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Formulation and evaluation of doxorubicin liposomal drug delivery system

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Abstract
The drug release from Liposomes depends on many factors including the composition of Liposomes, the type of drug encapsulated and nature of the cell. Once it is released a drug that normally crosses the membrane of a cell will enter the cell, other drugs will not enter. Doxorubicin is a drug with narrow therapeutic index and short biological half-life. This study aimed at developing and optimizing liposomal formulation of Doxorubicin in order to improve its bioavailability. In evaluation study the effect of the varying composition of lipids on the properties such as encapsulation efficiency, particle size and drug release were studied. Phase transition study was carried out to confirm the complete interaction of Doxorubicin with bilayer structure of liposome. Moreover, the release of the drug was also modified and extended over a period of 8 h in all formulations. F2 emerged as the most satisfactory formulation in so far as its properties were concerned. Further, release of the drug from the most satisfactory formulation (F2) was evaluated through dialysis membrane to get the idea of drug release.

Keywords: Liposomes, doxorubicin, bioavailability, thin film hydration technique, *in vitro* drug release studies

Introduction
Vesicles composed of a blazered phospholipids membrane surrounding water entrapped from the environment. Phospholipids form closed, fluid-filled spheres when they are mixed with water in part because the molecules are amphipathic; they have a hydrophobic “tail” and a hydrophilic or polar “head” [1]. Liposomes represent versatile Nano platforms for the improved delivery of pharmaceutical drugs and active compounds in a large variety of biomedical and nano medicine applications [2]. They are characterized by easily controllable properties such as lipid composition, size, structure and morphology, surface charge, and the possibility of functionalizing their surfaces with polymers or ligands [3]. The industrial applications of liposome Nano platforms include their use as drug-delivery vehicles in nano medicine, cancer, antimicrobial therapy, as signal carriers in biomedical diagnostics and biochemistry, as adjuvants in vaccination, and as solubilizes and support matrices for various active compounds and macromolecules [4]. Moreover, owing to their high biocompatibility and non-toxicity, liposomes are the most important category of clinically approved therapeutic drug nanocarriers for cancer treatment. Doxorubicin (DOX) is a very potent anticancer drug and has shown strong activity against various tumors. However, it shows many adverse effects such as cardio-toxicity and congestive heart failure limiting its use [5].

Materials
Doxorubicin was obtained from Micro lab. Phosphatidylcholine and cholesterol were procured from Synpharma Research Labs, Hyderabad, and other chemicals the reagents used were of analytical grade.

Methodology
FT-IR study [6]
Compatibility of the drug with excipients was determined by FT-IR spectral analysis, this study was carried out to detect any changes on chemical constitution of the drug after combined it with the excipients. The samples were taken for FT-IR study.

Preparation of liposomes [7]

Method

Liposomes were prepared by physical dispersion method using different ratio of lipids. In this method the lipids were dissolved in chloroform. This solution of lipids in chloroform was spread over flat bottom conical flask. The solution was then evaporated at room temperature without disturbing the solution. The hydration of lipid film form was carried out with aqueous medium phosphate buffer (pH 7.4). For this the flask was inclined to one side and aqueous medium containing drug to be entrapped was introduced down the side of flask and flask was slowly returned to upright orientation. The fluid was allowed to run gently over lipid layer and flask was allowed to stand for 2 h at 37 ^{0}C for complete swelling. After swelling, vesicles are harvested by swirling the contents of flask to yield milky white suspension. Then formulations were subjected to centrifugation. Different batches of liposomes were prepared in order to select an optimum formula. All batches of liposomes were prepared as per the general method described above.

Table 1: Composition of lipids for preparation of liposome

Ingredients	F1	F2	F3	F4
Phosphatidylcholine	100	200	300	400
Cholesterol	500	500	500	500
Solvent (Chloroform)	10	10	10	10
Doxorubicin	20	20	20	20
Phosphate buffer pH 7.4	10	10	10	10

Evaluations of liposomes [8, 9, 10]

Particle size analysis

All the prepared batches of liposomes were viewed under microscope to study their size. Size of liposomal vesicles from each batch was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles were determined.

Drug entrapment efficiency of liposomes

Entrapment efficiency of liposomes were determined by centrifugation method. Aliquots (1 ml) of liposomal dispersion were subjected to centrifugation on a laboratory centrifuge (Remi R4C) at 3500 rpm for a period of 90 min. The clear supernatants were removed carefully to separate non entrapped Doxorubicin and absorbance recorded at 245 nm. The sediment in the centrifugation tube was diluted to 100 ml with phosphate buffer pH 7.4 and the absorbance of this solution was recorded at 245 nm.

Amount of Doxorubicin in supernatant and sediment gave a total amount of Doxorubicin in 1 ml dispersion.

% entrapment of drug was calculated by the following formula.

$$\% \text{ Drug Entrapped (PDE)} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug}} \text{ X } 100$$

***In Vitro* Drug release study** [11]

The release studies were carried out in 10 ml Franz diffusion cell containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (10 ml) was placed in a 10 ml beaker. 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 liposomal dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1 sml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Stability studies [12]

The success of an effective formulation can be evaluated only through stability studies. The purpose of stability testing is to obtain a stable product which assures its safety and efficacy up to the end of shelf life at defined storage conditions and peak profile.

The prepared Doxorubicin liposomes were placed on plastic tubes containing desiccant and stored at ambient conditions, such as at room temperature, 40±2 ^{O}C and refrigerator 2-8 ^{O}C for a period of 90 days.

Results and Discussion

Drug - excipient compatibility studies (FT-IR)

The compatibility between the drug and the selected lipid and other excipients was evaluated using FTIR peak matching method. There was no-appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, lipid and other chemicals.

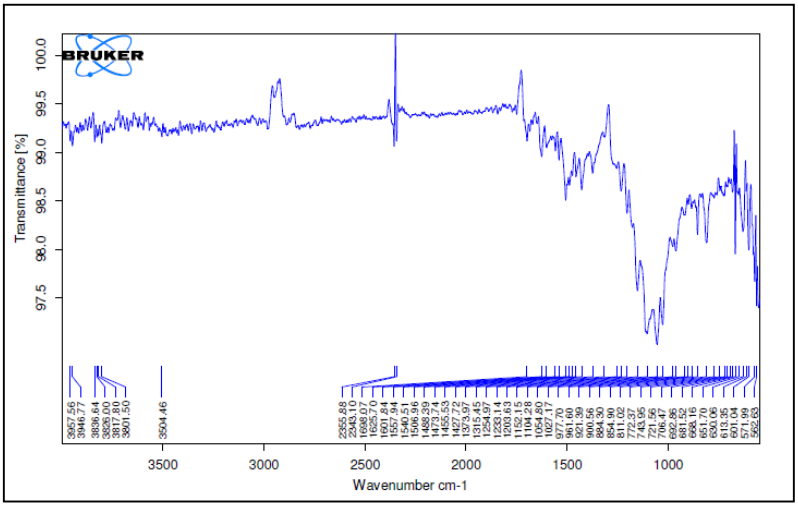


Fig 1: FTIR Studies of Pure Drug (Doxorubicin)

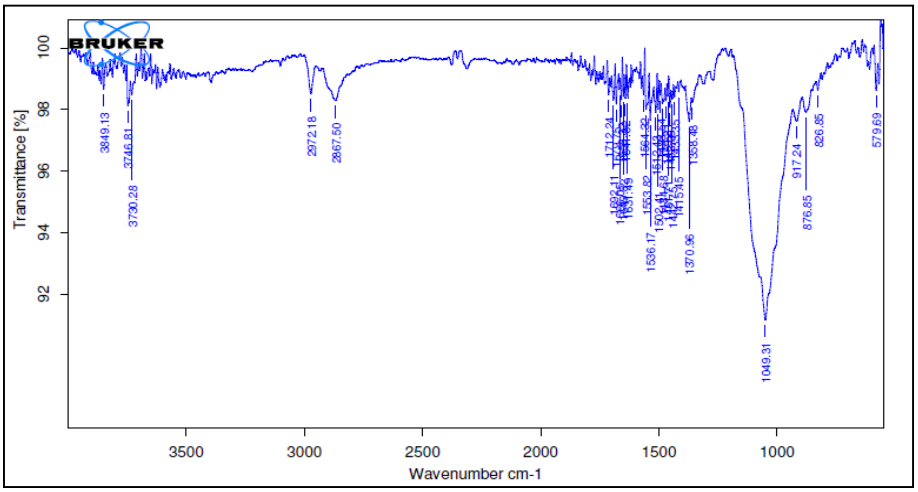
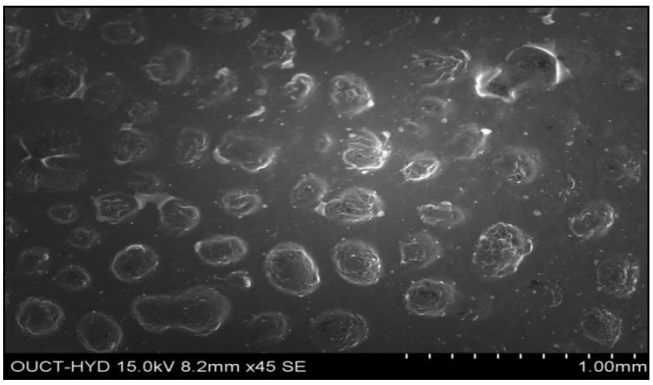


Fig 2: FTIR Studies of Optimized formulation

Particle size

1. Vesicle shape: Vesicle shape of the prepared formulation was found to be spherical from the SEM (scanning electron microscope) analysis at 15.00kV



2. Vesicle size

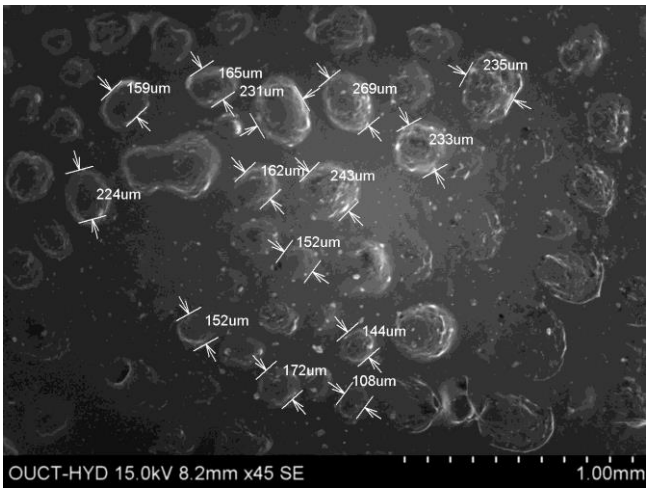


Fig 3: Particle size of Doxorubicin liposomes

Table 2: Particle of different formulation of liposomes

Sr. No	Formulation No.	Particle size
1	F1	95.03
2	F2	93.18
3	F3	101.4
4	F4	124.6

Drug entrapment efficiency

Table 3: Different batches of liposome made by using different ratio of lipids

Sr. No	Formulation no.	DEE
1	F1	83.56
2	F2	88.92
3	F3	79.11
4	F4	86.98

Drug release studies

Table 4: Cumulative percentage drug release from various formulation of liposomes

Time	F1	F2	F3	F4
0	0	0	0	0
1	17.35	19.85	22.6	18.35
2	23.08	23.63	34.18	27.09
3	35.15	36.1	47.3	35.13
4	40.39	49.32	51.35	46.38
5	51.81	61.52	63.24	54.51
6	62.47	74.25	75.54	64.4
7	76.16	85.15	83.08	72.16
8	85.5	97.43	95.1	88.74

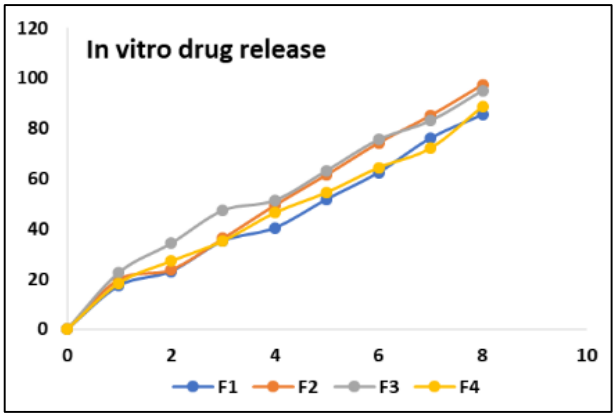


Fig 4: *In vitro* drug release of various formulations

All the three batches of formulation F2 were found to release the drug in 8 h. The cumulative percentage release was found to be 97.43%.

Stability studies

There was no significant change in physical and chemical properties of the tablets of formulation F-2 after 3 months. Parameters quantified at various time intervals were shown;

Table 5: Results of stability studies of optimized formulation F-2

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-2	25^0C/60%RH % Release	97.43	96.11	95.63	94.56	Not less than 85 %
F-2	30^0C/75% RH % Release	97.43	96.12	95.14	94.23	Not less than 85 %
F-2	40^0C/75% RH % Release	97.43	96.13	95.02	94.05	Not less than 85 %

Conclusion

Doxorubicin possesses all requisite qualities required for liposomal drug delivery. Among the various formulation, the combination F3 was found to be most suitable because of high encapsulation efficiency with smaller particle size. The formulation F2 comprising phosphatidylcholine, cholesterol, fulfills the requirement of good liposomal formulation. *In vitro* drug release up to 8 h and more than 97.43 % drug released. Follows Peppas model in release studies. It shows encapsulation efficiency of 88.92 % and particle size of 93.18 µm.

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