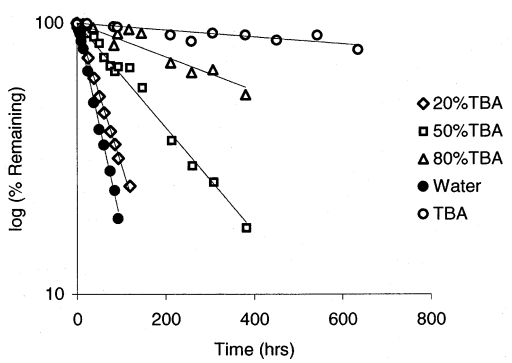

Fig. 4. Degradation of SarCNU in TBA/water mixture at 25 °C.

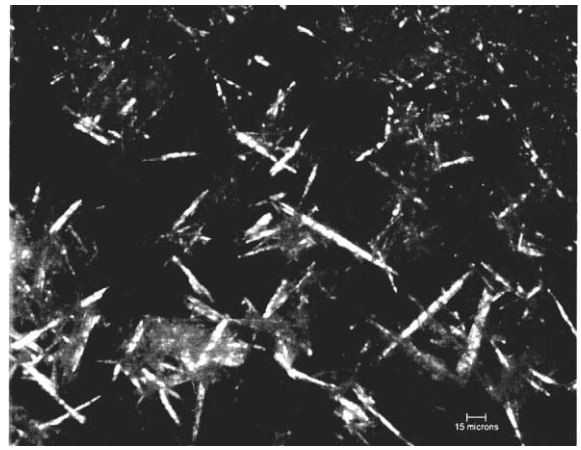

Fig. 5. Crystallinity of the cake.

2.2. Freeze-drying process

A laboratory freeze-drier (Virtis 15 SRC-X, The Virtis Co., Inc. Gardiner, NY) was used. The freeze-drying process was as follows: (1) freezing at −20 °C for 24 h; (2) primary drying at −20 °C for 10 h; and (3) secondary drying at 10 °C for 10 h and then 20 °C for 5 h. The cooling and heating rate was not controlled during freezing. The chamber pressure was maintained at 60 mTorr throughout the drying process. Solutions of SarCNU were prepared at a concentration of 5.0 mg/ml in TBA. The amber freeze-drying vials (Serum vials, Borosilicate Glass, Kimble) were 10.0 ml with a fill volume of 1.0 ml and were covered using rubber stoppers (Gray Buty Rubber Stoppers, Kimble).

2.3. Thermal Analysis

Differential scanning calorimetry (DSC) was conducted on 3–5 mg samples. The samples were placed in sealed aluminum pans and an empty sealed aluminum pan was used as a reference. They were heated at 10 °C/min using a TA Instruments DSC 910 (TA, Instruments, Inc., New Castle, DE).

Thermogravimetric analysis (TGA) was conducted on 3–5 mg samples in open aluminum pans at a heating rate of 10 °C/min using a TA Instruments TGA 951 (TA, Instruments, Inc.).

2.4. Analysis of sublimation of TBA and water during primary drying

Preweighed 10 ml freeze-drying vials were filled with 1 ml of TBA or water, reweighed and transferred to the freeze-dryer. The solutions were cooled at −20 °C over night to allow for complete solidification. Sublimation of the solutions was undertaken at a shelf temperature of −20 °C and a chamber pressure of 60 mTorr. Samples that were removed at various stage of drying were weighed and the weight loss was recorded.

2.5. Polarizing microscope and camera

Photomicrographs of the freeze-dried cakes ∈were taken using a SPOT camera and a Leica DMLP polarizing microscope (E. Licht Co., Denver, CO).

2.6. Gas chromatographic assay

A gas chromatographic assay (GC) with flame ionization detection (Hewlett Packard 5890, series II, Hewlett Packard Inc., Wilmington, DE) was used to measure the amount of residual TBA. The column used was a Supelco Simplicity-Wax Fused Silica Capillary Column, 30 m, 0.25 mm ID, 0.25 μm film (Lot no. 12130-08A) (Supelco Inc., Bellefonte, PA). Three aqueous solutions: TBA/SarCNU, pure drug, and the cake were prepared. A PIERCE Reacti-Therm™ Stirring/heating module (Pierce Inc., Rockford, IL) was used to heat the sample solution at 85 °C for 1 h, until a saturated vapor was obtained. The injection volume was 100 μl.

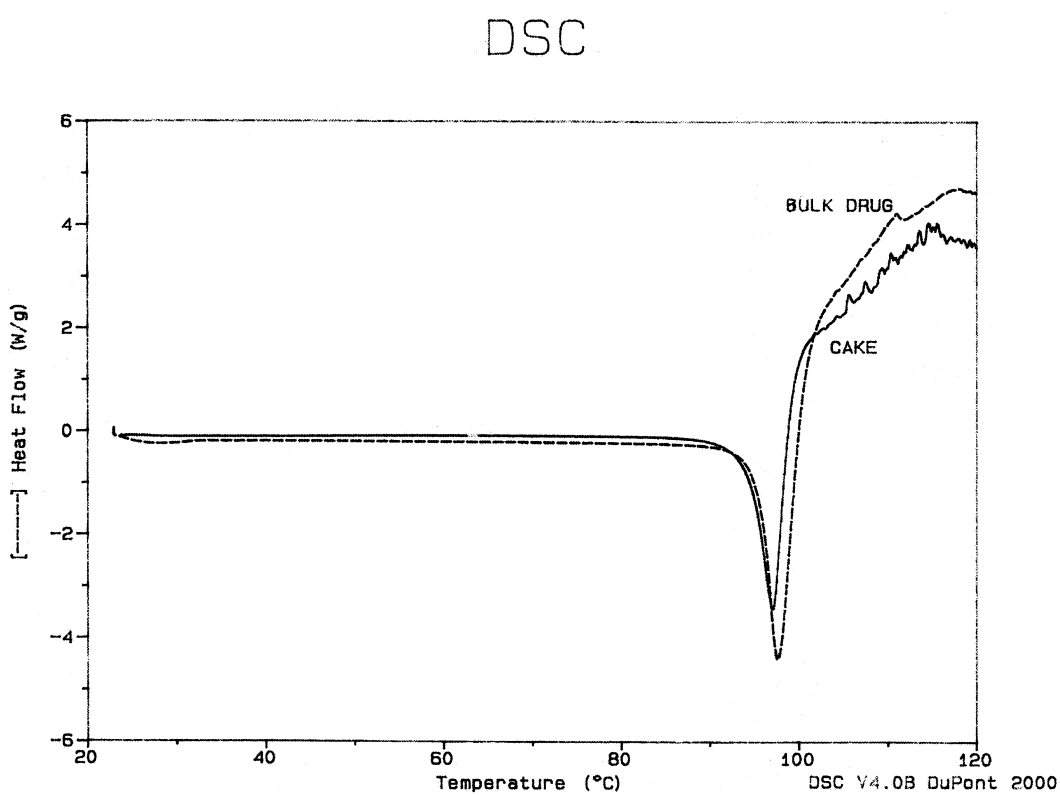

Fig. 6. DSC thermograph of SarCNU.

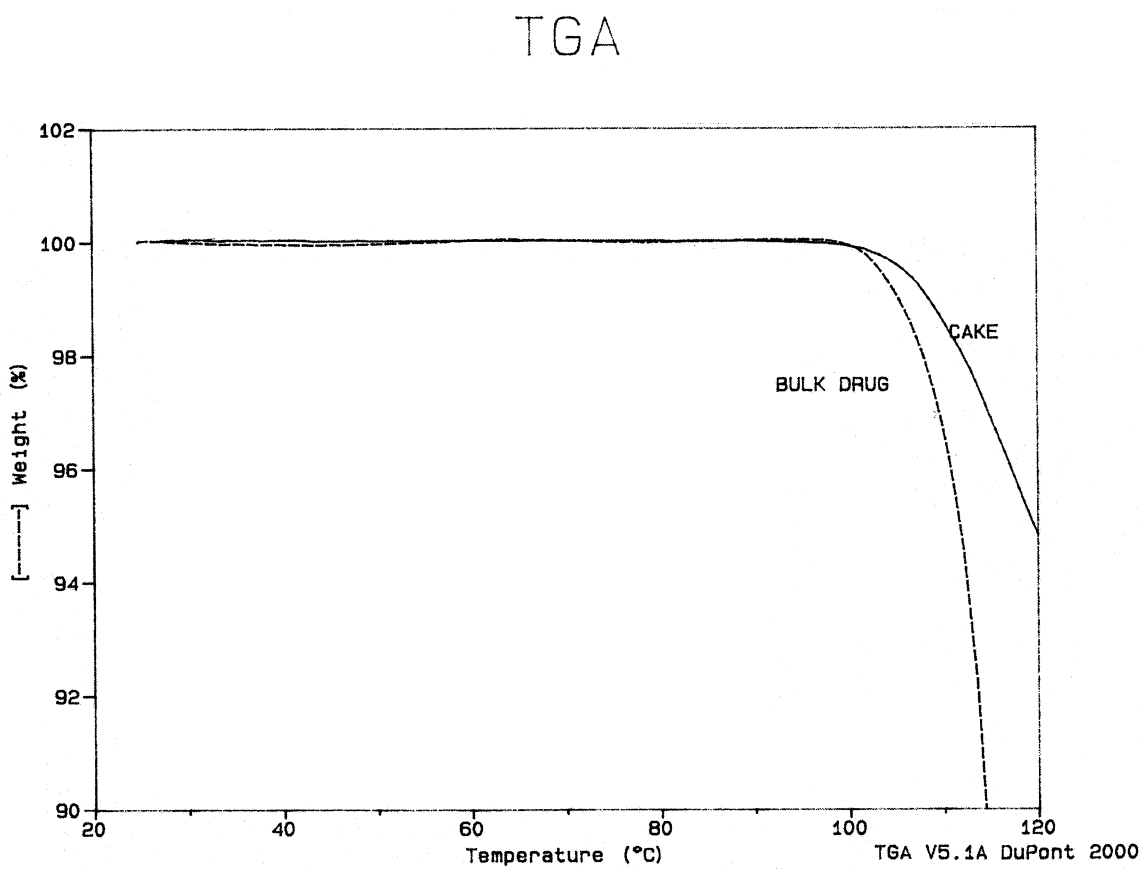

Fig. 7. TGA thermograph of SarCNU.

2.7. High pressure liquid chromatographic assay

The gradient high pressure liquid chromatography assay of Peninsula Laboratories has been modified for SarCNU as described below: A Beckman System Gold (Beckman Instruments Inc., Fullerton, CA) equipped with a model no. 167 detector at 254 nm was used for HPLC analysis. The injection volume was 20 μl. Separations were achieved on a Restek Pinnacle ODS Amine column (5 U, 4.6 × 250 mm^2) (Restek Inc., Bellefonte, PA) at room temperature with a flow rate of 1.0 ml/min. The initial composition of the mobile phases was 9 parts of 1% acetic acid and 1 part acetonitrile. This was changed over a period of 20 min to the final composition of 1 part of 1% acetic acid and 9 parts of acetonitrile. Samples were dissolved in methanol and filtered through a 0.45 μm nylon filter membrane (Alltech Associates Inc. Deerfield, IL) before injection. The observed relative retention time of SarCNU was 9 min.

3. Results and discussion

3.1. Selection of a freeze-drying medium

Water and three possible alternate freeze-drying vehicles: glacial acetic acid, TBA, and dimethyl sulfoxide (DMSO) were chosen for evaluation. Table 1 shows the physical properties of water and the three nonaqueous vehicles considered. Each of these relatively nontoxic solvents has a low molecular weight, a melting point that is only slightly lower than room temperature, and the

ability to readily sublime. Solvents with high vapor pressures sublime rapidly and thus accelerate the freeze-drying process. The cooling produced by rapid sublimation also helps to prevent the collapse of the cake by helping to keep the temperature of the cake below the collapse temperature. Solvents with a high viscosity are likely to reduce collapse or crystallization of amorphous drugs by inhibiting viscous flow during the formation of the cake. Table 1 shows that TBA has the highest viscosity as well as the highest vapor pressure. In addition, it was observed that TBA forms small loosely packed needle-shaped crystals. This further decreases the resistance of the partially dried solids to further drying, because the needle-shaped crystals sublime to leave a highly porous matrix. Since this matrix has a low resistance to vapor transfer and a large surface area, both primary and secondary drying are efficient and rapid. Thus, as a result of its high vapor pressure (Table 2) and its crystal morphology the sublimation rate of TBA is more than 2.5 times greater than that of water as shown in Fig. 2. Although TBA has been used as an aqueous cosolvent in freeze-drying (Kasraian and DeLuca, 1994; Wittaya-Areekul and Nail, 1998), it is rarely used as a neat vehicle. This may be due to the fact that it is solid at most ambient temperatures. However, Tesconi and Yalkowsky (Tesconi et al., 1999) have shown that solid vehicles can be successfully used for freeze-drying.

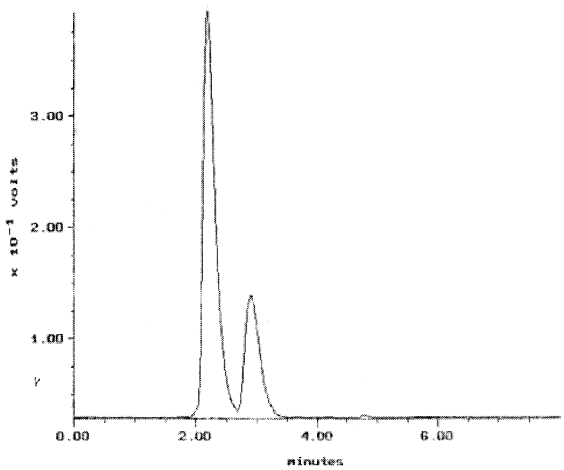

Fig. 8. GC of aqueous solution containing 0.7 g/100 ml SarCNU and 0.1 ml/100 ml TBA.

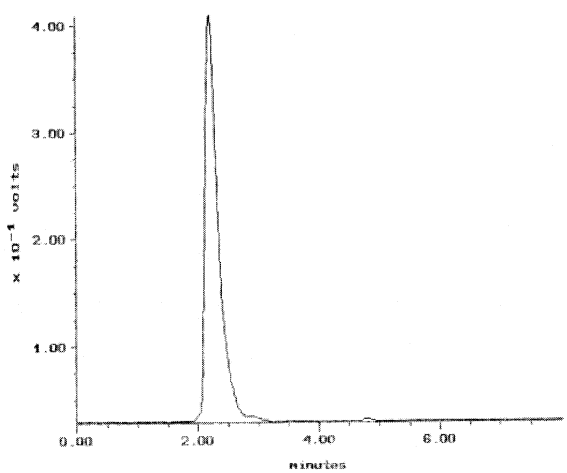

Fig. 9. GC of the cake aqueous solution containing 0.7 g/100 ml SarCNU cake.

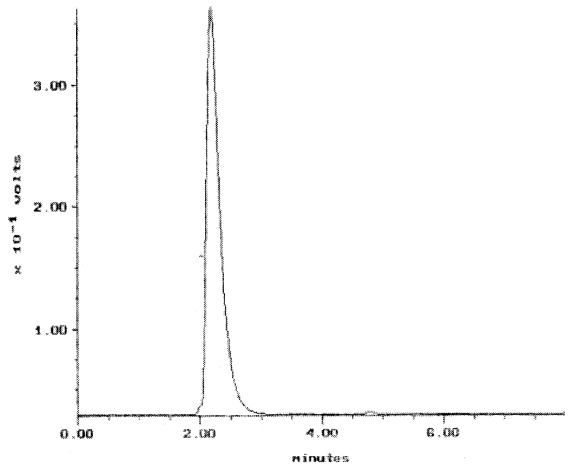

Fig. 10. GC of the pure drug aqueous solution containing 0.7 g/100 ml SarCNU.

3.2. Freeze-drying cake

No cake was formed when water was used to freeze-dry SarCNU. It was found that higher concentrations of TBA in TBA–water mixtures improve cake quality and the most uniform cake is produced from pure TBA. It is believed that the poor quality of cake formed in the presence of water is due to the lower vapor pressure and the

lower viscosity of water even though the primary drying temperature was maintained below the collapse temperature. The stronger interaction of water with SarCNU may also contribute to the reduction of cake quality. The rapid sublimation of TBA helps to keep the product temperature below the collapse temperature. (For a crystalline drug, the collapse temperature is the eutectic temperature and for an amorphous drug, the collapse temperature is the glass transition temperature.) The high viscosity of TBA is also believed to help to prevent collapse by reducing viscous flow during the formation of the cake. Table 3 and Figs. 3 and 4 show that SarCNU is more stable in pure TBA than in any of the other pure solvents or in any of the TBA–water mixtures.

Fig. 5 shows that needle-shaped crystals of SarCNU were obtained after freeze-drying with TBA. The DSC scans in Fig. 6 show that there is no significant difference between the crystals of the cake and those of the pure bulk drug. The high surface area of the crystals in the cake ensures rapid reconstitution. The drug can be stored at 4 °C over 6 months without any measurable physical or chemical change and at 25 °C with some cake collapse but without any chemical degradation. The drug also can be stored at 4 °C over 12 months with some cake collapse but without any chemical degradation. A possible reason for the collapse of the cake is that small amounts of SarCNU (which melts at 93 °C) slowly sublime and recrystallize in a process similar to caking or sintering. This process is faster at room temperature than in the refrigerator. The cake is readily reconstituted with water. However, the resultant SarCNU solution has a T_{90} of only 4 h. The cake can also be reconstituted with an aqueous solution of 40% propylene glycol (PG) and 10% EtOH. This solvent, which is less polar and has less water, was chosen to minimize hydrolysis. It gives a T_{90} of 13 h for SarCNU. In either case, both the 4 and 25 °C products can be reconstituted within 30 s with only gently shaking.

3.3. Residual solvent

The TGA scans in Fig. 7 show no detectable TBA in the SarCNU cake. The gas chromatograms of Figs. 8–10 confirm that there is less than 0.001% TBA in the cake. The absence of TBA in the cake results from its ability to form high surface area crystals and from the fact that the intermolecular forces among TBA molecules and between TBA and SarCNU are not as strong as those of water. This allows TBA to sublime more completely and easily than water. The detrimental effects of TBA on health are minimal (Faulkner et al., 1989). The 0.001% TBA (0.05 μg TBA per cake) that is left in the cake is much lower than the toxic level. It has also been reported that a volatile organic solvent such as TBA can significantly reduce microbial contamination during filling or drying (Olson, 1997).

3.4. Recovery

Samples were assayed by the HPLC method described above before freeze-drying and after reconstitution. Only about 4% of the drug was lost during the lyophilization process. The same recovery ratio was obtained by repeating the freeze-drying process. Most of the drug loss is believed to be due to sublimation as evidenced by the fact that the chromatograms show no evidence of degradation of SarCNU and that it can be detected on the condenser after the TBA is removed.

4. Conclusion

TBA can improve the solubility and stability of hydrophobic and/or water sensitive drugs that must be freeze-dried. The needle-shaped crystals of TBA allow it to freeze and dry quickly and completely. There is less than 0.001% residual solvent left in the cake after secondary drying. The rapid sublimation of TBA prevents the collapse of the cake. The high crystallinity of the drug formed after freeze-drying from TBA increases its stability and shelf life. The SarCNU cake is readily reconstituted with water or an aqueous solution containing 40% PG and 10% EtOH. Thus, based upon its high volatility, high viscosity, crystal morphology and low reactivity, TBA is determined to be a suitable freeze-drying

medium for SarCNU, and perhaps for other poorly water stable and poorly water-soluble drugs.

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References

Bosanquet, A.G., 1985. Stability of solutions of antineoplastic agents during preparation and storage for in vitro assays. Cancer Chem. Pharm. 14, 83–95.

Chemical Engineer's Handbook, fifth ed., 1973. R.C. Weast (Ed.) McGraw-Hill, New York, pp. 3–50.

Faulkner, T.P., Wiechart, J.D., Hartman, D.M., 1989. The effects of prenatal tertiary butanol administration in CBA/J and CS7BI/6J mice. Life Sci. 45, 1989–1995.

Handbook of Chemistry and Physics, 72nd ed., 1991–1992. Chemical Rubber Publishing Company.

Kasraian, K., DeLuca, P.P., 1994. The effect of tertiary butyl alcohol on the resistance of the dry product layer during primary drying. Pharm. Res. 12 (4), 491–495.

Lange's Handbook of Chemistry, 14th Ed., 1992. J.A. Dean (Ed.) McGraw-Hill, New York, pp. 5.26–5.29.

Olson, W.P., 1997. Volatile solvents for drying and microbial kill in the final container: a proposal and a review, with emphasis on tert-butyl alcohol. Pharm. Eng. July/August, 112–117.

Tesconi, M.S., Sepassi, K., Yalkowsky, S.H., 1999. Freeze-drying above room temperature. J. Pharm. Sci. 88 (5), 501–506.

Wittaya-Areekul, S., Nail, S.L., 1998. Freeze-drying of tert-butyl alcohol/water cosolvent systems: effects of formulation and process variables on residual solvents. J. Pharm. Sci. 87 (4), 491–495.