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DEVELOPMENT AND EVALUATION OF DOXORUBICIN CUBOSOMES FOR TARGETED CANCER THERAPY

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Abstract:

The present study was aimed at the formulation and evaluation of Doxorubicin-loaded cubosomes to enhance drug encapsulation efficiency, achieve sustained drug release, and improve formulation stability. Morphological evaluation by Scanning Electron Microscopy (SEM) revealed that the prepared cubosomes were discrete and nearly spherical in shape. Particle size analysis indicated that the formulations had particle sizes ranging from 165 to 183 nm, with formulation F4 exhibiting the smallest particle size (165 nm). Zeta potential values ranged from −25 to −30 mV, indicating good physical stability of the cubosomal formulations. Drug entrapment efficiency studies showed efficient incorporation of Doxorubicin into the cubosomes, with formulation F4 exhibiting the highest entrapment efficiency of 85.92%.In vitro drug release studies demonstrated a sustained release pattern over an 8-hour period, with the optimized formulation F4 exhibiting the highest cumulative drug release of 98.52%. Accelerated stability studies conducted as per ICH guidelines for three months showed no significant changes in the physicochemical characteristics or drug release profile of the optimized formulation, confirming its stability.The results of the present investigation suggest that Doxorubicin-loaded cubosomes represent a promising nanocarrier system for anticancer drug delivery, offering advantages such as high drug entrapment, sustained drug release, enhanced stability, and the potential to improve therapeutic efficacy while minimizing systemic toxicity. Among the developed formulations, F4 was identified as the optimized formulation based on its superior physicochemical characteristics and release profile. Further in vivo studies are warranted to establish the therapeutic potential and clinical applicability of the developed cubosomal formulation.

Keywords: *Doxorubicin, Cubosomes, Nanocarrier System, Entrapment Efficiency, Sustained Drug Release, Zeta Potential, Stability Studies.*

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INTRODUCTION:

Cubosome preparation can be achieved through two approaches: top-down (TD) and bottom-up (BU) techniques. In the TD route the bulk cubic phase is dispersed into an aqueous solution using high energy which can be created by either sonication, shearing or high-pressure homogenization. The TD method has been reported by Ljusberg- Wahren and is the most commonly used technique for the preparation of cubosomes. It is a rapid method which forms cubosomes with a size below 200 nm and low polydispersity. However, a drawback of this method is that it is only feasible in a small scale. Furthermore, this approach requires high thermal/mechanical energies 7 which in turn can cause problems in the incorporation of labile compounds such as proteins and peptides. In the BU method also referred to as the solvent dilution, liquid precursor or hydrotope method, a single-phase solution is diluted into a two phase system consisting of cubosomes and an aqueous phase. This is a more recently developed technique first introduced by Spicer and co-workers. It requires low energy and takes advantage of the miscibility of an auxiliary solvent (e.g., ethanol) in a continuous aqueous phase. Upon the addition of excess water, lipid droplet nucleation occurs and the mixture spontaneously phase separates and the cubosomes precipitate. The main advantage of this method compared to the TD technique is that less energy is required to form the dispersions, which allows for large scale preparation of cubosomes and is particularly preferred in case of incorporation of sensitive compounds. However, the main disadvantages of the BU method are the difficulties in controlling particle size and the possible presence of solvent in the final sample, which is often incompatible with solvent-sensitive compounds incorporated in the cubosomes such as proteins and may be unacceptable for medical applications. The most frequently investigated liquid crystal structures for biomedical applications are prepared by cubic mesophases of unsaturated glyceryl monoglycerides (GMO) and phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol) that are capable of forming viscous reverse phases (hexagonal and bicontinuous cubic) in excess water under physiological conditions and temperature. Phytantriol has gained more recent interest in the biomedical field compared to monoglycerides due to several reasons, such as superior chemical stability compared to monoglycerides due to the absence of the ester group and availability of commercial phytantriol with 95% purity, whereas monoglycerides are produced from various sources which have significantly different purities.

Cubosomes require the addition of another surfactant to kinetically stabilize these colloidal dispersions against aggregation. Pluronic®F127 is a nonionic linear triblock polymer that contains a polypropylene oxide block (PPO) between two polyethylene oxide blocks (PEO) and acts as a steric stabilizer via the adsorption and incorporation of its hydrophobic PPO blocks onto the surface of the nanoparticles while also maintaining the inner cubic phase structure.

In a number of studies, researchers have investigated the preparation of cubosomes via the TD and BU methods and aimed at optimizing the preparation procedure for producing cubosomes with consistent size and structure. Rizwan et al. first reported the preparation of phytantriol cubosomes using the BU method and compared them to cubosomes made by TD method

Definition

Cubosomes are discrete, sub-micron, nano-structured particles of bicontinuous cubic liquid crystalline phase. Cubosomes possess the same microstructure as the parent cubic phase but have larger specific surface area and their dispersions have much lower viscosity in comparison to the bulk cubic phase. Cubosomes are nanoparticles, more accurately nanostructure particles of a liquid crystalline phase with cubic crystallographic symmetry formed by the self assembly of amphiphilic or surfactant like molecules.

Advantages:

High drug payloads due to high internal surface area and cubic crystalline structures.

- Relatively simple method of preparation.
- Biodegradability of lipids.
- Capability of encapsulating hydrophilic, hydrophobic and amphiphilic substances.
- Targeted release and controlled release of bioactive agents.

MATERIALS:

Doxorubicin was procured from Synpharma Research Labs, Pluronic acid F-127, Glyceryl monostearate and Ethanol were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY:

Formulation development

Top-Down Method

Table-1: Formulation development

Ingredients	F1	F2	F3	F4
Doxorubicin	10	10	10	10
Glyceryl monostearate	100	200	300	400
Pluronic F-127	100	100	100	100
Ethanol	10	10	10	10
Water	q. s	q. s	q.s	q. s

Method of Preparation:

Top-Down Method

Doxorubicin and GMO was weighed into a glass vial and heated at 40°C until free flowing. Aqueous solution containing different concentrations of Pluronic F-127 dissolve in water, were added to the vial containing Doxorubicin and lipid solution. Subsequently, this mixture was homogenized by ultrasonication at 40°C, amplitude 80%, pulse cycle 1 for 30min until a milky dispersion was formed. To study the effect of ethanol on the cubosome formation, different amounts of ethanol were added to the F-127 aqueous solution. In order to examine the influence of the initial temperature on the cubosomes, they were also prepared at 60 and 70°C. By this method the formulations of various formulation ratios were determined.

Evaluation of Cubosomes:

Particle size

The cubosomes suspension (100 mg) was hydrated in a small glass test tube using 10 ml of pH 7.4 phosphate buffer solution. The dispersion was observed under optical microscope at 40X magnification. Size and size distribution of 200–300 niosomes were noted using calibrated stage and ocular micrometers (Elico Instruments, Hyderabad).

SEM analysis:

The shape, surface characteristics, and size of the cubosomes were observed by scanning electron microscopy.

Zeta potential

The zeta potential of a particle represents the overall charge of the particle and stability of the formulation. Zeta potential measurement was carried out using Zeta sizer Nano-ZS90, Malvern Instrument Ltd., UK by differential light scattering (DLS) technique. Nanoparticle samples redispersed in Milli-Q water. All measurements were carried out in triplicates at 25 °C.

Entrapment efficiency:

Entrapment efficiency (EE%) is a key parameter in the development of novel drug delivery systems such as liposomes, niosomes, cubosomes, nanoparticles, and other vesicular carriers. It represents the percentage of drug that is successfully enclosed (entrapped) within the carrier system compared to the total amount of drug initially used in the formulation.

In vitro drug Release Study:

In vitro release studies were carried out using unjacketed vertical Franz diffusion cells with a diffusional surface area of 6.154 cm^2 and 20 mL of receptor cell volume. Prior to the study, the dialysis membrane was soaked in phosphate buffer pH 7.4 Formulation equivalent to 100mg of Doxorubicin cubosomes was placed in the donor compartment. The receptor compartment consisting of PB pH 7.4 was maintained at 37±2°C under constant stirring up to 8 hrs. The donor chamber and the sampling port were covered with lid to prevent evaporation during the study. Aliquots of 5 mL were withdrawn periodically at different time intervals (1,2,3,4,5,6,7 and 8hrs) and replaced with equal volume to maintain constant receptor phase volume. At the end of the study, the samples were suitably diluted and the amount of drug was determined spectrophotometrically.

Stability Studies:

The formulations stored in glass vials covered with aluminium foil were kept at room temperature and in refrigerator (4◦C) for a period of 90 days, samples were withdrawn and hydrated with phosphate-buffered saline (pH 7.4) and observed for any sign of drug crystallization under optical microscope. Furthermore, the samples were also evaluated for drug release studies.

RESULT & DISCUSSION:

Determination of Vesicle morphology and Size

The morphological characteristics of formulated Cubosomes were carried by using Scanning electron microscopy (SEM). A small drop of Cubosomes was placed between two rivets fixed on a gold-plated copper sample holder. The whole system was subjected under vacuum in liquid nitrogen. The sample was heated to -85^0C for 30 min to sublime the surface moisture. Finally the sample was coated with gold and allowed the SEM to capture the images at a temperature of - 120^0c and voltage of 5kV.Scanning electron microscopy are the direct method to measure cubosomes, physical characterization of cubosomes with the former method being used for morphological examination.

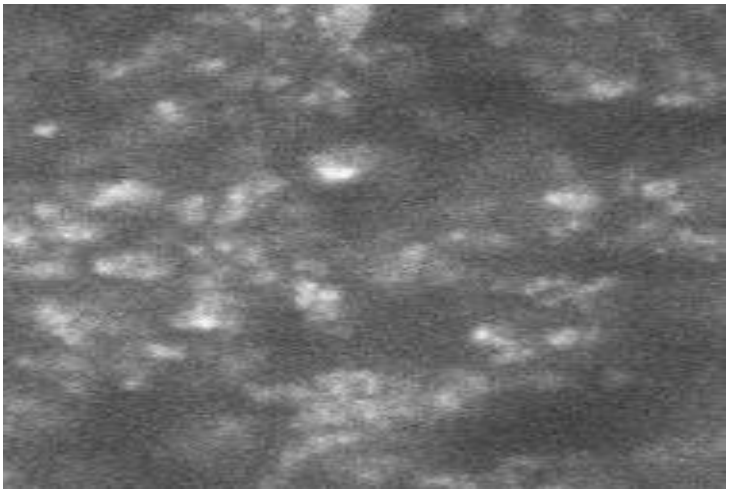


Fig-1: SEM Analysis of optimized formulation

Zeta potential:

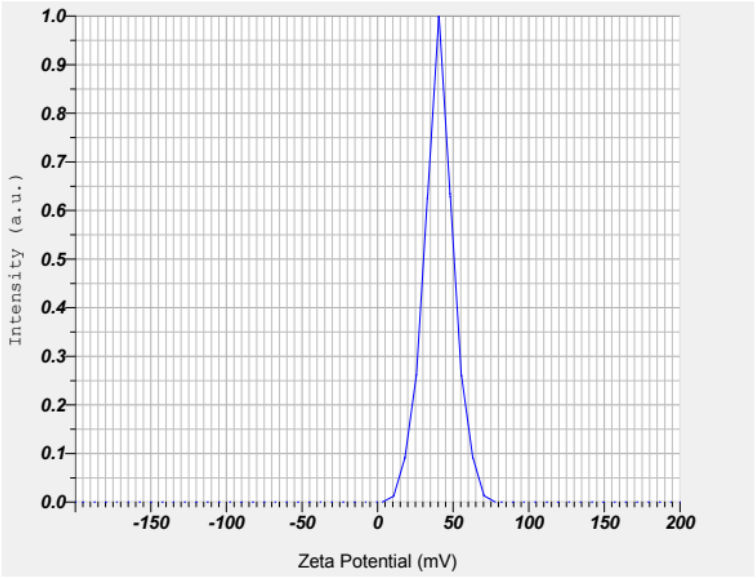


Fig-2: Zeta potential of optimized formulation

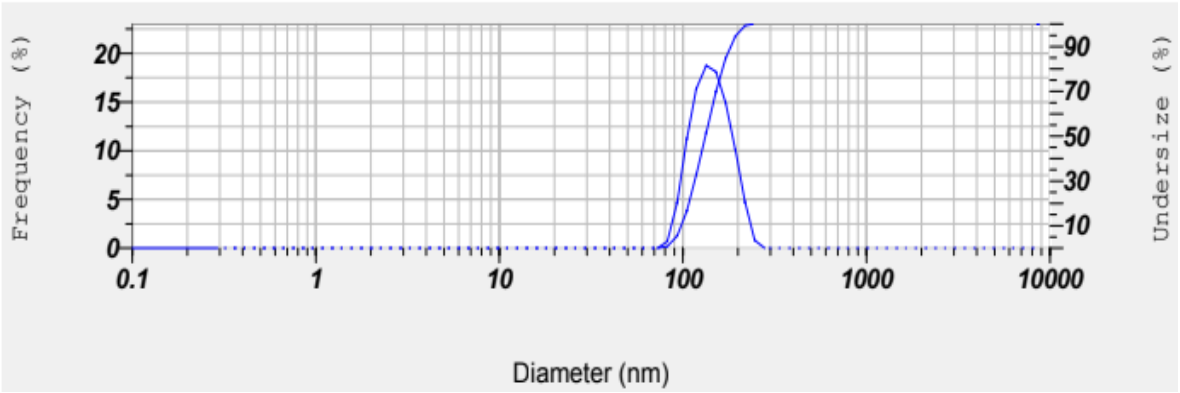


Fig-3: Particle size of optimized formulation

Table-2: Evaluation Studies of particle size and Zeta potential Cubosomes

F. No	Particle size (nm)	Zeta potential (mV)
F1	175	-25
F2	169	-29
F3	183	-26
F4	165	-30

Entrapment Efficiency:

Separation of unentrapped drug from Cubosomes was done by exhaustive dialysis method. A measured quantity of Cubosomes was placed in a dialysis tube to which osmotic cellulose membrane was attached securely on one side and the dialysis tube was suspended in 100 ml of phosphate buffer pH 7.4 which was stirred continuously using magnetic stirrer. Through the osmotic cellulose membrane, the unentrapped drug was separated into the medium. For every one hour the whole medium was replaced with same quantity of fresh medium and continued for about 9 to 12hrs till the absorbance of collected medium reaches a constant reading indicating complete separation of unentrapped drug. The Cubosomes in the dialysis tube was further lysed with propane–1–ol and the entrapped drug was estimated with the help of double beam UV spectrophotometer at 480 nm. The entrapment efficiency was measured in % with the help of following equation.

Table-3: Drug entrapment efficiency of all formulation

F.no	Drug entrapment efficiency
F1	82.39
F2	81.54
F3	79.80
F4	85.92

In vitro drug release studies:

Table-4: Results of Doxorubicin cubosomes of all formulations

Time(hr.)	F1	F2	F3	F4
0	0	0	0	0
1	17.12	18.15	19.25	19.62
2	24.69	31.98	36.88	37.55
3	39.86	45.86	47.55	44.78
4	47.96	52.68	58.75	50.27
5	59.45	67.85	69.38	65.28
6	70.25	71.25	75.86	72.85
7	81.26	80.45	84.40	84.64
8	93.68	94.50	95.28	98.52

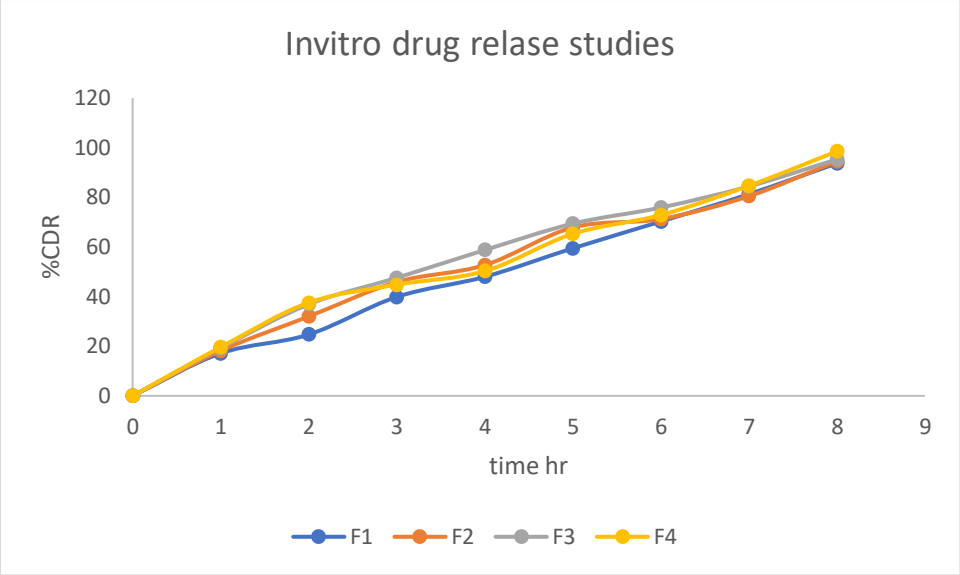


Fig-4: Drug release studies of (F1-F4) formulations

Most formulations reach >90% release, showing that the formulations are capable of complete drug release over the 8-hour period.
F4 reaches the highest release at 8 hr (98.52%), which may indicate a formulation optimized for maximum drug release.

Stability studies

Optimized formulations F4 was selected for accelerated stability studies as per ICH guidelines.

Table-5: Stability studies of optimized formulations at 40 ± 2 ^{0}C and 75 ± 5% RH for 3 months

Formulation Code	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	98.52	97.86	96.75	95.72	Not less than 85 %
F-4	98.52	97.52	96.25	95.36	Not less than 85 %
F-4	98.52	97.43	96.42	95.20	Not less than 85 %

SUMMARY AND CONCLUSION:

Summary

The present study was undertaken to formulate and evaluate Doxorubicin-loaded cubosomes using varying concentrations of formulation components. Four formulations (F1–F4) were successfully prepared and evaluated for their physicochemical properties, drug-excipient compatibility, particle size, zeta potential, entrapment efficiency, in vitro drug release, and stability. SEM analysis revealed that the prepared cubosomes exhibited discrete, nearly spherical morphology. Particle size analysis showed that the cubosomal formulations had particle sizes ranging from 165 to 183 nm, with formulation F4 exhibiting the smallest particle size (165 nm). Zeta potential values ranged from −25 to −30 mV, indicating good physical stability of the formulations.Entrapment efficiency studies demonstrated efficient incorporation of Doxorubicin into the cubosomes, with formulation F4 exhibiting the highest entrapment efficiency of 85.92%. In vitro drug release studies showed sustained drug release over an 8-hour period. Among all formulations, F4 demonstrated the highest cumulative drug release of 98.52%, indicating its superior release characteristics.Accelerated stability studies performed according to ICH guidelines for three months demonstrated no significant changes in the drug release profile of the optimized formulation. Formulation F4 retained more than 95% drug release, confirming its stability under storage conditions.

CONCLUSION:

The present investigation successfully developed and evaluated Doxorubicin-loaded cubosomes as a novel drug delivery system..Among the developed formulations, F4 was identified as the optimized

formulation based on its superior performance, including small particle size (165 nm), high entrapment efficiency (85.92%), satisfactory zeta potential (−30 mV), maximum cumulative drug release (98.52%), and excellent stability profile.The findings of this study suggest that cubosomes are a promising carrier system for Doxorubicin delivery, offering advantages such as enhanced drug encapsulation, controlled drug release, improved formulation stability, and the potential for enhanced therapeutic efficacy with reduced systemic toxicity.

Further in vivo pharmacokinetic and pharmacodynamic studies are recommended to establish the clinical applicability and therapeutic potential of the developed Doxorubicin-loaded cubosomal formulation.

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