AAPS PharmSciTech **2003; 4 (4) Article 52 (http://www.pharmscitech.org).**

Liposome Formulation of NSC-639829 Using Halothane as a Solvent: A Technical Note

Submitted: June 24, 2003; Accepted: August 19, 2003

Akash Jain,[1] Tapan Sanghvi,[1] and Samuel.H.Yalkowsky[1]

[1]College of Pharmacy, The University of Arizona, Tucson AZ 85721

KEYWORDS: halothane, chloroform, liposome, ether

INTRODUCTION

Liposomes are self-assembling vesicles with an inner aqueous compartment surrounded by a lipid bilayer that commonly consists of phospholipids[1] and cholesterol. Both hydrophilic and hydrophobic drugs can be effectively encapsulated into liposomes. Hydrophilic drugs can be encapsulated into the interior aqueous compartment, whereas lipophilic and amphiphilic drugs can be embedded in the liposome bilayers.[2] In either case, the systemic environment recognizes only the liposomes and not the free drug. The pharmacokinetic profile of the drug is determined by the physicochemical properties of the liposomes. In addition to low intrinsic toxicity and protection of drugs from degradation in the systemic circulation, liposomes accumulate in the tumor cells (enhanced permeation and retention effect)[3] making them a desirable delivery system for anticancer drugs. This enhanced permeation and retention of liposomes is due to the leaky blood vessels in the tumors, less dense cellular packing, and marginal expression of the lymphatic system (which removes liposomes from the tissues and organs) at tumor sites.[4]

The most traditional method of liposome formulation is the thin-film hydration method.[1] This method involves dissolving lipids and the lipophilic drug in a volatile solvent, evaporating the solvent to obtain a thin film, and hydrating the dried thin film with an aqueous vehicle to obtain the crude liposome formulation. Chloroform (vapor pressure 160 mm Hg, boiling point (BP) 61.7°C)[5,6] is the most widely used solvent for this method. But, there are potential problems of carcinogenicity[7] associated with its use and it is very difficult to get rid of chloroform entirely during the preparation. Another commonly used solvent is ether (vapor pressure 442 mm Hg, BP 34.8°C),[5,8] which is highly flammable and poses safety hazards. Ethanol can also be used as a solvent for liposome formulation. However, the drug loading cannot be achieved with thin-film hydration method because of the limited solubility of phospholipids in ethanol. As a possible remedy to these problems, a method for liposome formulation using halothane has been developed. Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) belongs to the class of inhalation anesthetics and is a highly volatile compound (vapor pressure 243 mm Hg, BP 50.2°C).[5,9] At the same time, it is nonflammable and is not classified as a carcinogen. Halothane can be easily and completely evaporated during the formulation process without any safety threats. Recently, Halothane was used for the liposome formulation of 2-4'-Amino-3'-Methylphenyl Benzothiazole (AMPB) (an anticancer compound).[7] **Table 1** lists the properties of the 3 solvents used in this study.

NSC-639829 (N-[4-(5-bromo-2-pyrimidyloxy)-3-methylphenyl]-(2-dimethylamino)-benzoylurea) (**Figure 1**) is an investigational antitumor agent that is believed to prevent the formation of the mitotic spindle during cell division by inhibiting tubline polymerization and causing microtubule depolymerization.[11] NSC-639829 demonstrates poor water solubility (30 ng/mL) and is highly lipophilic (CLOGP 6.21).[12] This study presents another example of a liposome formulation for an antitumor agent (NSC-639829) using halothane, thereby validating the use of halothane as an alternate solvent in liposome formulation.

Corresponding Author: Akash Jain, 1703 E Mabel Street, Room #441, Tucson, AZ 85721. Phone: (520) 626-4308; Fax: (520) 626-4063;
Email: akash.jain@pharmacy.arizona.edu

MATERIALS AND METHODS

NSC-639829 was provided by the National Cancer Institute and used as received. L-α-phosphatidylcholine (PC) (MW 760.09) was purchased from Avanti Polar-Lipids (Alabaster, AL). Cholesterol (Chol) (95%) was

Table 1. Solvent Properties

Properties	Chloroform	Ether	Halothane
Chemical name	Trichloromethane	Diethyl ether	2-bromo-2-chloro-1,1,1-trifluoroethane
Chemical formula	$CHCl_3$	$C_4H_{10}O$	$C_2HBrClF_3$
Molecular weight	119.38	74.12	197.38
Boiling point (°C)	61.7[5]	34.6[5]	50.2[5]
Vapor pressure (mm Hg @ 20°C)	160[6]	442[8]	243[9]
Flammability	Nonflammable[5]	Flammable[5]	Nonflammable[5]
Carcinogenicity	Carcinogen[10]	Noncarcinogen[10]	Noncarcinogen[10]

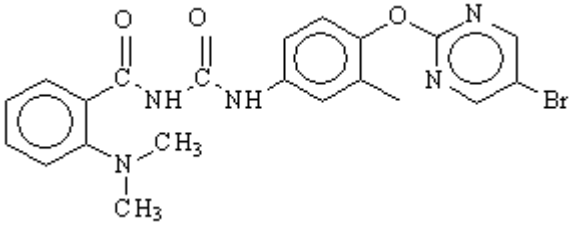

Figure 1. Chemical structure of NSC-639829.

purchased from Aldrich Chemical (Milwaukee, WI). Halothane was purchased from Sigma (St Louis, MO). Sodium chloride injection, 0.9% (normal saline) was purchased from Baxter Healthcare (Deerfield, IL). All other reagents used were analytical or high performance liquid chromatography (HPLC) grade.

Four different liposome formulations were prepared using halothane, chloroform and ether (**Table 2**).

Thin-Film Formation

The thin-film hydration method was used to prepare all liposomes. Solutions of drug, phospholipids, and cholesterol in chloroform, ether, or halothane were prepared and transferred to a 250-mL round bottom flask with a ground glass neck. The flask was attached to a rotary evaporator (Rotovapor-R 110, Brinkmann Instruments, Westbury, NY), immersed in a 45.0°C water bath, and rotated under vacuum at a speed of 90 rpm. This was continued until all the liquid evaporated and a dry thin lipid film was deposited on the walls of the flask. The flask was then left in a vacuum desiccator overnight to ensure complete removal of residual solvent.

Film Hydration

Hydration of the thin film with normal saline was done using a bath sonicator with mild heating. Complete hydration was achieved in 30 minutes to 1 hour (depending upon the film thickness and uniformity), resulting in a coarse liposome suspension.

Optical Microscopy

Formation of multilamellar vesicles was confirmed by examining the suspension under an optical microscope (Leica Microscopy and Scientific Instruments Group, Heerbrugg, Switzerland). Photographs of vesicles were taken using a SPOT camera (Spot Image Corp, Chantilly, VA).

Particle-Size Reduction

The desired particle-size (200-300 nm) was obtained by probe sonication (VIRSONIC 600 Ultrasonic Cell Disrupter, The VirTis Company, Gardiner, NY). The suspension was transferred to a 50 mL conical bottom plastic centrifuge falcon tube (Fischer Scientific, Pittsburgh, PA) and sonicated for 20 minutes using a 1/8-inch micro probe (The VirTis Company) at a power level of 4. All formulations were stored at 4°C.

Table 2. Compositions of the 4 Formulations*

Formulation	PC (mg)	Chol (mg)	Drug (mg)	Solvent	Solvent (mL)	Normal Saline (mL)
A	250	125	20	Chloroform	25	10
B	250	125	20	Ether	25	10
C	250	125	20	Halothane	25	10
D	250	125	20	Halothane	40	10

* PC indicates phosphatidylcholine and chol, cholesterol.

Particle-Size Measurement

Particle size measurements were performed using a Zetasizer 1000 (Malvern Instruments, UK). All measurements were carried out at 25°C with the following system: count rate, 150 ± 50 kilo counts per second; detector angle, 90.00; wavelength, 633.0 nm; and analysis mode, automatic continuous.

High Performance Liquid Chromatography Assay

The drug concentration in the formulation was determined by first separating the lipid and the aqueous phases by ultracentrifugation at 50 000 rpm and 10°C for 3.5 hours and measuring the drug concentrations in each phase using HPLC assay.[12] The HPLC system consisted of the following

- Beckman Gold HPLC system with a no. 168 detector (Beckman Coulter, Fullerton, CA);
- 250 × 4.6 mm Lichrosorb RP-18 column (Alltech Associates, Deerfield, IL) with a particle size of 10 μm;
- mobile phase, 90% methanol and 10% water;
- flow rate, 1.0 mL/min;
- detection wavelength, 254 nm; and
- injection volume, 20 μL

RESULTS AND DISCUSSION

The 4 liposome formulations obtained using chloroform, ether, and 2 halothane concentrations have similar physicochemical properties (ie, shape, size, incorporation efficiency). Multilamellar vesicles (MLVs) were obtained as a result of thin film hydration. Optical microscopy confirmed the formation of MLVs of similar morphology in all 4 formulations (**Figure 2**).

Probe sonication of these MLVs resulted in the formation of small unilamellar vesicles with particle sizes of 200 to 300 nm. Note that the differences seen in **Figure 2** are obliterated by the sonication, and the small unilamellar vesicles could not be seen under the optical microscope. As shown in **Figure 3**, the particle sizes for each of the 4 formulations remained within this range over a period of 4 months.

NSC-639829, a lipophilic compound, is expected to be incorporated within lipid bilayers. Incorporation efficiency (IE %) is a measure of the percentage incorporation of drug in lipid bilayers. It is calculated by the following equation:

$$IE\ (\%) = [C_{lipid} / (C_{lipid} + C_{aq})] \times 100 \quad (1)$$

Where C_{lipid} is the drug concentration in lipid phase and C_{aq} is the drug concentration in aqueous phase. Incorporation efficiencies of all 4 formulations were calculated to be about 100%.

Formulations A, B, and C were prepared using 25 mL of chloroform, ether, and halothane, respectively. Formulation D was prepared using a larger volume of halothane (40 mL) in order to obtain a thinner film. The excess amount of halothane was easily evaporated and a wider and thinner lipid film was produced that could be more easily dried and more readily hydrated. However, no significant differences were observed for the final products produced from the 2 halothane solutions.

CONCLUSION

Halothane has been successfully used as a solvent for the liposome formulation of NSC-639829. Liposomes with similar morphology, particle size, incorporation efficiency, and stability were obtained with halothane, chloroform, and ether. Halothane provides additional ease in formulation because of its higher volatility and safety as compared with chloroform and ether. Halo-

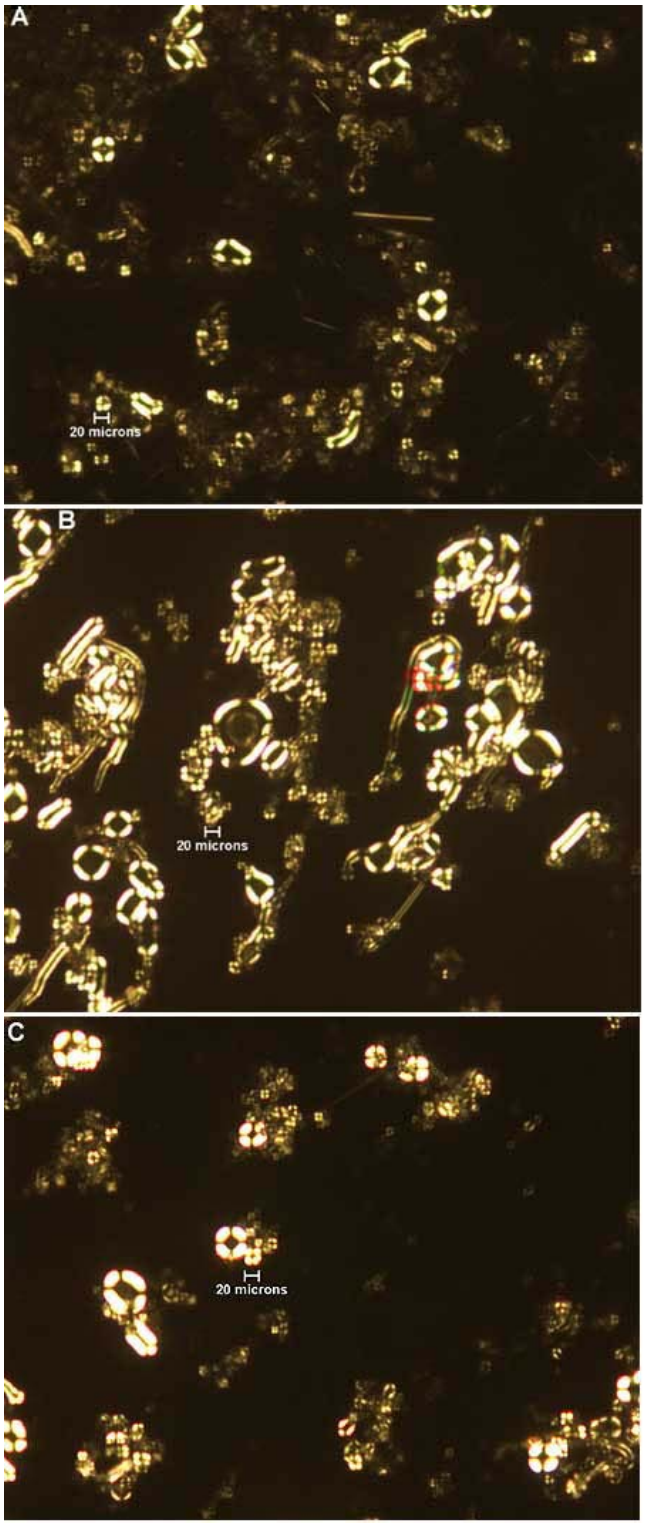

Figure 2. Liposomes containing NSC-639829 as seen under optical microscope (original magnification ×10): (A) chloroform, (B) ether, (C) halothane.

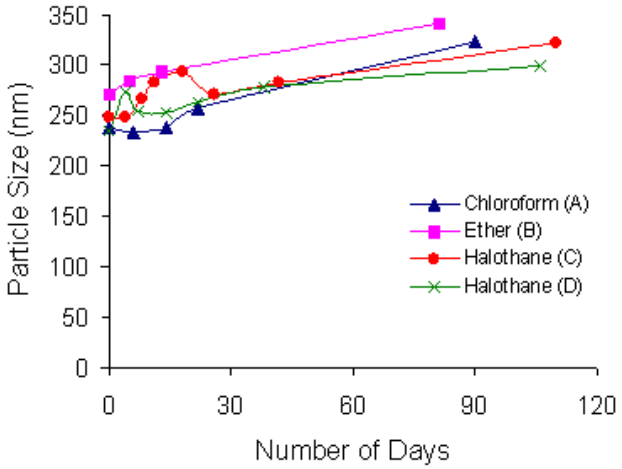

Figure 3. Particle size stability for the 4 formulations at 4.0°C.

thane can be regarded as a safe alternative to chloroform or ether in liposome formulation.

ACKNOWLEDGEMENTS

This study was supported by the National Cancer Institute contract number CM-77109.

REFERENCES

1. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol. 1965;13:238-252.
2. Massing U, Fuxius S. Liposome formulations of anticancer drugs: selectivity and effectiveness. Drug Resist Updat. 2000;3:171-177.
3. Jain RK. Transport of molecules in the tumor interstitium: a review. Cancer Res. 1987;47:3039-3051.
4. Gerlowski LE, Jain RK. Microvascular permeability of normal and neoplastic tissue. Microvasc Res. 1986;31:288-305.
5. Budavari S. The Merck Index. 12th ed. Whitehouse Station, NJ: Merck and Co; 1996.
6. Verschueren K. Environmental Data. Handbook of Environmental Data on Organic Chemicals. 2nd ed. New York, NY: Van Nostrand Reinhold; 1983: 178-179.
7. Ran Y, Yalkowsky SH. Halothane, a novel solvent for the preparation of liposomes containing 2-4'-amino-3'-methylphenyl benzothiazole (AMPB), an anticancer drug: a Technical Note. AAPS PharmSciTech. 2003;4(2):article 20.
8. Sax NI, Lewis RJ Sr. General Chemicals: Entries A-E. Dangerous Properties of Industrial Materials. 7th ed. New York, NY: Van Nostrand Reinhold; 1989: 1634-1635.
9. Medical Economics. Physicians' Desk Reference. 41st ed. Oradell, NJ: Medical Economics Co; 2002.
10. Occupational Safety and Health Administration. Chemical Sampling Information. Washington, DC: US Department of Labor; 1999.
11. Okada H, Kato M, Koyanagi T, Mizuno K. Synthesis and antitumor activity of water-soluble benzoylphenylureas. Chem Pharm Bull. 1999;47(3):430-433.

12. Jain N, Yang G, Tabibi ES, Yalkowsky SH. Solubilization of NSC-639829. Int J Pharm. 2001;225:41-47.