

INFLUENCE OF LIPOSOME COMPOSITION ON PACLITAXEL ENTRAPMENT AND pH SENSITIVITY OF LIPOSOMES

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Abstract: The objective of the present investigation was to study the effect of composition of phospholipids on drug entrapment efficiency and pH sensitivity. In the present study, paclitaxel containing liposomes of different phospholipid compositions were formulated and compared. The formulation composed of Phospholipon 90G/DOPE/CHEMS 8:2:2(D) containing paclitaxel and lipids in the molar ratio of 1:30 (drug: lipid) was found to have good incorporation efficiency(94%). The highest paclitaxel concentration achievable in the liposomal formulation was 1.5 mg/ml. Liposomes with phospholipon 90 G alone couldn't show pH sensitivity. Formulation B (Phospholipon 90G /DOPE 8:2) released drug at pH 5.5, but was unstable at pH 7.5. On inclusion of CHEMS, liposomes were stabilized at physiological pH, and released paclitaxel at lower pH. Thus including CHEMS into liposomal formulation of paclitaxel, composed of Phospholipon 90 G/DOPE has proved to be the most efficient pH sensitive system with 94 % entrapment efficiency. This formulation showed 96% drug release at pH 5 within 15 min.

Keywords : Paclitaxel, Liposomes, pH sensitivity, Entrapment efficiency

Introduction:

Paclitaxel, the first of a new class of microtubule stabilizing agents, has been hailed by National Cancer Institute (NCI) as the most significant advance in chemotherapy of the past 20-25 years.¹ Due to its low solubility in water, it is clinically administered after dissolving in cremophore EL and ethanol.^{2,3} One of the substantial problems associated with this formulation is that the ethanol: Cremophor vehicle is toxic.⁴⁻⁷ Inclusion of paclitaxel in liposomal formulations has proved to be a good approach to eliminating this vehicle and improving the drug's antitumor efficacy. pH sensitive liposomes have been suggested as a means to increase intracellular delivery of drugs. These liposomes are stable at physiologic pH (pH 7.4) but undergo destabilization and acquire fusogenic properties under acidic conditions, thus leading to the release of their aqueous contents. pH sensitivity of liposomes may mainly depend on their composition. Different classes of pH sensitive liposomes have been proposed in the literature, based on the mechanism of triggering pH sensitivity.⁸ The most

commonly established hypothesis involves the blend of phosphatidyl ethanolamine and its derivatives with compound containing an acidic group that acts as a stabilizer at neutral pH.^{9,12} Contemporary studies describe the use of novel pH sensitive lipids, synthetic fusogenic peptides/proteins either encapsulated or included in the lipid bilayer.^{13,15} The objective of the present investigation was to study the effect of composition of phospholipids on drug entrapment efficiency and pH sensitivity.

Materials and Methods

Paclitaxel was received as a gift sample from Naproid life sciences, Mumbai. Phospholipon 90 G(PL90G) was received as a gift sample from Phospholipid GmbH, Nattermannalle, Germany. Cholesteryl hemisuccinate (CHEMS) was received as a generous gift from Merk Eprova AG Switzerland. Diolyl phosphatidylethanolamine (DOPE) was received as a gift sample from Lipoid GmbH, Germany. HPLC grade

solvents and other chemicals were purchased from local supplier.

Preparation of liposomes

Liposomes were prepared by thin film hydration method. Paclitaxel and the required quantities of CHEMS, Phospholipon 90 G and DOPE were dissolved in chloroform. Chloroform was evaporated using rotary vacuum evaporator and kept overnight under vacuum. Nitrogen gas was passed over the thin film. The film formed was hydrated with 5% dextrose solution, above the phase transition temperature of lipids, using glass beads. The suspension of liposomes was sonicated for 2 min to reduce the size of liposomes. This was transferred to vials and stored at 4°C.

Percent Drug Entrapment

The amount of paclitaxel incorporated in liposomes was determined using HPLC (Perkin Elmer). 0.5 ml liposomal suspension was diluted with water and acetonitrile to 1 ml. Extraction of paclitaxel accomplished by adding 4ml of tert-butyl-methyl ether, vortex mixing for 1 min, and centrifuging the mixture for 15 min. 3ml of the organic layer was separated and evaporated to dryness. Residue was reconstituted with 1 ml methanol. 20 µl of above solution was injected into a C₁₈ column, 5µm. The column was eluted with acetonitrile /water (60/40). The drug was estimated by UV absorption measurement at 227 nm (flow rate 1ml/min).

Table 1. Formulations prepared using different Phospholipids and their entrapment efficiency.

Formulation	Phospholipid composition	Entrapment efficiency
A	PC 10	62
B	PC /DOPE 8:2	80
C	PC/CHEMS 10:2	90
D	PC/DOPE/CHEMS 8:2:2	94

In-vitro Release of Paclitaxel From pH Sensitive Liposomes:

Liposomal formulations were diluted 1:2 with buffers (pH 5, 5.5, 7.5) and incubated at 37°C. for 15 min. The drug released was separated from liposomal paclitaxel,

extracted and quantified using the same procedure described above for determination of incorporated drug.

Microscopy

The liposomes were mounted on glass slides and viewed under a microscope (Motic) for morphological observation after suitable dilution. Particle size was measured as average object perimeter.

Results and discussion.

Paclitaxel liposome formulations, A, B, C, D were prepared by using different phospholipids mixtures Table 1. Hydration of lipid film using glass beads was found to be a feasible preparation method for obtaining small vesicles. Photomicrograph of formulation D is shown in fig.1.

The formulation composed of Phospholipon 90G/DOPE/CHEMS 8:2:2(D) containing paclitaxel and lipids in the molar ratio of 1:30 (drug: lipid) was found to have good incorporation efficiency (94%). The highest paclitaxel concentration achievable in the liposomal formulation was 1.5 mg/ml.

Results of % drug release at pH 5, 5.5 and 7.5 are shown in fig.2. Liposomes with phospholipon 90 G alone couldn't show pH sensitivity. Formulation B (Phospholipon 90G /DOPE 8:2) released drug at pH 5.5, but was unstable at pH 7.5. On inclusion of CHEMS, liposomes were stabilized at physiological pH, and released paclitaxel at lower pH. CHEMS acts as an amphiphilic stabilizer which was shown to be resistant to extraction by albumin. And therefore this formulation is expected to exhibit higher stability in biological fluids.

Conclusion

Inclusion of CHEMS into liposomal formulation of paclitaxel, composed of Phospholipon 90 G/DOPE proved to be the most efficient pH sensitive system with 94 % entrapment efficiency. This formulation showed 96% drug release at pH 5 within 15 min. To confer clinical advantage, additional work is required to obtain the sterically stabilized liposomes with increased incorporation efficiency.

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Fig. 1. Photomicrograph of liposomes (formulation D)

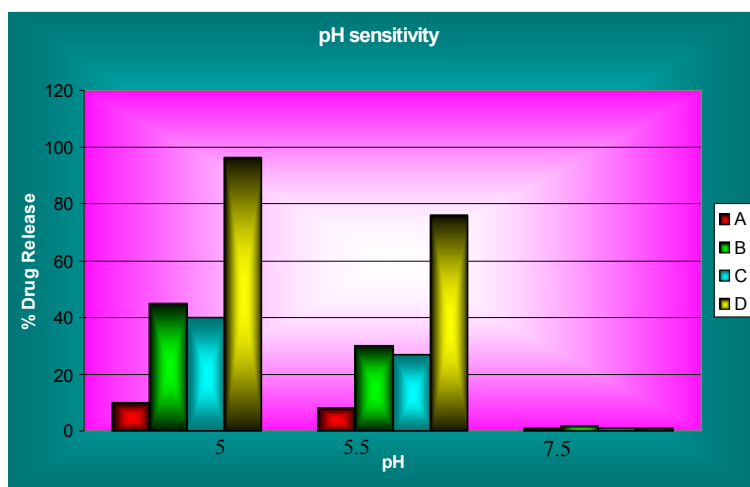


Fig 2. pH sensitivity expressed in terms of % drug release at various pH.

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