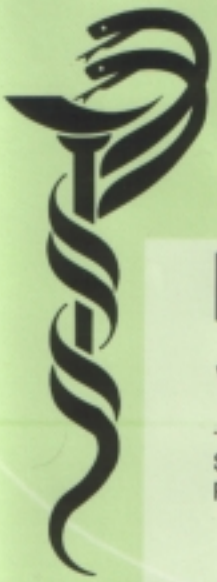

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The effects of lyophilisation on the stability of liposomes containing 5-FU

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Introduction

In recent years liposome preparations have been increasingly used as drug carrier systems for controlled drug delivery. A disadvantage of these preparations is their chemical and physical instability in aqueous dispersions (I). Lyophilisation or freeze-drying is commonly used method to achieve long-term stability of liposomes. To maintain the same particle size distribution and to avoid leakage of the encapsulated drug from liposomes after the freeze-drying-rehidration cycle, a crioprotectant (usually sugars) needs to be added. The protective effect of sugars has been related to their ability to interact with the polar head groups of the phospholipids and to stabilise the membranes when the bilayer stabilising water is removed by sublimation (2).

The purpose of this study was to investigate the effect of lyophilisation on the stability of liposomes loaded with hydrophilic drug substance 5-FU. Both size, leakage from the bilayer and physical state of it were investigated.

Materials and Methods

For the preparation of liposomes the following materials were used: Phospholipid PL 90H (Nattermann Phospholipid, Germany), cholesterol (Galerika, Yugoslavia), 5-FU (Ebewe Arzeneimittel, Austria) and saccharose (Merck, Germany).

Liposomes containing an antineoplastic agent 5-FU were prepared by modified lipid film hydration method. Lipid phase containing different quantities of PL 90H and cholesterol (9:1, 12:1, 15:1; samples 1, 2 and 3) was dissolved in chloroform and evaporated to dryness under reduce pressure at 65 °C. The thin lipid film was hydrated and gently shaked above the phase transition temperature with an aqueous phase, 5-FU in phosphate buffer pH 7.4 (drug/aqueous phase mass ratio 1:60). In an order to remove the free drug substance, the resulting liposome dispersions (series Ld) were ultracentrifugated (20000 rpm, 3x45 min.) by washing the liposomes with phosphate buffer pH 7.4. Liposome dispersions were freeze-dried (temperature -40 °C, pressure 2 mBar) in a presence of saccha-rose as a cryoprotectiv agent, incorporated on the both si- des of the phospholipids lamellae (lipid phase:saccharose =1:1.25). The lyophilisated cakes were rehydrated to its original volume **(series** Lr) and separated from the leaked drug by ultracentrifugation.

The mean particle size of the prepared liposome dispersions and rehidrated liposomes after lyophilisation were determined by the laser diffraction technique (Fritsh particle size analysette D LAB/22). The spherical form is based on optical micrographs (Nikon E-800, Japan) of liposomes using method by Nomarsky.

Quantity of entrapped drug before and after lyophilisation/rehydration was determined UV spectrophotometricaly (266 nm. Perkin Elmer, Lambda 16, USA), after dissolving of liposomes in chloroform: methanol mixture.

Results and Discussion

Microscopic observations confirmed the formation of spherical vesicles. The liposomes were 5.21 μm in average and showed an encapsulation efficiency of 7.69 % - 12.5 %. The effects of the lyophilisation on the liposome size and percentage of entrapped drug are shown in Table 1. The process of freeze-drying caused an increase in particle size and was harmful for the liposome integrity, as freezing caused a pronounced increase in the release of the encapsulated drug. Briefly, freeze-drying tended to destroy the membrane function of the phospholipid bilayer. Particle size increase after freeze-drying was in good agreement with drug leakage. Incorporation of cholesterol is known to cause strong reduction in the permeability of the liposome bilayer and thus reduce leakage of the drug from liposomes (3). However, incorporation of cholesterol was shown no effect on the percentage of drug remaining entrapped. This gives an understanding that reduction in the permeability of the membrane may not be the only mechanism acting, because in the anhydrous state there is no possibility of drug diffusion, therefore, drug retention cannot be increased by reducing the permeability alone.

Conclusion

As a conclusion it becomes clearly evident that liposome stability is a complex parameter. Since liposomes are very sensitive to rehydration it is very important to optimize the freeze-drying protocol and to select optimal crioprotectiv agent.

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Table 1. The effect of lyophilisition on the particle size and % of entrapped 5-FU into liposomes.

Samples	Particle size ((μm)		% of entrapped drug	
	Before lyophil. (Ld)	After lyophil. (Lr)	Befor luophil. (Ld)	After lyophil. (Lr)
1. Pl 90H : chol. =9: 1 saccharose	5. 56 ± 1. 68	4. 88 ± 0. 13	7. 69	5. 98
2. PL 90H : chol. = 12 : 1 saccharose	4. 53 ± 1. 95	4. 70 ± 0. 63	9. 35	7. 53
3. PL 90H : chol. = 15 : 1 saccharose	4. 96 ± 1. 57	5. 24 ± 0. 25	12. 5	10. 02
1. Pl 90H : chol. = 9 : l	5. 48 ± 1. 25	5. 98 ± 0. 10	7. 69	3. 54
2. PL 90H : chol. = 12 : 1	4. 85 ± 1. 11	6.70 ± 0. 11	9. 35	6. 86
3. PL 90H : chol. = 15 : 1	5. 21 ± 1. 65	6. 34 ± 0. 13	12. 5	8. 62

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