DEVELOPMENT OF A DEPOFOAM TECHNOLOGY FOR THE SUSTAINED DELIVERY OF DESFERRIOXAMINE MESYLATE

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ABSTRACT

Encapsulation of drugs into multivesicular liposomes (Depofoam) offers a novel approach to sustained release drug delivery. Depofoam –encapsulation has been shown to result in sustained-release lasting over several days to weeks after non-vascular administration. In this paper we describe encapsulation of desferrioxamine in a multivesicular (MVL) depot –delivery system. The Depofoam particles were characterized by their morphology, particle size and capture volumes. The effects of various concentrations of components and two manufacturing methods on capture volumes of these particles were studied. The light micrograph showed that particles were smooth and multivesicular without any debris. The particle size of these liposomes were between 15-35 µm with capture volumes about 27% . The results show that the concentration of triolein in lipid combination and lysine in the second aqueous solution have significant effects on the captured volume.The in vitro studies in 0.9% NaCl at 37 ºC showed that the multivesicular liposomes released desferrioxamine slowly over several days, and 57% desferrioxamine was released in 9 days.

Key words: Multivesicular liposome, Depofoam , Desferrioxamine mesylate, Controlled drug release, Sustained delivery

INTRODUCTION

Liposomes can act as a depot from which the entrapped compound is slowly released. Such a sustained release process can be exploited to maintain therapeutic levels of drug in the blood or at the site of administration for a long period of time. Thus benefical consequences of these formulations are an increase in duration of action and a decrease in frequency of the drug administration. Various types of liposomal formulations have been utilized as drug delivery vehicles for sustained release of drugs (1-2).

Multivesicular liposomes (Depofoam particles) have a highly characteristic physical structure, which differentiate them from other types of liposomes and other lipid-based drug delivery systems. Depofoam particles are microscopic and spherical, and each particle encloses multiple non-concentric aqueous chambers bounded by a single bilayer lipid membrane which appears like a foam under microscopic examination. Multivesicular liposomes (MVL) have advantages over other types of liposomes such as good stability during storage, slow release of drugs, and efficient entrapment of hydrophilic molecules (3) including a variety of therapeutic proteins (4), analgesics (5), anticancer (6-10), antimicrobial (11-13) and antiviral agents (14). Desferrioxamine mesylate (DFO) remains the only first-line iron-chelating agent. Since it has a short half-life and is poorly absorbed by the gastrointestinal tract, DFO must be administered parentrally, usually by daily subcutaneous continuous infusion (15). Which is uncomfortable for patients. Therefore, a slow-release depot preparation of DFO is desirable (16). This paper describes preparation and characterization of depofoam particles as a new slow-release delivery system for subcutaneous administration of DFO.

MATERIALS AND METHODS

Materials:

Phosphatidyl Cholin (ScPc-1, Sigma Type IV-S), Triolein (To, Sigma 65%), Cholesterol (Chol), L-lysine free-base and Ferric chloride were obtained from Sigma (St. Louis, MO); Soy lecithin Lipoid S75 (ScPc-2) was from Lipoid GMBH, Ludwigshafen, Switzerland; Desferrioxamine Mesylate was from Novartis, Switzerland; methanol, dextrose, sodium chloride, sucrose, and chloroform were obtained from Merck, Darmastadt, Germany.

Equipments:

Ultraviolet Spectrophotometer (Shimadzu 160A, Tokyo, Japan), Vortex Mixer (Heidolph,

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Germany), Particle Sizer (Malvern Master Sizer X, Malvern, UK), Centrifuge (Sigma 101) and Light Microscope (Zeiss, Germany), Mixer(IKA Werk,Germany) were used in this investigation.

Preparation of MVL liposomes:
The multivesicular Depo-DFO particles were prepared by modification of the method of Kim et al(18).

Vortex Method:
The lipid phase was made by addition of the lipids (Table1) to chloroform and the aqueous phase was prepared by dissolving of desferri-oxamine mesylate (100 mg/ml) in 1% sucrose solution. One ml of the aqueous solution phase was added slowly into a vial containing 1 ml of lipid phase while the vial was gently shaken by hand. The vial was capped and then attached to the head of a standard vortex machine with an adhesive tape and shaken for 12 minutes. Then each half of the resulting "water-in-oil" emulsion was individually squirted rapidly through a narrow-tip of pasteur pipette into vials containing 2.5 ml of aqueous solution containing dextrose (3.2% w/v) and free-base lysine (second aqueous solution in Table 1) and the mixture was shaken on the vortex mixer for 8 seconds in order to form chloroform spherules. The chloroform spherule emulsion in the two vials was transferred to the bottom of a 250-ml Erlenmeyer flask containing 5 ml of the second aqueous solution. Chloroform was removed by flushing the nitrogen gas at 8 lit/min through the surface of the mixture at 37 ºC±2 ºC for 15 minutes. The multivesicular liposomes (Depo-DFO) were then isolated by centrifugation at 600g for 5 minutes and washed with 0.9% NaCl solution three times. The liposomes were used within 24 hours.

Standardization of vortex mixer:
The intensity of mechanical shaking on the vortex mixer was standardized with a 1-dram vial containing 0.1 ml chloroform and 1 mg of lipids(ScPc1:Chol:To) in 8:1:1 weight ratios and 4 ml of 0.2 M sucrose in water. The vial was attached horizontally to the machine head with a piece of adhesive tape.The machine was set in a way that chloroform particles of 7.8±2.9 µm diameter were produced after 60 s shaking of the standardization vial (18).

Mixer Method:
Ten ml of desferrioxamine mesylate (100 mg/ml) in 1% sucrose (w/v) was emulsified with an equal volume of chloroform solution containing 20.5 mg of ScPc-1, 20.5 mg of ScPc-2, 19 mg of cholesterol, 26 mg of triolein at ambient temperature (23-28 ºC) for 30 minutes at 3500 rpm. Then each half of the resulting "water-in-oil" emulsion was expelled rapidly through a narrow-tip Pasteur pipette into 25 ml of an aqueous solution containing dextrose (3.2%, w/v) and L-lysine (40 mM), and the mixture was then shaken for 20 seconds at 3500 rpm. The resulting emulsions were added to 1 lit baffled flask containing 25 ml of aqueous solution containing dextrose (3.2%, w/v) and L-lysine (40 mM). After removal of the chloroform by flushing the surface of mixture with nitrogen (8 lit/min) at 37±2 ºC for 15 min, the resulting multivesicular liposomes were isolated by centrifugation at 600 g for 5 minutes and washed with 0.9% NaClsolution 3 times.

Characterization of the Depofoam particles

Particle Size:
The size distribution of the Depo-DFO particles were determined with a Malvern Mastersizer Particle sizer. Particle sizing was determined by addition of 10-15 µl of depofoam suspension to 10 ml of 5% dextrose and measuring the volume-adjusted distribution of size.

Microscopy:
Depo-DFO suspensions were observed with a Zeiss light microscope and images were recorded photographically.

Quantification of Encapsulated DFO:
Ten ml of Depo-DFO suspension was centrifuged at 630 g for 10 minutes. The supernatant was aspirated from the particle fraction and the pellet was washed with 0.9% NaCl solution three times. Then 8 ml of methanol was added to the pellet in order to break the Depo-DFO particles. The resulting mixture was diluted to 10 ml with methanol and was stored at 4ºC until analysis. A 2 ml sample was added to a 3 ml of $FeCl_3$ solution (75 mg/ml) and the mixture was diluted to 25 ml with water. The amount of desferrioxamine mesylate was measured colorimetrically at 485 nm (17). The volume of trapped desferrioxamine mesylate solution in liposomes was calculated from the total amount of desferrioxamine mesylate in the liposomal pellet and the concentration of original desferrioxamine solution.The captured volume was calculated as the volume of the original solution trapped per unit quantity of the total lipid that was used initially (µlit/mg) (18).

In vitro release studies:
To estimate the *In vitro* release rate of desferrioxamine mesylate from multivesicular liposomes, washed Depo-DFO(F_2,F_3,F_4) were diluted by 100 times with NaCl solution (0.9%) (22). Ten ml of the suspension were pipetted into 15 ml centrifuge tubes in a way that each tube represents one time point, and tubes were incubated at 37 °C. All in vitro release studies were set up in triplicate. At each point, tubes were centrifuged for 10 minutes at 600 g, and supernatants and pellets were separated. Desferrioxamine was extracted from the pellets as described above.

Statistical analysis:
Comparison of different formulations were performed using a single factor analysis of variance (ANOVA) followed by a Tukey multiple comparison test. A p value of less than 0.05 was regarded as an indication of statistical significance.

RESULTS AND DISCUSSION

Figure 1 depicts a light micrograph of a representative Depofoam formulation containing DFO. The picture shows the smooth, spherical and multivesicular nature of Depofoam particles.

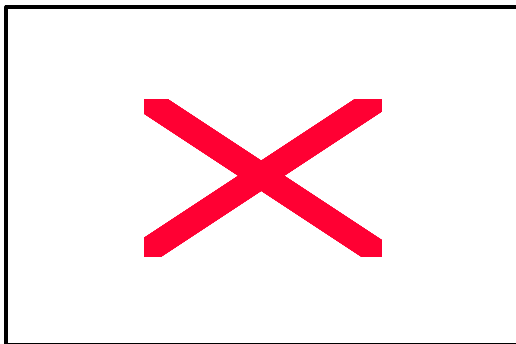

Figure 1. Light Micrograph of Depo-DFO (X100,F_3), showing the smooth, spherical and multivesicular nature of Depo-DFO particles.

Depofoam particles have a typical size range of 1-100 µm in diameter(23).The particle size distribution of a typical Depo-DFO formulation is shown in figure 2 .The volume weighted size determination shows a narrow and monomodal distribution of particles with a median size of 24.77±10.47 µm. No particles of the size of <8 µm or >70 µm were observed. Table I represents the various formulations of Depo-DFO. The amount of two lipids in the lipid mixture (ScPc-1: ScPc-2) was kept constant. Triolein a liquid-like phase acts as a hydrophobic space filler at bilayer intersection points and stabilize these junctions. As a result it should have an optimum concentration in the lipid concentration (18,19,23).

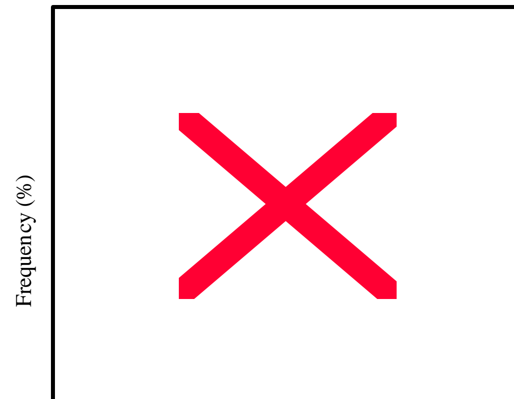

Figure 2. Particle size distribution determined by laser light scattering of a typical Depo-DFO suspension(F_3). The measurement is a volume-weighted distribution of size.(n=2)

The effect of the amount of triolein upon captured volume is depicted in Figure 3. As the amount of triolein was increased from 1.7 to 2.6 mg/ml, the captured volume increased until it reached a plateau (from 2.6 to 3.4 mg/ml). Kim and co-workers have reported similar results (18). They have shown that when the concentration of triolein was reduced to less than a mole freaction of 0.01 in lipid combination , capture efficacy decreased markedly.Moreover higher concentrations of triolein aggregates and thus forms a triolein oil phase (24).
Figure 4 shows that when the concentration of L-lysine in second aqueous phase was increased to more than 40 mM, the capture volume decreased markedly and when it was decreased to less than 20 mM, liposomes aggregated.The charge on the MVLs arises from the lecithin which is a mixture of phospholipids whose extract composition depends on its source . Phosphatidic acid and phosphatidylglycerol comprise 2-5% of total lipid and are negatively charged at pH 7 . These ionized minor components usually contributes a major proportion of the surface charge(20).At pH 7 , L-lysine would be positively charged ,thus supporting the hypothesis that L-lysine stabilizes Depofoam particles via its effect on the electrostatic potential. This efficient inter-action takes place at constant surface

Table 1. Various composition of Depo-DFO.

Number	Lipids	mg of respective lipids	lysine conc in sec. aq. phase(mM)	Captured volume (µ l/mg)	Method of manuf.
F_1	ScPc-1: ScPc-2:Chol:To	2.05:2.05:1.9:0	40	0	Vortex
F_2	ScPc-1: ScPc-2:Chol:To	2.05:2.05:1.9:1.7	40	15.45 ±2.97	Vortex
F_3	ScPc-1: ScPc-2:Chol:To	2.05:2.05:1.9:2.6	40	22.28 ±2.79	Vortex
F_4	ScPc-1: ScPc-2:Chol:To	2.05:2.05:1.9:3.4	40	23.51 ± 2.9	Vortex
F_5	ScPc-1: ScPc-2:Chol:To	2.05:2.05:2.8:2.6	40	21.54 ± 2.5	Vortex
F_6	ScPc-1: ScPc-2:Chol:To	2.05:2.05:3.8:2.6	40	21.28 ± 2.29	Vortex
F_7	ScPc-1: ScPc-2:Chol:To	2.05:2.05:1.9:2.6	0	0	Vortex
F_8	ScPc-1: ScPc-2:Chol:To	2.05:2.05:1.9:2.6	20	26.55 ±1.57	Vortex
F_9	ScPc-1: ScPc-2:Chol:To	2.05:2.05:1.9:2.6	30	22.21 ± 2.72	Vortex
F_{10}	ScPc-1: ScPc-2:Chol:To	2.05:2.05:1.9:2.6	50	9.51 ± 2.12	Vortex
F_{11}	ScPc-1: ScPc-2:Chol:To	2.05:2.05:1.9:2.6	40	24.12±1.7	Mixer

Capture efficiencies expressed as µ lit/mg, and is the volume of the original aqueous solution trapped per unit quantity of the total lipids initially used.The procedure described in the text, using the amount of lipids indicated in the table.

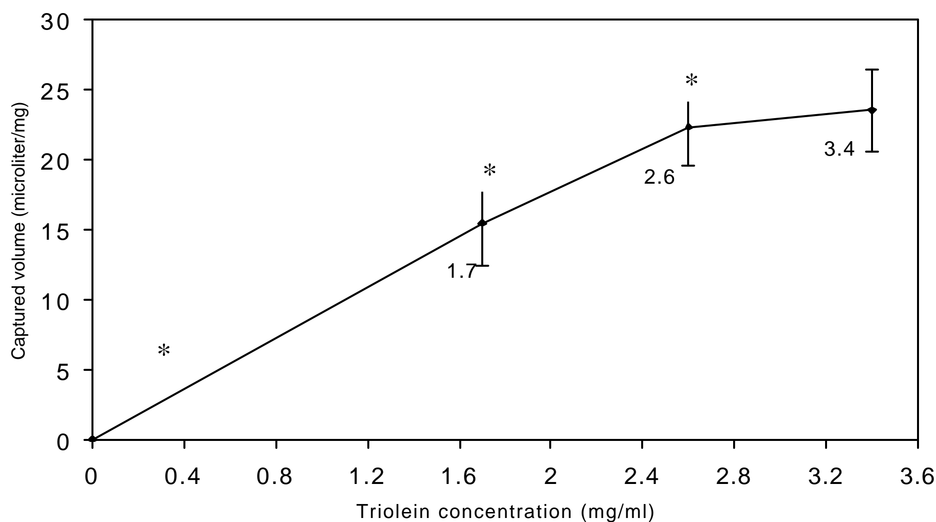

Fig.3. Effect of triolein concentration on captured volume (F_1-F_4). The lipid combination of Scpc-1: ScPc-2:Chol: To (2.05:2.05:1.9:X mg), respectively were used. Each point represents mean±standard deviation of three independent experiments.(* Significant, $p<0.05$, one way ANOVA)

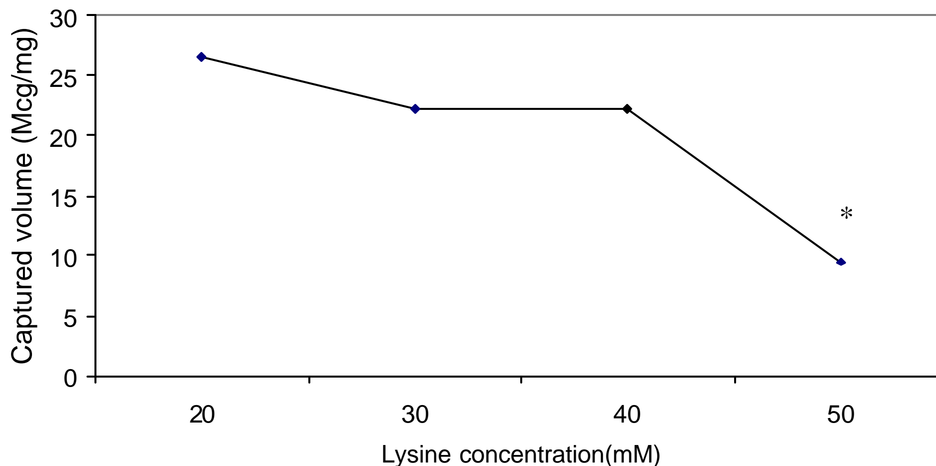

Fig.4. Effect of lysine concentration upon captured volume(F_3,F_8,F_9,F_{10}).The lipid combination of ScPc-1: ScPc-2:Chol: To (2.05:2.05:1.9:2.6 mg) were used. Each point represents mean± standard deviation of three independent experiments.(* Significant, p<0.05, One Way Anova).

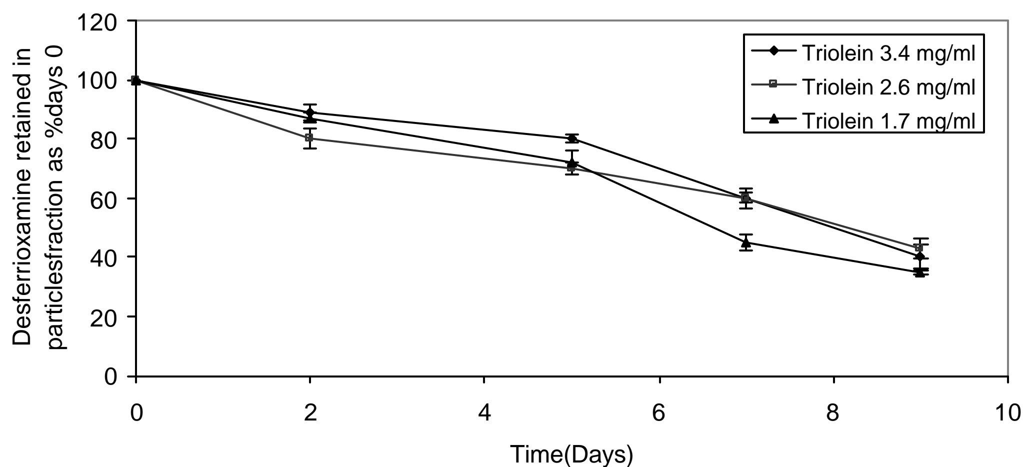

Fig .5. In vitro release profile of three formulations($F_2F_3F_4$) of Depo-DFO[ScPc1:ScPc2:Chol:To (2.05:2.05:1.0:X), Vortex method].Particles incubated in 0.9% Nacl. The amount of deferrioxamine mesylate retained in the Depo-DFO particle fractions over 9 day time period ,expressed as percent of day of 0, is shown versus days of incubation. Each point represents the mean of three independent experiments.

potential via a specific concentration of L-lysine Higher concentrations of L- lysine inhibit packing the internal vesicules to form multivesicular liposomes during evaporation of organic solvent.Also L-lysine stabilizes fat emulsions by decreasing interdroplet Hamaker constant in the system (21).

Effect of different concentrations of cholestrol were studied, and results show that the capture volume didn't alter significantly. The mixer method of multivesicular preparation has given approximately an equivalent captured volume (24.12±1.7 µl/mg) as well as an equivalent particle size (27.77±12.55 µm) with vortex method (22.28±2.79 µl/mg, 24.77±10.47 µm).In addition while the volume of each batch is significantly larger than vortex method, the release pattern is simillar to that method.

The capture volume for the best formulation of Depo-DFO was 22.28±2.79 µlit/mg of lipids. The capture volume for Depo-DFO (22.28±2.79 µlit/mg) is relatively lower than glucose (73

µlit/mg) and sucrose (81 µlit/mg) (18) , thus supporting the hypothesis that it is possible to use salt solutions to form multivesicular liposomes although at reduced capture efficiency (18). Figure 5 illustrates the *in vitro* release of desferrioxamine mesylate from depo formulation in 0.9% NaCl at 37ºC. This figure shows that there is no rapid initial release upon incubation at 37 ºC. The *in vitro* release data also shows that in this formulation 57% of encapsulated DFO was released in a sustained manner over a 9 day period.

CONCLUSION

The results of this investigation demonstrate that depofoam technology can be used successfully for desferrioxamine mesylate.The results show that the concentration of triolein in lipid combination and lysine in the second aqueous solution, have significant effects on the captured volume.Probabely the effective concentration range for triolein in the lipid combination is between 1.7 to 3.4 mg/ml and for lysine in the second aqueous phase is between 20 and 40 mM.Determintation of accurate effective ranges needs more investigation.The concentration of cholestrol in lipid combination doesn't have a significant effect on the captured volume. The mixer method of multivesicular preparation has given approximately an equivalent captured volume and particle size distribution and release pattern with the vortex method and the volume of each batch is significantly larger than vortex method. Multivesicular liposomes are capable of providing a sustained manner in *in vitro* without a rapid initial release.

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