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# FORMULATION DEVELOPMENT AND INVITRO EVALUATION OF LIPOSOMAL DRUG DELIVERY SYSTEM FOR DAUNORUBICIN HYDROCHLORIDE

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## ABSTRACT

The present work was done on the formulating of daunorubicin liposomal formulation using Soybean lecithin, Cholesterol, Dicetyl phosphate, Stearyl amine. Ammonium sulphate polymers in different ratios and physical and particle size analysis and SEM analysis, invitro studies were performed for all formulations in that F2 formulations shows better kinetic propertied compare with the all the formulations.

**KEYWORDS:** Daunorubicin, liposomal kinetic studies, bioavailability.

## INTRODUCTION

Nano-based drug delivery system (NDDS) in this case has been proved to be essentially efficient choice line of treatment. It has ability to overcome many of the pharmaceutical and clinical hurdles which are faced during the chemotherapy. NDDS exhibits improved drugs stability, desired Pharmacokinetics patterns, targeted delivery system.[5,6,7] It also increases possibility of combination of different type of drugs in a single formulation, thus holds a big share in developing effective chemoimmunotherapy regimens. Liposome is one of the most widely explored area in NDDS.[5] It has huge applicability by inducing different manipulations in the structure as well as the excipients used. The spectrum of variations in this delivery system that are possibly makes it more viable formulations for many drugs as well as treatments. The other reason for its acceptability is the structure of liposomes. As, it can carries both hydrophilic and lipophilic type of drugs in one closed vesicle.[8] This is possible due to its two-layered structure with phospholipids and cholesterol.[9] This combination facilitates higher encapsulation, targeting ability, low toxicity and primarily its feasibility to produce at industrial level. Liposomes possess advantage over others are enhanced solubility of drug which is encapsulated, prevent the drug from biological and chemical degradation, decrease the toxic effect of drug, enhance therapeutic index and efficiency of drug entities, compatibility of drug increases with non-toxic and biodegradable materials.[10] The separation of inner core from outer phase improves the stability of the drug enclosed inside the formulation. Encapsulation of drug leads to gain controlled or sustain release of drug. The bioavailability of poorly soluble drugs can be improved using this technique. The change in the biological characters can be achieved by modifying the ligands on the surface of liposomes.[11] There are various methods for formulating liposomes. The selection of the technique is based on the following factors: 1) the physicochemical properties of the components of liposome and the drug to be loaded; 2) amount and toxicity of drug to be loaded; 3) form of medium which is used to dispersed the liposomes; 4) the additional processes during the application/delivery of the liposomes; 5) the size and the half-life desired for the successful application; and 6) the costs, reproducibility, and applicability regarding large-scale production for clinical purpose and good manufacturing practice-relevant issues.[10,11] The methods employed to formulate liposomes includes supercritical fluid technology, dual asymmetric centrifugation, membrane contactor technology, cross-flow filtration technology and freeze drying technology. The more commonly used method to prepare liposome is Bangham or thin film hydration method.[12,13] The method involved the solubilization of lipid in organic solvent, evaporation of the solvent and dispersion of the lipid film in aqueous medium. The techniques such as solvent injection and reverse- phase evaporation provide hydration of lipids from organic solvent and produce unilamellar vesicles (ULVs) and multilamellar vesicle (MLVs).[14,15] The advantage of solvent injection and reverse- phase evaporation method is to produce high encapsulation efficiency than thin film hydration technique. It has been documented by many researchers that liposomes formulated with these two methods are very stable and also give higher encapsulated yield.

Daunorubicin is a drug used in cancer chemotherapy, it is an anthracycline topoisomerase. Daunorubicin is commonly used in the treatment of a wide range of

cancers, including hematological malignancies, many types of carcinoma, and soft tissue sarcomas. It is chemically called as (7S,9S)-9-acetyl-7-[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-4-methoxy-8,10-dihydro-7H-tetracene-5,12-dione.

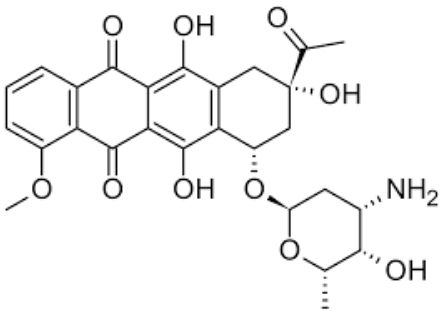


**Figure 1: chemical structure of daunorubicin.**

## MATERIAL AND METHODS

All materials were AR and LR graded used for the study. The following chemicals and drugs Daunorubicin hydrochloride Soybean lecithin, Cholesterol, Dicetyl phosphate, Stearyl amine. Ammonium sulphate, Sucrose, Histidine, Chloroform, Sodium hydroxide, Acetonitrile, Methanol, Sodium lauryl sulphate, Isopropyl alcohol were collected and purchased from Sipra private limited Hyderabad.

### Methodology

### Standard Calibration Curve

Standard calibration curve of Daunorubicin hydrochloride was developed using phosphate buffer pH 7.4 and estimated by UV-Visible spectrophotometer at 254nm.

### General Procedure for the preparation of calibration curve by UV

A stock solution of (1mg/ml) of standard drug was prepared, later required dilutions were made with a phosphate buffer pH 7.4. To a series of 10ml volumetric flasks aliquots standard solutions were taken and the volume was made up using a phosphate buffer pH 7.4. The absorbance of these solutions was measured at respective wave length of maximum absorbance, using 1cm quartz cuvette in UV- Visible spectrophotometer. Absorbance values were plotted against respective concentration to obtain standard calibration curve.

### Compatibility Studies

IR spectroscopy can be used to investigate and predict any physicochemical interactions between different components in a formulation and therefore it can be applied to the selection of suitable chemically compatible excipients.

### Preparation Of Daunorubicin Liposomes

### Procedure for the preparation of Daunorubicin liposome

The preparation of liposomes with Soybean lecithin was prepared by dried thin film hydration technique using rotary evaporator.

Accurately weighed quantities of Soy lecithin, cholesterol, Stearylamine and Dicetylphosphate are dissolved in chloroform and rotated in a rota-vap by applying vaccum of about 25mmHg at $25^0$c, until it forms a thin film. Required quantities of ammonium sulphate and sucrose (0.3%) are dissolved in W.F.I and it is added to the above thin film in R.B flask and rotated until it forms a milky white suspension. The above solution is homogenized for 15 cycles to reduce particle size of liposomes. The above solution is undergone for 25 cycles of dialysis, by using sucrose solution (10%) to remove free ammonia and sulphate from the lipid solution. Drug solution is prepared by adding the required quantities of Drug and Histidine in a W.F.I and pH is adjusted to 6.4 to 6.7 and this drug solution is added to the solution in a R.B flask (lipid solution) and rotated for 1hr.

### In-process Checks

**RPM:** 65-70rpm (Film formation), 50-55rpm (Hydration), 60-65rpm (DrugLoading).

**Temperature:** 40-45°C (Film formation), 65-70°C (Hydration), 65°C (DrugLoading).

The composition and ratios of lecithin, cholesterol and stabilizers for different types of Liposomes were mentioned in Table No: 1.

**Table 1: Daunorubicin liposomal Formulation table.**

| Formul ation code | Drug (mg/ml) | Soy lecithin (mg/ml) | Cholesterol (mg/ml) | Stearylamine (mg/ml) | Dicetyl phosphate (mg/ml) | Ammonim Sulphate (mg/ml) |
|---|---|---|---|---|---|---|
| F1 | 2 | 7 | 3 | - | - | 30 |
| F2 | 2 | 7.5 | 2.5 | - | - | 30 |
| F3 | 2 | 7 | 3 | 1 | - | 30 |
| F4 | 2 | 7.5 | 2.5 | 1 | - | 30 |
| F5 | 2 | 7 | 3 | - | 1 | 30 |
| F6 | 2 | 7.5 | 2.5 | - | 1 | 30 |

### Physical Characterization of Liposomes

All the liposomal formulation was evaluated by studying their physicochemical properties like

- Particle size analysis

- Polydispersity index
- Zeta potential analysis
- SEM analysis

**Determination of particle size distribution**
Determination of average vesicle size of Daunorubicin hydrochloride liposomes with carrier was very important characteristic.

**Polydispersity Index**
Polydispersity was determined according to the equation,
Polydispersity = D (0.9) - D (0.1) / D (0.5)
Where, D (0.9) corresponds to particle size immediately above 90% of the sample.D (0.5) corresponds to particle size immediately above 50% of the sample. D (0.1) corresponds to particle size immediately above 10% of the sample.

**Scanning Electron Microscopy**
Determination of surface morphology (roundness, smoothness and formation of aggregates) of Daunorubicin hydrochloride Liposomes with carrier was carried out by scanning electron microscopy (SEM). Samples for by SEM were mounted on metal studs and were magnified to X 2000.

**In Vitro Characterization**
**Estimation of free ammonia**
Added 1ml of lipid solution to 1ml of barium chloride solution and mixed together in a centrifuge tube and centrifuged for 10 minutes at 5000rpm, it forms a precipitate of barium sulphate, the formed precipitate is dried and weight of barium sulphate is noted.

Free ammonia = weight of barium sulphate ×0.56590

**Percent free drug: (Howard G *et al.*, 1977)**
Measure the absorbance of solution at 590nm using sucrose Histidine solution as blank.

1. Transferred 0.1ml of sample to a 20ml stoppered test tube, add 8ml of Sucrose-Histidine solution to it, mix well, measure the absorbance at 590nm using calibrated UV spectrophotometer. Transfer the solution from the cell to test tube ($A_1$).
2. To the above test tube containing solution, added 1ml sodium hydroxide solution, mix well measure the absorbance at 590 nm using UV transfer the solution from the cell to test tube ($A_2$)
3. To the above test tube containing solution, add 1ml of Triton X-100 solution, mix well measure the absorbance at 590 nm using calibrated UV ($A_3$)

Percent Free Daunorubicin Hcl = $[(A_2 \times 1.125) – A_1/A_3 \times 1.25] \times 100$

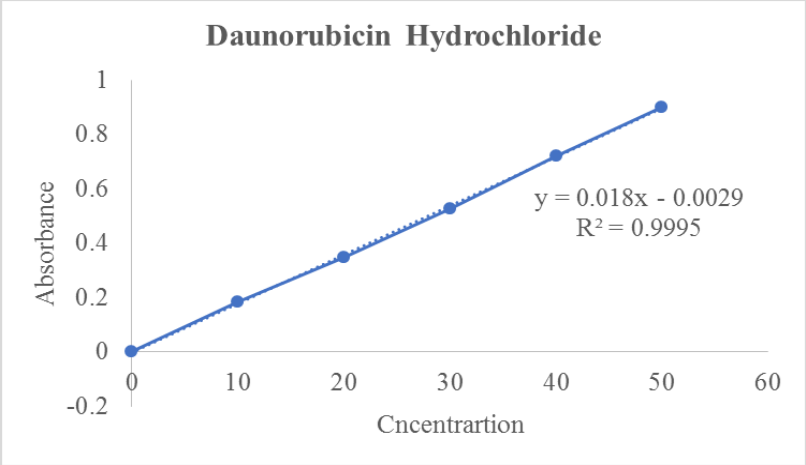
**In vitro dissolution studies of Daunorubicin hydrochloride liposome**
The *in vitro* release of drug from the liposomal formulation was carried out by using dialysis membrane employing in two sides open ended cylinder. 4 ml of liposomal suspension containing known amount of drug was placed in a dialysis membrane previously soaked overnight. The two sides open cylinder was placed in 200ml of PBS (pH 7.4), maintained at 37$^{o}$ C and stirred with the help of a magnetic stirrer. Aliquots (4ml) of release medium were withdrawn at different time intervals and the sample was replaced with fresh PBS (pH 7.4) to maintain constant volume. 1 ml of acetonitrile was added to each aliquot to precipitate the lipids and dissolve the entrapped Daunorubicin hydrochloride and then the samples were analyzed by UV spectrophotometry at a λ max of 254nm.

**Release kinetics (**Harris shoaib *et al.*, 2006)
Zero-order
Fist-order
Higuchi
Korsmeyer –Peppas (n)

**RESULTS AND DISCUSSION**

**Table 2: Standard readings of Daunorubicin hydrochloride in UV.**

| S. No. | Concentration (µg/ml) | Absorbance at 254nm |
|---|---|---|
| 1. | 0 | 0 |
| 2. | 10 | 0.184 |
| 3. | 20 | 0.348 |
| 4. | 30 | 0.526 |
| 5. | 40 | 0.721 |
| 6. | 50 | 0.901 |




**Graph No: 1 Standard graph of Daunorubicin hydrochloride in phosphate buffer of pH 7.4.**

**Table 9: Physicochemical characteristics of Daunorubicin hydrochloride Liposomes for Optimized Batches.**

| S. No. | Formulation code | Average vesicular size (nm) | Zeta Potential(mV) | Poly dispersive index (Pdi) |
|---|---|---|---|---|
| 1. | F2 | 356nm | 5.21 | 0.635 |
| 2. | F4 | 564nm | 24.66 | 0.762 |
| 3. | F6 | 317nm | -23.4 | 0.645 |

**Scanning Electron Microscopy**

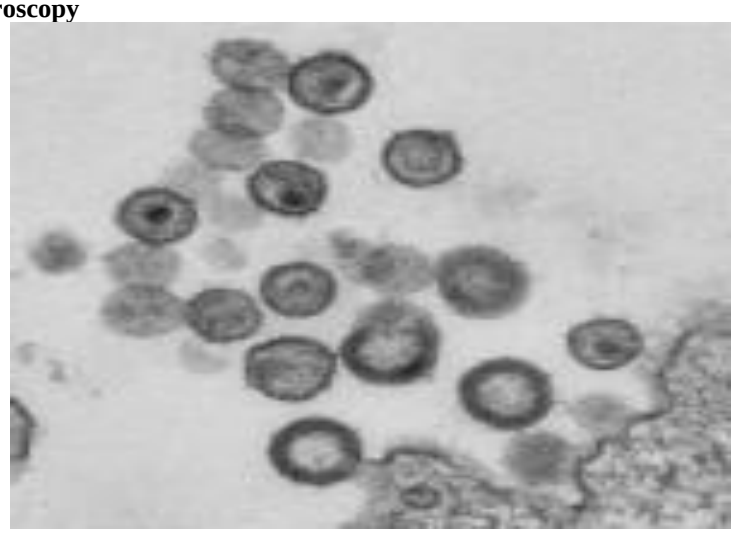


**Fig. 7: SEM photography of Liposomal solution for F2 formulation.**

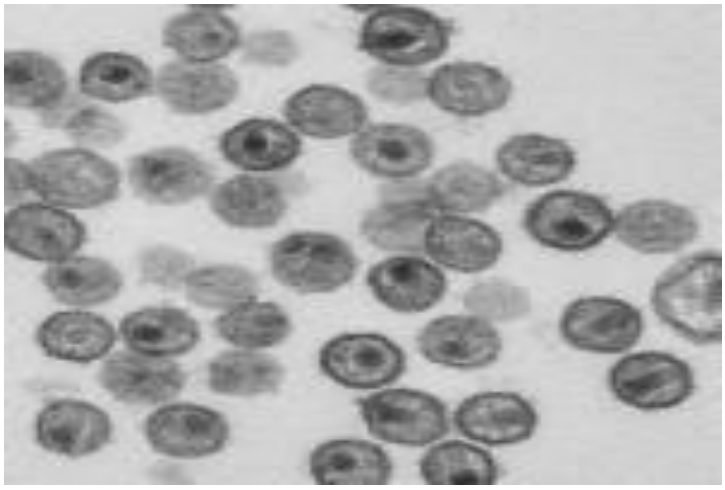
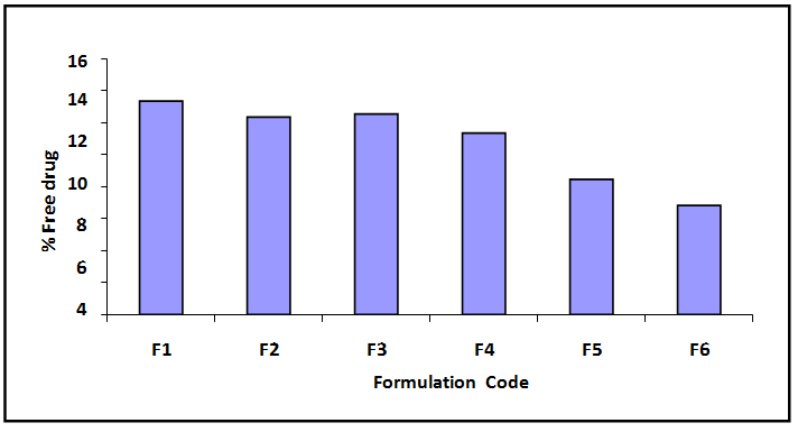


**Fig. 8: SEM photography of Liposomal solution for F6 formulation.**

**Table 10 Percent free drug of Daunorubicin liposomal solution for F1, F2, F3, F4, F5 and F6 Formulations. (n=3).**

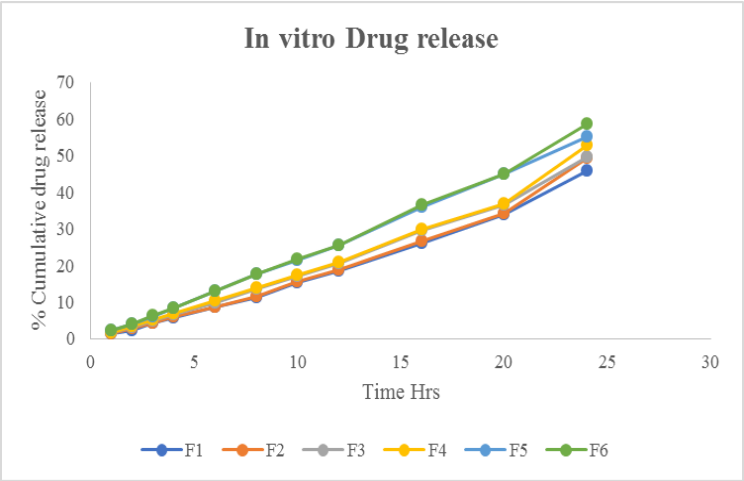
| S. No. | Formulation code | Percentage of free drug |
|---|---|---|
| 1. | F1 | 13.36±1.1% |
| 2. | F2 | 12.36±1.1% |
| 3. | F3 | 12.56±4.6% |
| 4. | F4 | 11.36±1.3% |
| 5. | F5 | 8.45±5.9% |
| 6. | F6 | 6.83±0.8% |




**Graph 2: Percent free drug plot for F1, F2 F3, F4, F5, F6 Formulations.**

**Table 12: *In vitro* cumulative % drug release profile of Daunorubicinhydrochloride liposomal formulations.**

| Time (hrs) | Cumulative % drug release | | | | | |
|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 |
| **1** | 1.29 | 1.38 | 1.65 | 1.74 | 2.12 | 2.15 |
| **2** | 2.36 | 2.92 | 3.21 | 3.37 | 4.02 | 4.07 |
| **3** | 4.32 | 4.41 | 5.01 | 5.11 | 6.12 | 6.19 |
| **4** | 5.65 | 5.93 | 6.56 | 6.82 | 8.32 | 8.43 |
| **6** | 8.53 | 8.77 | 9.85 | 10.5 | 12.92 | 13.01 |
| **8** | 11.21 | 11.65 | 13.56 | 13.9 | 17.53 | 17.62 |
| **10** | 15.25 | 15.63 | 17.04 | 17.38 | 21.45 | 21.62 |
| **12** | 18.65 | 18.91 | 20.53 | 20.86 | 25.35 | 25.56 |
| **16** | 25.98 | 26.73 | 29.62 | 29.82 | 35.95 | 36.47 |
| **20** | 33.85 | 34.25 | 36.56 | 36.78 | 44.86 | 45.07 |
| **24** | 45.85 | 49.31 | 49.59 | 52.84 | 55.25 | 58.67 |




**Graph 2: In vitro cumulative % drug release profile of Daunorubicinhydrochloride liposomal formulations.**

**Table Kinetic release rate profile of Formulations F2, F4, F6.**

| Type of Formulation | Zero-order ($R^2$) | First-order ($R^2$) | Higuchi ($R^2$) | Korsmeyer –Peppas (n) |
|---|---|---|---|---|
| F2 | 0.978 | 0.9758 | 0.9053 | 1.0890 |
| F4 | 0.9818 | 0.9761 | 0.9162 | 1.0483 |
| F6 | 0.9953 | 0.9869 | 0.9437 | 1.0502 |

## DISCUSSION

The UV absorbance's of Daunorubicin hydrochloride standard solution in the range of 10-50 µg/ml of drug in buffer, pH 7.4 showed linearity at λ max 254nm. The linearity was plotted for absorbance against concentration with R2 value 0.9995 and with the slope equation y=0.0179x-0.003. The absorbance values and standard curve shown in Table No:1 and Graph No:1 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.

### Daunorubicin liposomal formulation

The Liposomes were prepared by dried thin film hydration technique using rotary evaporator with drug and carrier (soybean lecithin) The formulation containing Daunorubicin were prepared with differentstabilizers like Dicetylphosphate and Stearylamine and all other parameters like temperature, vacuum and RPM were kept constant. The composition and ratios of compounds showed in Table No: 7. among those compositions 6 Formulations are selected as optimized batches for further evaluation.

### Particle size distribution

The particle size distribution was analyzed for F2, F4, F6 formulations of Daunorubicin Liposomes by wet method. The particle size was optimum in F6 Formulation, When compared to F2 and F4.

### Scanning Electron Microscopy

The Morphology and surface appearance of Liposomes were examined by using SEM. The SEM photographs of F2 and F6 formulation showed that the particles have smooth surface.

### Zeta Potential analysis

The zeta potential report of liposomal solution for F2, F4, F6 formulations are 5.21mV, 24.66mV, -23.4 which lies near to the arbitary value. The report shows good stability value for formulated liposomal solution.

### In Vitro Characterization

### Percent free drug

The percent free drug is determined for all the formulations F1to F6. The percent free drug was optimum in F6 formulation, which is within the limit (10%).

### *In vitro* Dissolution data

The *in vitro* dissolution profile of prepared formulations was determined by membrane diffusion method. The dissolution was carried out for a period of 24 hrs in 7.4 pH phosphate buffer. The cumulative percent release of F1 to F6 formulations at various time intervals was calculated and tabulated in Table No: 12 The cumulative percent drug release in all formulations was plotted against time in Graph No: 4, 5, 6, 7, 8, and 9. The Maximum percent of drug release was found in F6 formulation which contains maximum drug entrapment.

### Release Kinetics

The release kinetics of F2, F4, F6 formulations were studied. All formulations follow Zero order release kinetics and follow case II transport when it applied to the Korsmeyer-Peppa's Model for mechanism of drug release. F6 formulation has better kinetic results when compared to F2 and F4 formulations.

## CONCLUSION

Based on the results it concluding that the. F6 formulation has better kinetic results when compared to F2 and F4 for Daunorubicin liposomal formulation.

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