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FORMULATION AND EVALUATION OF DOXORUBICIN LIPOSOMES

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ABSTRACT

Doxorubicin is an effective anticancer drug used in the treatment of several cancers such as osteosarcoma, kaposis sarcoma. The usage of the drug is limited because of its adverse effects on the heart. To reduce the adverse effects and to increase the release rate doxorubicin is formulated into liposomal dosage form. The liposomes are prepared by the thin film hydration method.Using soyalecithin as the phospholipid. This study mainly explains about the effect of concentration of soyalecithin, cholesterol and DSPE-$MPEG_{2000}$ on the particle size of formulated liposomes which ranges in between 0.766 ± 0.03µm to 13.56 ± 0.10 µm , drug entrappment efficiency of different formulations in which maximum entrapment efficiency was determined as 96.45 ± 0.95 % and minimum was 24.89 ± 1.18 % , zeta potential which is determainedd as -0.271 mV, *in vitro* drug release in which the the maximum sustain release was found as 41.45 ± 1.06 % and stability studies at different temperatures and maximum drug retention was found in refrigerated temperature 2-8 ^{o}C.

Keywords: Liposomes, Doxorubicin, Film hydration method, Soyalecithin, *In vitro* release

INTRODUCTION

In the current drug delivery systems there are many drug delivery systems which releases the drug in a specified period af time to the diseased organs. Paul Enrich in 1906 intiated an era for the development of the targeted drug delivery systems, which include immunoglobulins, serum proteins, lipidvesciles (liposomes), microspheres, reseled erythrocytes, niosomes etc. Among all the delivery systems liposomes show effective drug delivery.These liposomes are biologically inert in nature, devoid of any antigenic, pyrogenic reactions. As the components of liposomes are same as the components of cellmembrane / biological membrane these delivery systems are more acceptable. [1] These are the new trend formulations for effective results, which were discovered 40 yrs ago by (Bangham *etal).* Liposomes are spherical microscopic vesicles composed of one or more concentric lipid bilayers separated by water or aqueous buffer compartments with a diameter ranging from 25 nm-10000 nm. [2]

Doxorubicin is an anthracycline antibody which was proved effective in the treatment of murine tumors. Even though many anticancer drugs were available; doxorubicin is the most preferred anticancer drug. But its therapeutic usage was restricted because of high cardio toxicity. Among many formulations, the liposomes are considered as best formulations which encapsulate the doxorubicin and reduce the uptake of the drug by heart and prevent the cardio toxicity. Doxorubicin is used in the treatment of several types of cancer such as breast cancer, osteosarcoma, Kaposi's sarcoma. [3, 4]

Doxorubicin acts by intercalation of DNA, lipid per oxidation and inhibition of topoisomerase II. [5] Doxorubicin entrapped in the liposomes containing PEG (polyethylene glycol) chain on the surface has proved best results as; they prevent recognition by the endothelial cells and also increase permeation. [6] In this present work doxorubicin liposomes are formulated to study the effect of various concentrations of soya lecithin, cholesterol, DSPE-$MPEG_{2000}$ on the drug content and *in vitro* release studies.

MATERIALS AND METHODS

Materials: Doxorubicin is obtained as gift sample from the Strides Arcolabs, Bangalore, India. Soya lecithin, cholesterol and DSPE-$MPEG_{2000}$ were procured from the Sigma Aldrich, USA. All other solvents and chemicals used are of analytical grade.

Methods: Doxorubicin liposomes were prepared by thin film hydration method by using rotary flask evaporator. [7, 8] The compositions of various formulations are shown in the **Table 1**. Different weight ratio of phospholipids: cholesterol and DSPE-$MPEG_{2000}$ were weighed and dissolved in chloroform: methanol mixture (2: 1 v/v) in 250 mL round bottom flask. A thin film was formed on the inner side of round bottom flask by evaporating organic solvent under vacuum in rotary evaporator at 45-50 ℃. Subsequently, the flask was kept for overnight under vacuum to ensure the complete removal of residual solvent. The dry lipid film was hydrated with 20 mL phosphate buffer solution (pH 7.4) containing doxorubicin at a temperature of 60 ± 2 ℃. The dispersion was left undisturbed at room temperature for 2-3 h to allow complete swelling of the lipid film and hence to obtain vesicular dispersion.

EVALUATIONS

Particle size determination: Particle sizes of the formulated liposomes are determined by using particle size analyzer (Sympatec HELOS, Germany (H1004), which measures particle size based on the laser diffraction theory. In this method the liposomal suspension was diluted up to 100 times and the diluted suspension was added to the simple dispersion unit and stirred at 2000 rpm to reduce the inter particulate aggregation and the laser beam was focused, and the experiment was repeated in triplicate. [9]

Drug entrapment efficiency or Drug content: Doxorubicin associated with liposome was separated from unentrapped drug using centrifugation method. Liposomes were centrifuged at 20000 rpm for 1h at controlled temperature of 4 $^{\circ}$C. Supernatant containing unentrapped doxorubicin suspension was withdrawn and diluted with phosphate buffer saline (pH 7.4). The amount of drug present in the liposomes was determined spectrophotometerically at 254nm (Shimadzu 1800, Japan). Entrapment efficiency was obtained by repeating the experiment in triplicate and the values were expressed as mean standard deviation. [10]

Zeta potential determination: This method is used to determine charge on empty and drug loaded vesicles surface using Zetasizer 300HSA (Malvern Instruments, Malvern, UK). Analysis time was kept for 60 seconds and average zeta potential and charge on the liposome was determined. [11]

In-vitro drug release study: The *in vitro* diffusion studies were carried out by using 500 ml beaker containing 250 ml Phosphate buffer pH 7.4. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37±5 ^{0}C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped doxorubicin liposome dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. The assay process was done by withdrawing 1 mL of sample at regular intervals for 49 hours and immediately replaced by the same quantity of buffer in to the donor compartment. The received samples were diluted with phosphate buffer saline (pH 7.4) and assayed UV spectrophotometrically (Shimadzu 1800, Japan) at 254nm. [12] Drug release mechanism was determined by Higuchi and Korsmeyer-Peppas plots. [13, 14, 15]

Stability of liposomes: Stability studies were performed to inspect the leakage of the drug from the liposome during storage. Liposomal suspensions of doxorubicin of optimized formulations were sealed in 20 mL glass vials and stored at refrigeration temperature (2–8 °C) and room temperature (25 ± 2 °C / 60 ± 5 % R.H) for a period of 3 months. Samples from each liposomal formulation which are kept for examination were withdrawn at definite time intervals. The withdrawn samples were assayed for drug content at 254 nm. [16]

RESULTS AND DISCUSSION

Doxorubicin liposomes were formulated by thin film hydration method by varying the composition of the soya lecithin, cholesterol and DSPE- $MPEG_{2000.}$ In this study effect of different compositions of lipids on

the doxorubicin liposomes were studied. The results show that the increase in the concentration of the soya lecithin and DSPE- $MPEG_{2000}$ had increased the entrapment efficiency and *in vitro* release was sustained. The increase in the concentration of cholesterol had shown minimum entrapment efficiency and it has proved that the cholesterol involves only in the stabilizing the lipid membrane, filling the empty spaces between the phospholipid and anchoring of the structure.

Particle size and Drug entrapment efficiency: The particle size of the formulated liposomes containing doxorubicin was determined using method described previously. The particle size of different formulations was in the range between 0.766 ± 0.03µm to 13.56 ± 0.10 µm. Drug entrapment efficiency was also determined by the method explained previously. Maximum entrapment efficiency was found in F9 formulation which is 96.45 ± 0.95 % and minimum in F10 formulation which is 24.89 ± 1.18 %. **Table 2** shows the particle size and drug entrapment efficiencies of different formulations. By these results we can conclude that as the concentration of cholesterol increases the particle size was increased and entrapment efficiency was decreased and simultaneously as the concentration of the soya lecithin and DSPE- $MPEG_{2000}$ increases entrapment efficiency increases. It was shown that the maximum entrapment efficiency was found to be in formulations (F9), (F3), (F8), (F2) which are considered for the in vitro release and F9 was considered as the optimized batch for determining the zeta potential based on the drug content / drug entrapment efficiency.

Zeta potential: The zeta potential of optimized formulation (F9) which is selected based on entrapment efficiency is shown in **Figure 1.** The value was -0.271 mV which indicates that the surface of liposomes is dominated by the anions and proved that prepared liposome have sufficient charge to avoid aggregation of vesicles.

In vitro drug release: The cumulative drug release of the different formulations had indicated that the drug release depends up on the variables used and each batch had shown different drug release rate based on the composition used in the formulation process which was showed in **Figure 2**. Formulation F9 shows more sustain release as it has more concentration of soya lecithin, cholesterol and DSPE-$MPEG_{2000}$. Increase in concentration of soya lecithin and DSPE-$MPEG_{2000}$ increases the entrapment efficiency and sustains the *in vitro* drug release. The r^2, *'k'* and '*n'* values of selected formulations are shown in **Table 3.** According to this all the formulations are best fitted to Korsmeyer-Peppas plots. Formulations F9, F8 and F3 showed non-fickian drug release and the drug release varies with time (t) according to the power law. The formulation F2 followed Super case II transport could be due to increased plasticization at the relaxing boundary.

Stability studies: Stability studies of selected formulations were performed for the selected formulations. The studies are carried for about 3 months in different temperatures; refrigeration temperature (2-8°C) and room temperature (25 ± 2°C / 60 ± 5 % R.H) and results indicated that the maximum drug was retained in the refrigeration temperature (2-8°C) which is shown in **Table 4.**

CONCLUSION

The different formulations of liposomes containing doxorubicin are prepared by the thin film hydration method. The percentage entrapment efficiency was determined and maximum entrapment efficiency was found to 96.45 ± 0.95 %. The prepared optimized liposomes are found to have good mean particle size and it is proved that the as the concentration of the cholesterol increases the particle size increases and entrapment efficiency decreases. The surface charge was determined for the optimized F9 formulation which was selected based on the drug content. The maximum sustain release was found with formulation F9. From the above study it is concluded that doxorubicin can be formulated as liposomes with phospholipids such as soya lecithin and cholesterol and DSPE- $MPEG_{2000}$ as polymer.

Figure 1: Zeta Potential of selected formulation (F9)

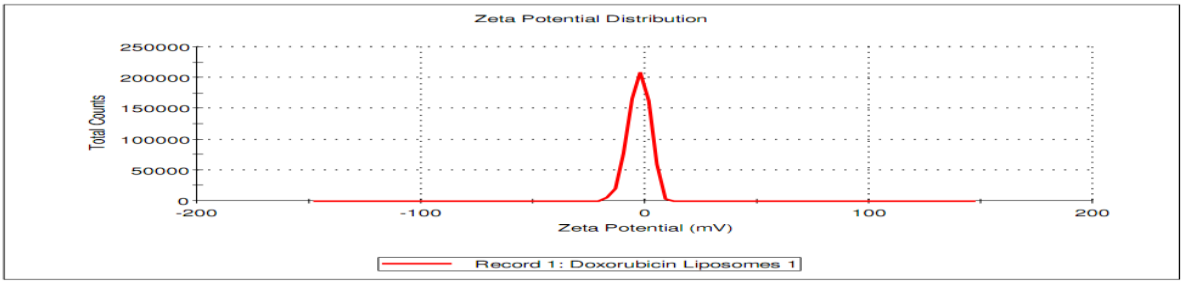

Figure 2: *In vitro* drug release of selected formulations

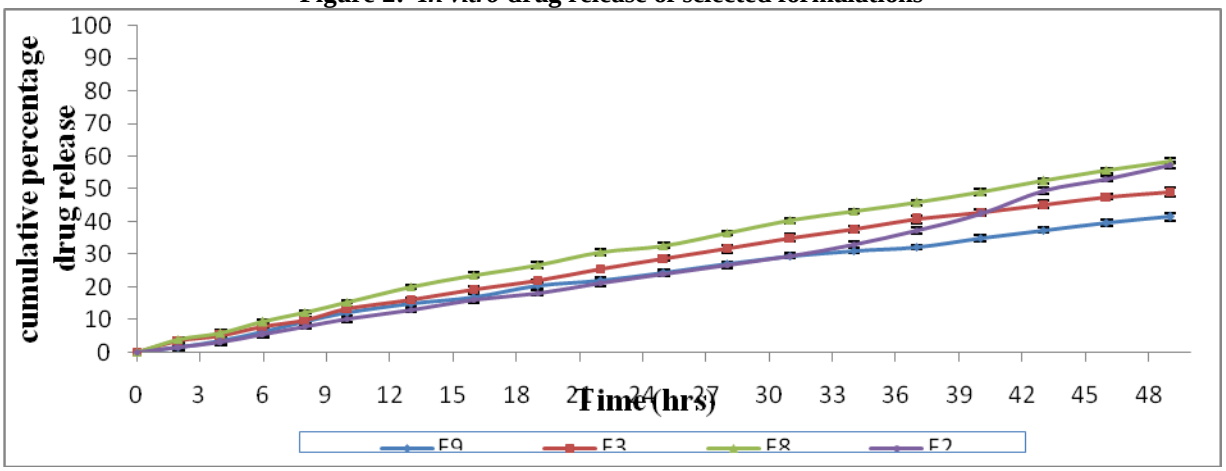

Table 1: Formulation of doxorubicin liposomes

Formulations	Doxorubicin (mg)	Cholesterol (mg)	DSPE-MPEG$_{2000}$ (mg)	Soya lecithin (mg)
F1	20	100	100	200
F2	20	100	100	400
F3	20	100	100	600
F4	20	100	200	100
F5	20	100	400	100
F6	20	100	600	100
F7	20	100	100	200
F8	20	100	200	400
F9	20	200	300	600
F10	20	200	100	100
F11	20	400	100	100
F12	20	600	100	100

Table 2: Drug entrapment efficiency and particle size of different formulations

Formulation Code	Drug Entrapment Efficiency (%)	Particle Size (µm)
F1	30.27 ± .892	1.84 ± 0.16
F2	85.6 ± 1.11	1.54 ± 0.19
F3	89.75 ± 1.97	1.35 ± 0.22
F4	30.48 ± 1.5	1.77 ± 0.26
F5	32.24 ± 1.99	1.83 ± 0.14
F6	36.97 ± 1.7	1.85 ± 0.28
F7	40.09 ± 2.0	1.91 ± 0.17
F8	86.56 ± 1.92	1.17 ± 0.19
F9	96.45 ± .95	0.766 ± 0.03
F10	24.89 ± 1.18	6.08 ± 0.31
F11	27.08 ± 0.945	9.17 ± 0.25
F12	28.96 ± .765	13.56 ± .10

Mean ± SD, n=3

Table 3: r^2, '*k*' and '*n*' values of selected formulations

Formulations	Higuchi		Korsmeyer's and Peppas		Mechanism of drug release
	r^2	*k*	r^2	*n*	
F9	0.969	6.605	0.968	0.986	Non-fickain/Diffusion
F8	0.963	9.183	0.984	0.960	Non-fickain
F3	0.960	7.947	0.989	0.941	Non-fickain
F2	0.888	8.501	0.994	1.062	Super case II transport

Table 4: Stability studies of selected formulations

Temperature	Periods (months)	F9	F8	F3	F2
2-8 °C	1	96.33 ± 1.04	85.6 ± 1.43	89.34 ± 1.09	85.26 ± 1.15
	2	95.40 ± 0.96	85.2 ± 1.0	89.27 ± 1.15	84.79 ± 1.16
	3	94.85 ± 1.06	84.46 ± 1.1	88.89 ± 0.99	84.66 ± 1.25
25 ± 2 °C / 60 ± 5 % R.H	1	82.08 ± 1.05	69.19 ± 0.99	72.62 ± 1.16	68.57 ± 1.09
	2	79.22 ± 1.004	66.66 ± 1.22	70.26 ± 1.10	66.72 ± 1.11
	3	77.08 ± 0.966	65.65 ± 1.27	68.15 ± 1.05	66.52 ± 1.39

Mean ± SD, n=3

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