DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 8(4), 465-473 (1982)

LYOPHILIZATION - A MEANS OF INCREASING SHELF-LIFE OF PHOSPHOLIPID BI-LAYER VESICLES

Roger E. Gordon, Philip R. Mayer and Dane O. Kildsig
Department of Industrial and Physical Pharmacy
School of Pharmacy and Pharmacal Sciences
Purdue University
West Lafayette, IN 47907

ABSTRACT

The release rates of sodium stibogluconate were followed in lyophilized and non-lyophilized phospholipid bi-layer vesicles. The release rates were followed under the following environmental temperatures: $4^{o}C$, $19^{o}C$, $29^{o}C$, $35^{o}C$, $45^{o}C$, $50^{o}C$ and $55^{o}C$. Lyophilization did not significantly effect release rates within temperature groups 4^{o}, 19^{o}, 29^{o}, 35^{o} and $45^{o}C$. When the environmental temperature was varied above the tranisition temperature of the dipalmitoyl lecithin, release rates of sodium stibogluconate from lyophilized and non-lyophilized liposomes were found to be independent of temperature.

INTRODUCTION

Phospholipid bi-layer vesicles, or liposomes, have received considerable attention as potential carriers of drugs since thier discovery in 1962[1-3]. Research during the early 1970's on lipsomes climaxed with the conception that liposomes would not only function as carriers of toxic drugs but could also function as homing devices[4].

Thus, liposome drug delivery systems could render therapeutically toxic drugs, e.g., 5-Flurouracil, tolerable to the immune system while still eliciting toxic effects to tissue specific cells[5,6]. Conceptually, this idea was a major breakthrough in cancer treatment; however, as soon was discovered, the liposomes were concentrated in the liver and spleen. In addition, the entrapped drugs did not remain passively encapsulated by the phospholipids but slowly diffused across the bi-layer. While this latter problem may pose some difficulty from a dosage form development standpoint, it may be overcome by lyophilization of the liposome suspension.

The objective of this paper is to demonstrate that lyophilization of L-αPhosphatidyl choline dipalmitoyl liposomes does not significantly alter the physical arrangement of the liposome or the in vitro release of liposome-entrapped drug. The drug used in this study was sodium stibogluconate which is used in the treatment of leishmaniasis, a liver parasite. As such, it is an ideal candidate for a liposome delivery system as liposomes are concentrated in the liver. Sodium stibogluconate-liposome systems have been studied in hamsters and have been shown to effect a 700 fold reduction in the required dose[7].

MATERIALS AND METHODS

Liposomes

Lipids were purchased from the following sources: Synthetic L-α Phosphatidyl choline dipalmitoyl (DPPC) 98% pure from Sigma Chemical Company and cholesterol from Scientific Products. Sodium stibogluconate (Pentostam) powder and injection were graciously supplied by the Wellcome Foundation Ltd., Beckenham, Kent, England.

Unilamella liposomes were prepared by the chloroform-film method[8]. This method consists of the following steps. DPPC and

cholesterol were weighed out such that a total 150 mg of a 7:3 μmolar ratio, DPPC to cholesterol was obtained. The DPPC and cholesterol powder were dissolved in 3.0 ml of chloroform and allowed to form a thin lipid film by slowly evaporating off the chloroform at 46°C on a Buchii Rotavapor-M. With the lipid film completely dried, 2.0 ml of injectable sodium stibogluconate heated to 46°C was added to the Buchii flask. The dry film was collapsed into multi-layer liposomes with the aid of 0.5 mm glass beads and vortexed for 10 minutes. After 10 minutes of vortexing, the multi-layer liposome dispersion was sonified on a Heat System Ultrasonil Sonicator at a setting of 3 for approximately 3 minutes maintained at 46°C by a water bath to obtain what is considered to be unilamella liposomes.

The unilamella liposomes were separated from the unentrapped sodium stibogluconate on a Beckman L2-65B ultracentrifuge. The centrifuge was run at 40,000 rpm for 15 minutes at 4°C with a T65 rotor. The supernatant containing the free sodium stibogluconate was decanted. In order to minimize residual errors due to the physical entrapment of free sodium stibogluconate in the liposome pellet, the pellet was resuspended in 10 ml of sterile water and recentrifuged. The resulting pellet was again resuspended in 10 ml of sterile water and frozen immediately in a dry ice-acetone mixture. The frozen liposome system was lyophilized in a Virtis freeze dryer and the resulting powder was placed in a desiccator. The desiccator was evacuated and placed in a freezer until reconstituted with triple distilled water.

Alterations in the liposomal system resulting from lyophilization, if any, were determined by analyzing drug release rates from

lyophilized and control liposome systems at various environmental temperatures under sink conditions. The environmental temperatures were selected such that the lyophilized and control liposomal systems would experience release over a broad spectrum of its physical state. Thus, release studies were investigated at 4^{o}, 19^{o} and 29^{o}C which corresponds to a solid DPPC-cholesterol liposome system, and 35^{o}, 45^{o}, 50^{o} and 55^{o}C where the liposome system is in a liquid crystal state. Also, a scanning electron microscope equipped with an EDAX analyzer was employed to further investigate the effects of lyophilization on liposome structure.

Antimony Assay

Liposome samples and sodium stibogluconate standards were diluted with triple distilled water and assayed with a Perkin-Elmer Model 5000 atomic absorption apparatus. An antimony lamp operated at 15mA, wavelength of 217.6 nm, slit width of 0.2, and a sampling time of two seconds was used for analysis. The phospholipids did not interfere with the analysis of antimony. Total antimony was determined as it has also been reported that Sb^{3+} cannot be separated from Sb^{5+} via atomic absorption[9].

RESULTS

Drug Release From Lyophilized and Non-lyophilized Lipsome Systems

Figure I shows the kinetics of sodium stibogluconate release from liposomes in both lyophilized and non-lyophilized forms. The data points represent the means of three experimental determinations at time interval and temperature. The biphasic release profile characteristic of liposome systems was obtained. The initial fast release is assumed to be due primarily to surface desorption with diffusion through the lipid bilayers responsible for the second phase of the release curve.

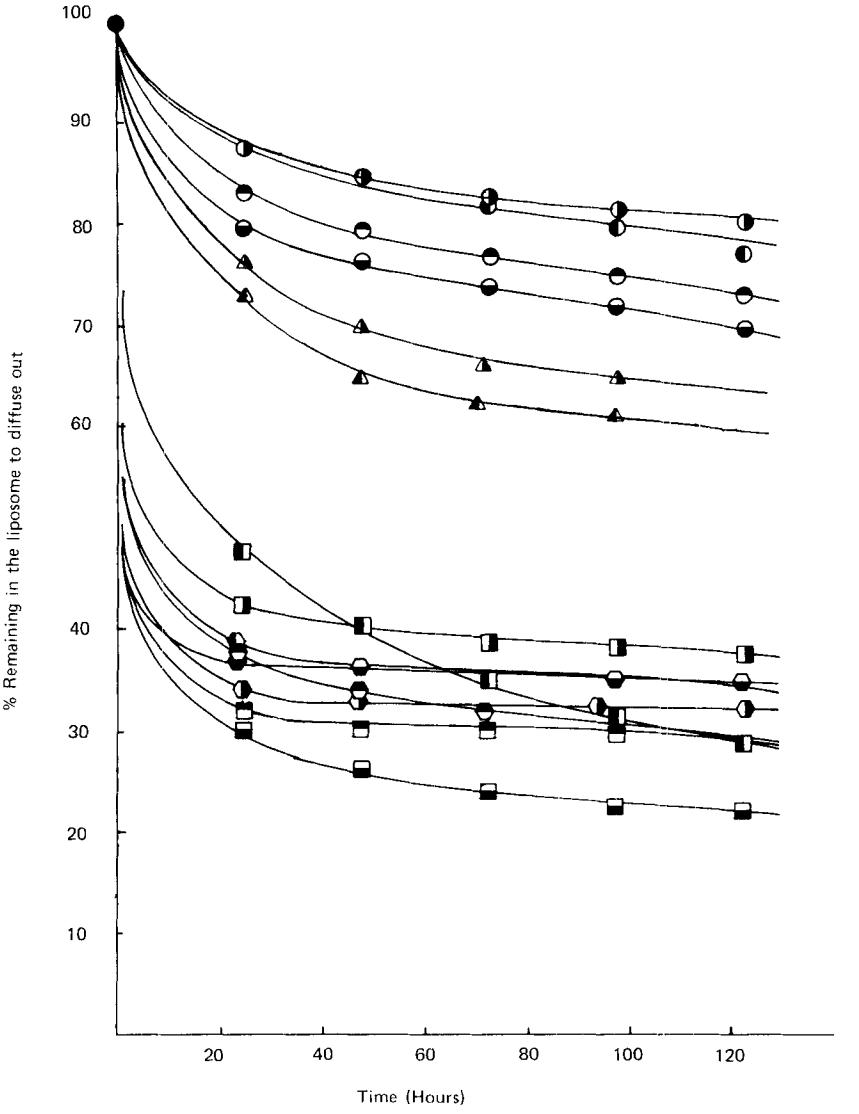

FIGURE I

The Release of Pentostam from Liposomes Prepared by the Film Method as a Function of Environmental Temperature.

◑ -4°C not lyophilized, ◐ -4°C lyophilized, ◓ -19°C not lyophilized, ◒ -19°C lyophilized, ◭ -29°C not lyophilized, ◭ -29°C lyophilized, ◨ -35°C not lyophilized, ◧ -35°C lyophilized, ⬒ -45°C not lyophilized, ⬓ -45°C lyophilized, ◑ -50°C not lyophilized, ◐ -50°C lyophilized, ◓ -55°C not lyophilized, ◒ -55°C lyophilized.

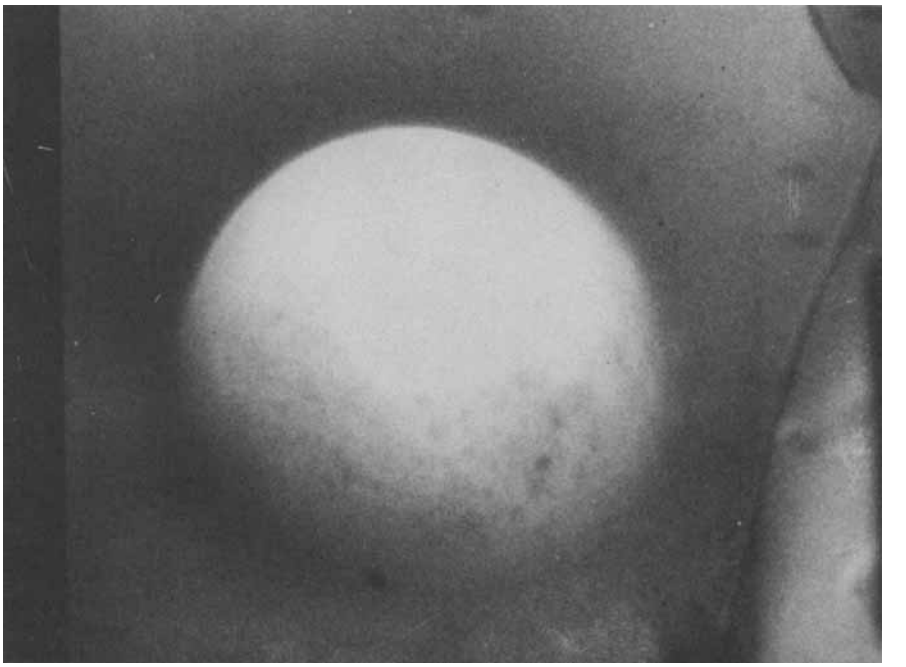

FIGURE II

Dipalmitoyl Phosphatidyl Choline-Cholesterol Liposome (7:3 μmolar ratio) Not Lyophilized. 23 μ in diameter.

Statistical testing of the kinetic data at each temperature (4^{o}, 19^{o}, 29^{o}, 35^{o}, 45^{o}, 50^{o}, 55^{o}) on the basis of release differences determined that lyophilization did not significantly alter the release of sodium stibogluconate from the liposomes. Actually, there did not appear to be a significant difference between any of the release profiles at 35^{o} and above. As the critical temperature for the DPPC-cholesterol liposome system is approximately 32^{o} [10], the liposomes at 35^{o}-55^{o} are in a fluid state. The relase of sodium stibogluconate from this fluid state does not appear to be affected by

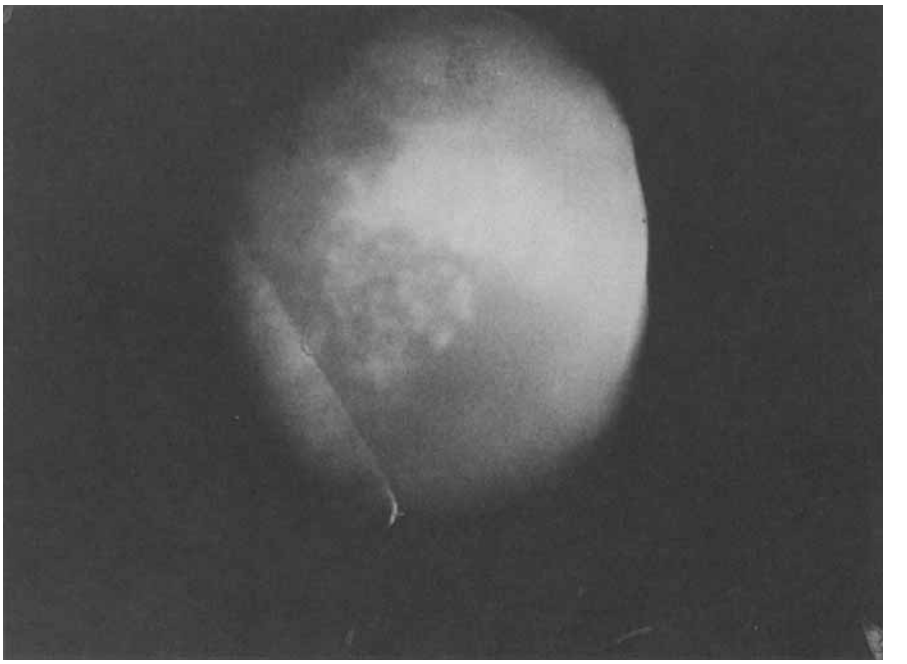

FIGURE III

Dipalmitoyl Phosphatidyl Choline-Cholesterol Liposome (7:3 μmolar ratio) Lyophilized and Reconstituted with Triple Distilled Water. 20 μ in diameter.

the 20° temperature change between 35° and 55°. However, the release of sodium stibogluconate from the liposomes is temperature dependent at temperatures below the transition temperature where the liposomes have solid character. This temperature dependence was observed only for the surface desorption step with little difference in the diffusion processes.

Scanning Electron Microscope Images

Figure II is an electron-micrograph of a DPPC-cholesterol liposome while Figure III is an electron micrograph of a lyophilized DPPC-

cholesterol liposome. Each figure was first analyzed with an EDAX system to insure image validity before photographing. Not only is no structural difference observed in the lyophilized liposome in Figure III,but there did not appear to be structural damage to any liposomes observed with the SEM.

DISCUSSION

It is obvious from Figure I that a substantial percent of the initial sodium stibogluconate was released within the first 10 hours of 35^{o} and above. This initial release ranged from approximately 40% to over 60%. Thus, a liposome delivery system for sodium stibogluconate would not be feasible unless it could be used immediately after preparation. This would obviously not be possible for extensive human use. Lyophilization of the sodium stibogluconate-liposome system does, however, appear to provide a means of stabilizing the system and preventing drug release during normal pharmaceutical processing and handling. The resulting lyophilized liposmes showed no structural damage and had similar release characteristics. While the initial work seems promising, additional studies are certainly required on the lyophilization process itself and certainly on the ease of reconstitution of the lipid system.

REFERENCES

1. A.D. Bangham, in "Liposomes and Their Uses in Biology and Medicine," Vol. 308, D. Papahadjopoulos, eds, New York Academy of Sciences, New York, 1978, p. 1.

2. G. Gregoriadis, FEBS Lett., 36, 292 (1973).

3. M. Colley and B.E. Rymer, Biochem. Soc. Trans., 3, 157 (1973).

4. G. Gregoriadis, Biochem. Soc. Trans., 3 613 (1975).

5. G. Gregoriadis and N. Neerunjun, Biochem. Biophys. Res. Commun., 65, 537 (1975).

6. Y.E. Rahman, W.R. Hanson, J. Bhanucha, E.J. Ainsworth and B.N. Jaroslow, in "Liposomes and Their Uses in Biology and

Medicine," Vol. 308, D. Papahadjopoulos, eds, New York Academy of Sciences, New York, 1978, p. 325.

7. C.R. Alving, E.A. Steck, W.L. Chapman, V.B. Waits, L.D. Hendricks, G.M. Swartz and W.L. Hanson, Proc. Natl. Acad. Sci. USA, 75, 2959 (1978).

8. A.D. Bangham, M.W. Hill and N.G.A. Miller, in "Methods in Membrane Biology," Korn, eds, Plenum Press, New York, , p. 1.

9. C.D. Black, G.J. Watson, Trans. Royal Soc. Tropical Med. Hygiene, 71, 550 (1977).

10. B.D. Sadbrokke, R.M. Williams and D. Chapman, Biochimica Et Biophysica Acta, 150, 333 (1968).