INFLUENCE OF DIFFERENT SUGAR CRYOPROTECTANTS ON THE STABILITY AND PHYSICO-CHEMICAL CHARACTERISTICS OF FREEZE-DRIED 5-FLUOROURACIL PLURILAMELLAR VESICLES

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ABSTRACT

Lyophilization increases the shelf-life of liposomes by preserving it in a dry form as lyophilized cake to be reconstituted with water immediately prior to administration. Aiming at increasing stability and availability of 5-Fluorouracil liposomal products, 5-Fluorouacil Stable Plurilamellar Vesicles were prepared. Freeze dried liposomal dispersions were prepared with or without cryoprotectants. The cryoprotectants used were glucose, mannitol or trehalose in 1, 2 and 4 grams per gram phospholipids. The results showed that lyophilized cake of liposomes without cryoprotectants was compact and difficult to reconstitute, in comparison with fluffy cakes which reconstituted easily and quickly when using cryoprotectants. The percentage of 5-Fluorouracil retained in liposomes freeze-dried without cryoprotectants was 18.29% ± 0.96% and the percentage of 5-Fluorouracil retained in stable plurilamellar vesicles was 31.22% ± 0.62% using 4 grams trehalose as cryoprotectant per gram of lipid. Physico-chemical and release stability studies showed superior potentials of the lyophilized product after reconstitution in comparison to dispersion product. It may be concluded that all tested sugars have cryoprotectant effects that stabilized liposomes in the freeze dried state, where trehalose offered the most superior cryoprotectant effect for freeze dried 5-fluorouracil liposomes.

Keywords: 5-Fluorouracil, Trehalose, Release stability, Stable plurilamellar vesicles, Lyophilization, Cryoprotectants.

INTRODUCTION

5-Fluorouracil is an antineoplastic agent used in the treatment of carcinomas of the breast, colon, head and neck, pancreas, rectum or stomach. The drug is also used topically for the management of various skin conditions, mainly actinic or solar keratoses (1, 2) and has been investigated for the use in genital precancerous and cancerous lesions (3, 4).

The use of microspheres, nanoparticles and liposomes as particulate delivery systems for 5-FU has been the subject of extensive *in vitro* research (5, 6). However, encapsulation of 5-FU in conventional liposomes has been challenging to investigators because of the drug hydrophilicity, a property which results in poor interaction with liposomal bilayers and consequently relatively poor entrapment and fast drug release (7). As different methods of liposome preparation and different lipid compositions could greatly influence the characteristics of liposomes including drug entrapment and release, nonconventional liposomes have been developed to enhance the performance of hydrophilic-drug loaded liposomal delivery systems. Positive results have been obtained with stable plurilamellar vesicles and vesicular phospholipids gels (8, 9).

Liposomal aggregation, bilayer fusion and drug leakage are the main problems of physical stability encountered in any liposomal formulation which could greatly affect the shelf life of liposomes.

Drug leakage depends on both liposome composition and drug characteristics. Larger polar or ionic, water soluble drugs will be retained much more efficiently than low molecular weight, ampiphilic compounds. In general, membranes composed of saturated phospholipids (with acyl chain of C≥ 16) and/or membranes which contain a sufficient amount of cholesterol are the least permeable ones.

From the pharmaceutical point of view, the physical and chemical properties of liposome particles are critical parameters affecting the performance of the drug loaded liposomes in vitro and in vivo. Unfortunately, liposomal formulations do not meet the required standards for long term stability of pharmaceutical preparations if they are stored as aqueous suspensions (10). The encapsulated drug tends to leak out of the bilayer structure and the liposomes might aggregate or fuse on storage. These processes can cause a

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change in the pharmacokinetic profile of the encapsulated drug and therefore reduce the reproducibility of the therapeutic effect which shortens the storage time for the liposomal preparations, where an acceptable shelf life is prerequisite for the successful introduction of liposomes into therapy.

As an alternative to storing aqueous dispersions, freeze dried liposome are proposed. A number of articles and patents have been published on this subject which indicate that liposomes containing encapsulated drugs can be stored and distributed in the dry form as lyophilized cake (10, 11). Lyophilization increases the stability and the shelf life of the finished product by preserving it in a relatively more stable dry state, especially if the drug is not stable in the aqueous suspension. Some liposomal products in the market or clinical trials are provided as lyophilized powder forms (12), accompanied with labeling that calls for reconstitution (with water or saline for injection) (13).

Freeze drying is a technique that consists of 3 separate steps. First the product is frozen, then sublimation (primary drying) starts which followed by secondary drying process (10).

To maintain the same particle size distribution after freeze drying rehydration cycle, a cryoprotectant should be added. Liposome integrity on freeze drying may be protected against leakage, aggregation and fusion in the presence of properly selected cryoprotectants which is an important factor governing the efficiency of the process of freeze drying of liposomes. For liposome stabilization, usually sugars such as glucose, sucrose, lactose and trehalose are used as cryoprotectants, although other types of excipients have also been reported to exert cryoprotective effects (13). The effect of cryoprotectants (glycerol and mannitol) on the leakage of 5-fluorouracil liposomes after freeze drying has been reported previously (14). Freeze-drying conditions in the presence of other cryoprotectants especially trehalose should be investigated in detail in order to increase physical stability of 5-fluorouracil liposomes.

The objective of this study was preparation and characterization of liposomal 5-fluorouracil lyophilized powder, and to investigate the physical stability of the prepared powder using different type and concentration of cryoprotectants.

MATERIALS AND METHODS

Materials

5-Fluorouracil USP 25 was obtained from Nantong General Pharmaceutical Factory (China), Cholesterol 99% Extra pure was purchased from S.d.Fine-Chem Ltd. (Mumbai, India). Lipoid E 80 [Phosphatidylcholine 80% fat free egg lecithin] was purchased from LIPOID GmbH (Germany). Mannitol and β-glucose were purchased from BDH Chemicals Ltd (Poole, England) and trehalose 98% was obtained from NutriScience Innovations Inc (USA).

Spectra/Por® dialysis membrane 12,000-14,000 molecular weight cut off was received from Spectrum Laboratories Inc. (USA).

All other materials and solvents were of analytical grade and double-distilled water was used.

Equipments

A Shimatzu U.V. visible spectrophotometer, model UV-1601 PC (Japan) was used for analysis. Other instruments were Rotavapor, Typ For analysis, e R 110 (Buchi, Switzerland), sensitive electric balance model 310 (A&D Company Ltd., Japan), Sigma Laborzentrifugen refrigerated centrifuge 3K-30 (GMBH, Germany), JEOL scanning electron microscopy, model JEM-100S (Jeol, Japan), Cilas Laser diffraction particle size analyzer (Model 1064 Liquid), Electrolab tablet dissolution tester USP 24, model TDT-06N (Electrolab gansons engineering, PVT Limited, Mumbai, India), Freezone 4.5 Freeze drying system (Labconco Corporation, Kansas city, Missouri, USA) and Julabo sonicator, model USR-3 (Ceelbach, Germany).

Methods

Liposome preparation

Stable plurilamellar vesicles (SPLVs) were prepared by the previously reported technique of "hydration from organic solvents" (15). The lipid components (Lipoid E-80) with cholesterol (CHOL) in a molar ratio of 1 : 1 (SPFU-10) were weighed into long-necked pear-shaped quick-fit round bottom flask and dissolved in chloroform / methanol 7/3 v/v mixture. The organic solvent was then removed under reduced pressure at 55 ^{0}C using a rotary evaporator. The resulting thin lipid film was redissolved in 8 ml of diethyl ether. The aqueous phase containing 5-fluorouracil was added in successive portions to the lipid solution and the mixture was sonicated. This resulted in the formation of a homogeneous opalescent dispersion. Sonication was continued for about 15 minutes until the organic solvent was evaporated and could no longer be smelled(15). The resulting viscous gel was redispersed in isotonic phosphate buffer saline of pH 7.4 (IPBS pH 7.4). The liposomal dispersion was kept for 24 hours in the refrigerator to mature(16).

Separation of liposomes from unentrapped 5-Fluorouracil

This was achieved by centrifugation at 16500 rpm (27800 X g) for 90 minutes at -5 ^{0}C. The liposomal concentrate was washed with IPBS of pH 7.4 twice and recentrifuged for further 90 minutes(17). The resulting liposomal concentrates were refrigerated.

Entrapped 5-FU was determined by lysis of liposomes with chloroform : methanol (7:3 v/v) mixture. The concentration of 5-FU was determined spectrophotometrically at 266.5 nm (Table 1). Empty liposomes with the same composition were dissolved in the same solvent and used as controls(9, 18).

Freeze drying of 5-fluorouracil liposomal dispersion

The SPFU-10 liposomal dispersion in IPBS pH 7.4 either as such or mixed with equal volume of cryoprotectant (mannitol, glucose or trehalose) buffered solution (IPBS pH 7.4), were first frozen slowly at -10 °C and then freeze dried for 48 hours under vacuum at -40 °C. Cryoprotectant concentrations which were investigated were 1, 2 and 4 gm cryoprotectant/gm lipid. Assigned codes for 5-FU freeze dried liposomal preparations are shown in Table 2.

The resulting lyophilized cakes were either rehydrated to their original dispersion volumes at room temperature with IPBS pH 7.4 or stored in the dry form at room temperature for further experiments.

Measurement of drug leakage after freeze drying

Liposome-entrapped 5-fluorouracil was determined after freeze drying and reconstitution of liposomes with IPBS pH 7.4. Liposomes were separated from the unentrapped drugs by ultracentrifugation and the entrapment efficiency was determined. The percentage of drug which was retained inside the liposomes after the freeze drying rehydration process was calculated relative to the initial percent drug entrapped in liposomes before freeze drying. Entrapment efficiency data were used to estimate the rate of drug leakage as a result of freeze drying.

Vesicle size analysis

The vesicle size of 5-FU liposomes (SPFU-10) following freeze drying and reconstitution was determined using the laser diffraction particle size analyzer operated at a wavelength of 780 nm, and at a measuring range of 0.1-150 μm. The sample was diluted with IPBS of pH 7.4, and was measured while stirring (19). The results were compared with those obtained for the same liposomal formulation (SPFU-10) before freeze drying.

Transmission electron microscopy

Selected freeze dried liposomal formulations F-0 and T-4 were reconstituted with IPBS pH 7.4 and examined by transmission electron microscopy. Negative staining with 2% uranyl acetate followed by dehydration was used to visualize the liposomes. The samples were examined at 20,000 X magnification power at 80 kV(19).

Stability testing of 5-fluorouracil freeze dried liposomal powder stored at 25 °C in sealed glass bottles

The selected parameters for assessment were extent of drug leakage and release stability.

Effect of storage of 5-FU freeze dried liposomal powder on drug leakage(20, 21)

The freeze dried liposomal formulations (G-4 and T-4) were stored in sealed glass vials at room temperature at 25 °C. At predetermined time intervals of seven, fourteen and thirty days, liposomes were analyzed for the percent of drug which was entrapped using the "sample and separate" technique(20, 21).

An amount of freeze dried powder was reconstituted with IPBS pH of 7.4 and centrifuged. The entrapment efficiency was determined and used to calculate the percentage of drug leakage.

Release stability testing of 5-fluorouracil lyophilized liposomal powder and aqueous liposomal dispersion

Freeze-dried liposomal formulations (G-4 and T-4) were stored in sealed glass vials at room temperature (25 ^{0}C). At predetermined time intervals (one, two and four weeks), they were reconstituted in IPBS pH of 7.4 and subjected to a release study using the dialysis method. Dialysis bags were spectra/Por® 2 of 12,000-14,000 Dalton molecular weight cut off which were fitted on a modified tablet dissolution tester paddle (Figures 1 and 2).

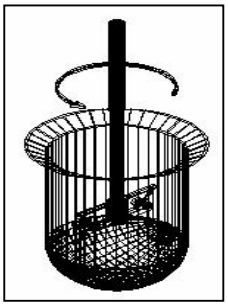

Figure 1. Vessel and paddle of the USP tablet dissolution tester with stainless steel part attached to paddle.

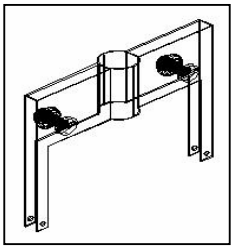

Figure 2. Stainless steel part attached to the paddle of the tablet dissolution tester.

The reconstituted 5-FU liposomes dispersed in IPBS pH 7.4 was filled in a 10 cm length, 6.4 mm diameter dialysis bag. The final length of the bag was 8±0.2 cm. The dialysis bag in fully stretched manner was attached to the modified paddle and then was immersed in the beaker of the tablet dissolution tester. The release medium was 150 ml of IPBS pH 7.4 to provide sufficient sink conditions. The bag was fully immersed under the surface. The temperature was set at 32±0.2 ^{0}C when final liposomal dispersion was intended to be used topically (22) and the speed of rotation of the paddles was 100 rpm.

Zero time data were obtained by subjecting fresh lyophilized liposomal G-4 and T-4 to a release study under similar conditions.

Aliquots of the release medium were withdrawn for analysis at different time intervals and replaced with fresh medium. Release runs were continued for 12 hours. The absorbance of the collected samples, after necessary dilution with release medium, was measured at λ_{max} 266.5 nm. Also, for comparison, twelve-hour release profiles were obtained directly after preparation (zero time) of the aqueous liposomal dispersion (SPFU-10) and after storage for one, two and four weeks in well closed tubes at 4 ^{0}C using the same technique.

RESULTS AND DISCUSSION

This investigation focused partly on freeze drying as a possible means of presenting 5-fluorouracil liposomes in a stable powder form for storage. The selected liposomes were the stable plurilamellar vesicles and study was designed to look into possible effects of freeze drying on drug leakage from the vesicles.

The entrapment efficiency of all liposomal dispersions under study were almost similar with values ranging from 30.69% to 33.22%. Freeze drying without cryoprotection decreased the entrapment efficiency to 18.29% ± 0.96%. Incorporation of cryoprotectants reduced drug leakage upon freeze drying of liposomes, the effect being dependent on the type and concentration of cryoprotectant. Cryoprotectants exert their action via one or more of the following mechanisms(13, 23):

-Amorphous glass formation, which provides an amorphous matrix between the vesicles prevent fusion or bilayer damage by crystal formation.

-Interaction with the phospholipids, through a hydrogen bond between hydroxyl groups of the sugar molecules with the phosphate moiety of the phospholipid head groups in the dry state, replace water which is the base of "water sublimation theory". These interactions result in membrane fluidization of dehydrated lipids, which without sugars would undergo phase transitions and phase separations.

Thus, damage of membranes may be prevented due to pseudohydration. Entrapment efficiency data were processed to estimate the percentage of drug retention and drug leakage following freeze drying and reconstitution of 5-FU SPLVs (Figure 3).

The use of cryoprotectants increased the resistance of liposomes to the damaging effect of freeze drying. The protective effect of the cryoprotectants was in the order trehalose > glucose > mannitol. In all cases, the protective effect increased with the cryoprotectant concentration. Moreover, the entrapment efficiency of the relative low molecular weight, hydrophilic drug, 5-fluorouracil, could be further improved through polymer coating of the liposomal dispersion[24].

Values of median diameter based on weight fraction of the vesicles of 5-FU SPLVs freeze dried in the absence and presence of cryoprotectants (mannitol, glucose and trehalose) are shown in Table 3 and Figure 4. Vesicle size histogram and frequency under size of 5-FU liposomal dispersion before freeze drying (SPFU-10) and after freeze drying without cryoptoyectants (F-0) are shown in Figure 5.

Freeze-drying in the absence of cryoprotectants resulted in increased vesicle size compared to unlyophilized liposomes. The increase in liposome size was more limited in the presence of cryoprotectants. It is worth noting that 5-FU SPLVs lyophilized in the presence of trehalose preserved their original sizes, prevented any sort of aggregation between liposomal vesicles and showed a superior cryoprotective effect of trehalose.

Transmission electron micrographs of 5-FU SPLVs (SPFU-10) which were freeze dried in the absence of cryoprotectants and in the presence of trehalose are shown in Figure 4 a and b respectively.

Increased liposome size and irregular bilayer structure are obvious in photomicrographs of liposomes freeze dried without a cryoprotectant. Trehalose preserved the original liposomal integrity during freeze drying.

The results indicate the feasibility of applying freeze drying to 5-fluorouracil liposomes and were in accordance with related published data concerning the necessity for the use of a cryoprotectant to maintain vesicle integrity during freeze drying / rehydration process(25-27). The extent of 5-fluorouracil leakage from the vesicles in absence of cryoprotectant exceeded 40% and decreased in the presence of mannitol, glucose and trehalose as cryoprotectants and the least

Table 1. Characteristics of ultraviolet absorption of 5-fluorouracil in different media.

Agent	*medium*	*pH*	λ_{max} *(nm)*	*Linear regression (y = ax + b)*				
				a	*b*	*R*	R^2	*Range*
5-FU	Isotonic Phosphate Buffer Saline (IPBS)	7.4	266.5	-3.3×10^{-3}	0.048	0.999	0.999	2 < [ ] < 18 µg/ml
	Chloroform/Methanol Mixture (7/3)	-	266.5	2×10^{-3}	0.056	0.999	0.999	2 < [ ] < 18 µg/ml

Table 2. Composition of the 5-FU SPLVs dispersions based on formula SPFU-10 subjected to freeze-drying.

Code	Cryoprotectant	g Cryoprotectants per g Lipids
F-0	no cryoprotectant	0.00
M-1	Mannitol	1.00
M-2	Mannitol	2.00
M-4	Mannitol	4.00
G-1	Glucose	1.00
G-2	Glucose	2.00
G-4	Glucose	4.00
T-1	Trehalose	1.00
T-2	Trehalose	2.00
T-4	Trehalose	4.00

Table 3. median vesicle diameter of 5-fluorouracil liposomal dispersions, freeze dried without and with different cryoprotectants, determined after reconstitution with IPBS pH 7.4. (Results represent the mean ± standard deviation of three independent experiments)

Code	Median vesicle diameter (µm)
SPFU-10	0.09 ± 0.02
F-0	3.34 ± 0.11
M-1	2.62 ± 0.14
M-2	2.42 ± 0.19
M-4	1.59 ± 0.12
G-1	0.66 ± 0.21
G-2	0.58 ± 0.18
G-4	0.55 ± 0.09
T-1	0.07 ± 0.02
T-2	0.06 ± 0.01
T-4	0.06 ± 0.01

Table 4: Slope and intercept of Higuchi's release plots of freeze dried 5-fluorouracil liposomes as a function of storage time at 25 ^{0}C.

Code		Intercept (% released)*	Slope ($)^*\%/\sqrt{t}$
G-4	**Zero time**	41.32	3.88
	One week	38.45	5.71
	Two weeks	38.95	5.38
	Four weeks	36.81	6.99
T-4	**Zero time**	38.63	5.36
	One week	38.88	5.42
	Two weeks	38.89	5.53
	Four weeks	38.81	5.74
SPFU-10**	**Zero time**	42.15	2.66
	One week	42.79	4.32
	Two weeks	45.9	4.98
	Four weeks	50.55	6.75

* Of linear plot of release data from 2-12 hours, ** Stored as dispersion

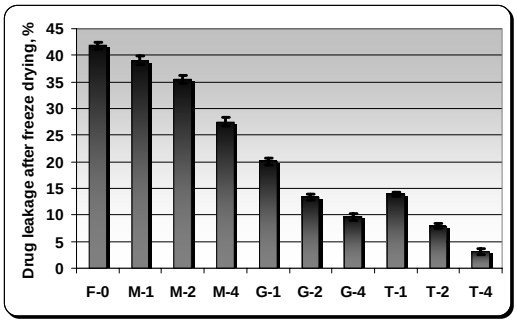

Figure 3. Effect of freeze drying, in the absence and presence of cryoprotectants, on drug leakage from 5-FU SPLVs (SPFU-10) after reconstitution (Each result represents the mean ± standard deviation of three independent experiments

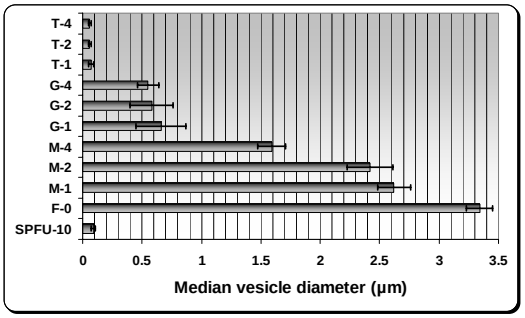

Figure 4. Effect of freeze drying, in absence and presence of cryoprotectant, on vesicle size of SPFU-10 liposomes. Cryoprotectants were trehalose, glucose and mannitol. (Each result represents the mean ± standard deviation of three independent experiments)

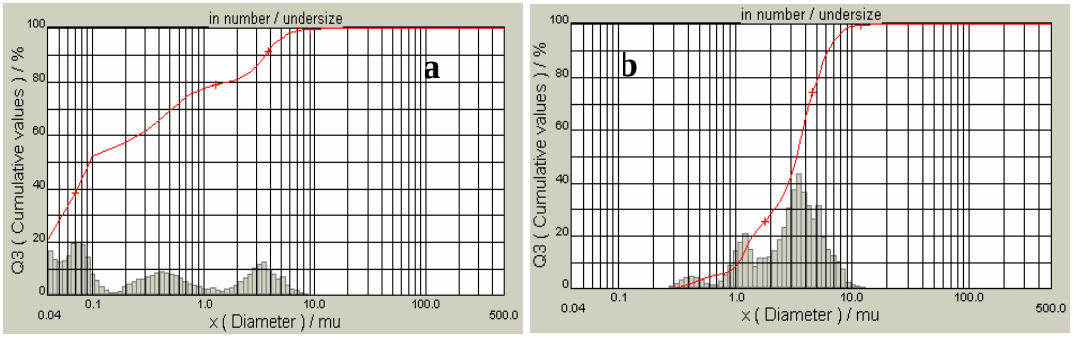

Figure 5. Vesicle size histogram and frequency under size of 5-FU liposomal dispersion: a) Before freeze drying (SPFU-10), b) After freeze drying without cryoptoyectants (F-0).

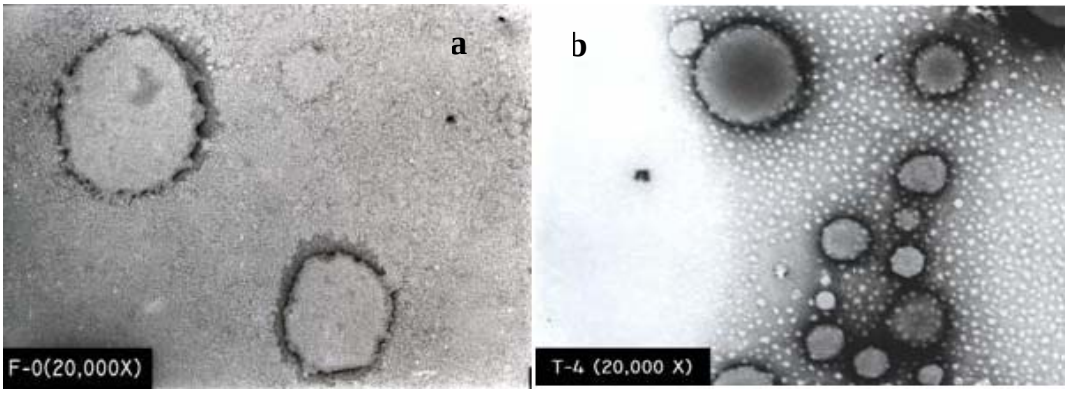

Figure 6. Transmission electron photomicrographs of 5-FU SPLVs (SPFU-10): a) Freeze dried in the absence of cryoprotectants (F-0), b) In the presence of trehalose in a cryoprotectant / lipid mass ratio 4 / 1 (T-4).

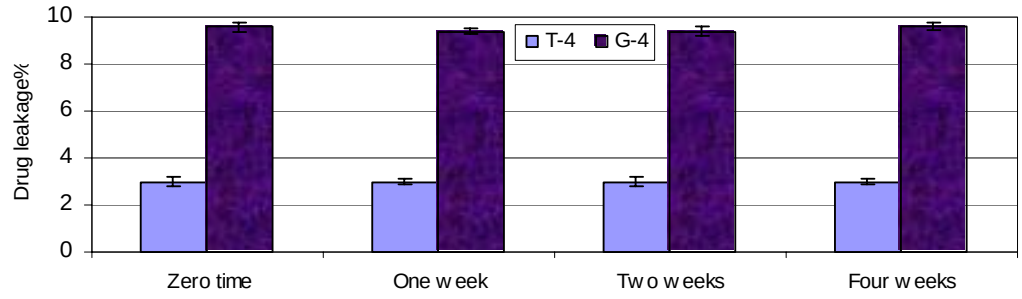

Figure 7. 5-Fluorouracil leakage out of freeze dried SPFU-10 liposomes during storage for one month as a lyophylized powder form. Cryoprotectants used in freeze drying were glucose and trehalose. (Each result represents the mean ± standard deviation of three independent experiments)

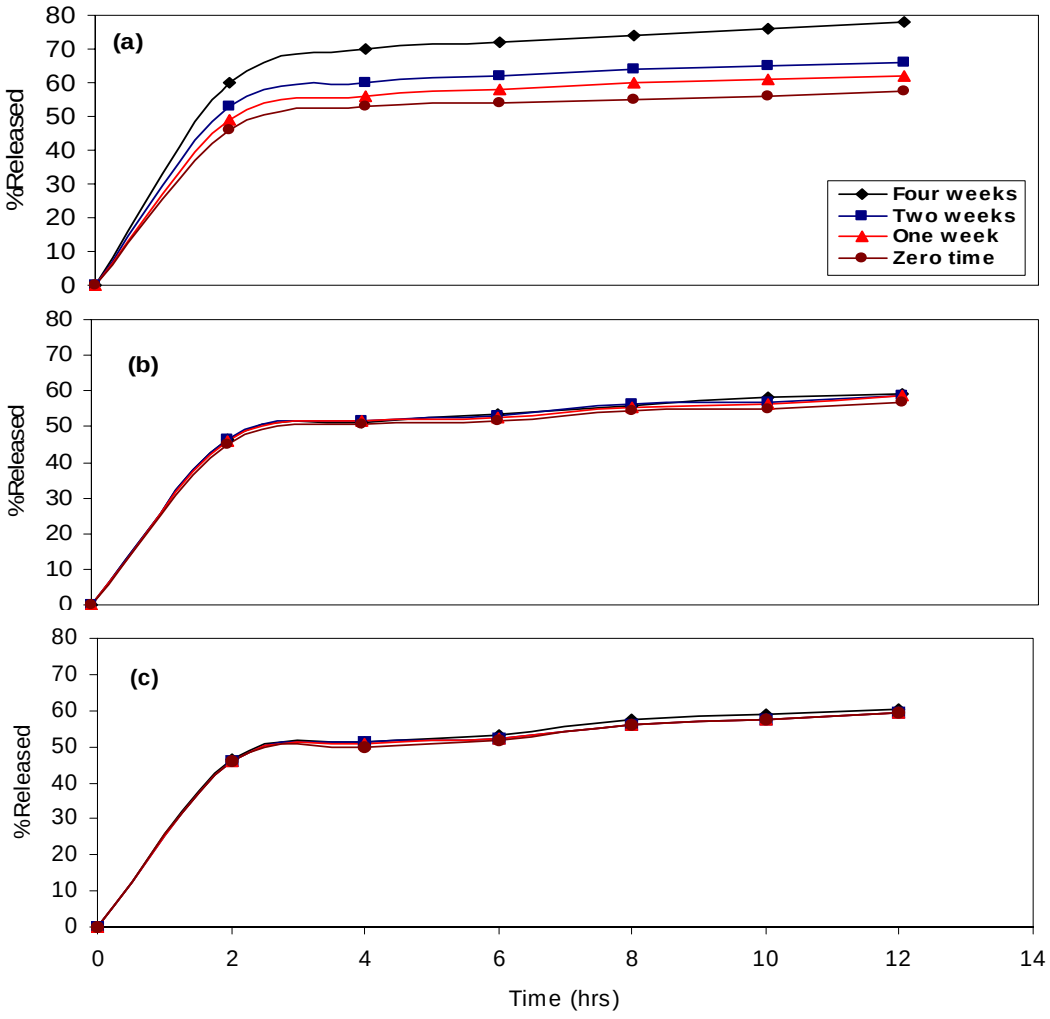

Figure 8. Release stability profiles at 32 ^{0}C of: a) 5-FU liposomal dispersion (SPFU-10) as a function of storage time at 4 ^{0}C, b) Lyophilized 5-FU liposomal powder (G-4) as a function of storage time at 25 ^{0}C, c) Lyophilized 5-FU liposomal powder (T-4) as a function of storage time at 25 ^{0}C (Each point represents the mean ± standard deviation of three independent experiments).

protection was provided by mannitol and maximum protection of vesicles was exhibited by trehalose. The success of glucose and trehalose as cryoprotectants in the process of lyophilization could be related to the proper spacing between the phosphate head groups, in a way that a full potential of hydrogen bond interactions can be realized, while correct spacing between the phospholipid head groups were maintained (28, 29). It has been reported.(30, 31) that almost 100% of entrapped solute was retained in rehydrated vesicles which was previously freeze dried with trehalose.

Previous studies also demonstrated the superiority of trehalose compared to other sugars in preventing the fusion and leakage of entrapped drug where it has the ability to preserve biological membranes in absence of water. It has been suggested that the presence of selected concentration of trehalose during the freeze drying process preserves the structure of drug loaded liposomes in the anhydrous state by replacing the water molecules normally which are bound to the lipid head group (30). Upon rehydration, water molecules quickly replace sugars and the liposomes appear to reseal before significant leakage occurs(13, 32).

The concentration of the cryoprotectant is also recognized as an important factor in the efficiency of the lyophilization process (26, 33). The results obtained indicated that by increase in concentrations of the added cryoprotectants, increased retention during lyophilization steadily up to 4 g trehalose per gram lipid. Data obtained with mannitol as cryoprotectant indicated that it did not provide the required stability to the liposomal formulation judging by percentage of drug retained inside the vesicles using mannitol which increased only slightly compared to corresponding value in absence of cryoprotectant. This could be explained by the fact that mannitol crystallizes during the freeze drying process (23). These results agree with the previous work of previous work (14). Although it has been reported (10) that during the freezing step in the freeze drying process of liposomes, the rupture of liposomes and leakage from them were considerably reduced in the presence of glycerol mannitol mixture as a cryoprotectant; but this could not be attributed either to the presence of glycerol or mannitol which was proposed to protect the liposomes through direct interaction with the lipid bilayer structure, reducing the ice crystallization rate because of an increased viscosity or the decreased efflux which can be ascribed to the lower eutectic temperature of the aqueous system with glycerol compared to the situation without cryoprotectant(10).

Freeze dried liposomal powders show maximum resistance to drug leakage during the freeze drying rehydration process (T-4 and G-4) were challenged in this stability study.

Entrapment efficiency data and the corresponding drug leakage data generated over a storage period of four weeks are shown in Figure 7. The data indicate that the leakage occurring during the freeze drying / rehydration cycle (zero time data after freeze drying) did not progress any further during storage (one to four weeks data) as evidenced by the lack of change in the percent of drug leakage with time.

Apart from percent leakage calculated from entrapment efficiency, release stability was investigated as an indicating parameter for the stored lyophilized liposomal powders. The results of monitoring of release at 32 ^{0}C of the lyophilized liposomes (G4 and T4) as a function of storage time at 25 ^{0}C for 4 weeks indicated superb stability as evidenced by the lack of change in release profile of the stored powders (Figure 8 (a-c)). This in contrast to the relative instability of the same type of liposomes (SPFU-10) which were stored as aqueous dispersion (Figure 8 c). The freeze dried liposomal powder form after stability and delays or prevents drug leakage from the liposomes.

The release process was characterized by treating release data mathematically using zero order, first order and Higuchi equations. Larger correlation coefficients were obtained for the Higuchi equation(34) indicating a diffusion controlled release model.

Higuchi diffusion model parameters (slope and intercept), recorded in Table 4, give further evidence of the stability of freeze dried powders particularly T-4 powder where only a minor change in the slope and intercept was observed compared to G-4 powder and SPFU-10 dispersion. Also included in table 4 are the corresponding release parameters for SPFU-10 liposomes monitored during storage at 4 ^{0}C in the form of dispersion. Progressive increase in Higuchi intercept values which were noted in this case ascertain the relative instability of this system compared to lyophilized powders.

Apart from monitoring drug leakage and changes in drug release as stability indicators for the stored freeze dried powders (T-4 and G-4), these powders showed no signs of color change for two month-storage at 25 ^{0}C and remained fluffy and redispersable in aqueous medium during this period.

CONCLUSION

The results obtained indicated the ability of lyophilization process with the proper choice of cryoprotectants to maintain the integrity, particle size distribution and stability of liposomal dispersions. Lowest drug leakage was observed with lyophilized 5-fluorouacil stable plurilamellar vesicles using trehalose and glucose as cryoprotectants with lipids: cryoprotectant molar ratio of 1: 4.

The results suggest that liposomal dispersions could be stored at room temperature in the freeze dried form with minimum effect on stability, drug content and physicochemical properties. The freeze dried lyophilized powder could be used for incorporation of liposomes in topical gel formulations (in the powder form or reconstituted dispersion form) or could be reconstituted immediately prior to injection or further formulation processing.

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