Formulation and Evaluation of Turmeric Extract Loaded Cubosomes

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ABSTRACT :
The present study was focused on develop and evaluating Turmeric extract containing Cubosomes formulation for invitro studies. Cubosomal formulations were prepared by using bottom up method and were evaluated for invitro characteristics, stability studies. Cubosomal formulation displayed highest entrapment efficiency with desired particle size. SEM analyses showed that cubosomal formulation was spherical in shape. Cubosomes containing Poloxamer 407 percentage of drug release after 8h as compared to other formulations.F-5 formulation was found to be stable at the end of the study on storage condition. The present study suggested that cubosomes formulations provide sustained and prolonged delivery of drug with enhance bioavailability.
Keywords: Cubosomes, Turmeric extract, bioavailability, bottom up method, in vitro drug release studies.

I. INTRODUCTION

Nanotechnology-based drug delivery systems have emerged as a powerful strategy to overcome the biopharmaceutical limitations associated with poorly soluble phytoconstituents.[1] Cubosomes are nanostructured particles formed from amphiphilic lipids, such as glyceryl monooleate or phytantriol, stabilized by suitable surfactants. Their three-dimensional lipid bilayer network with interconnected aqueous channels enables the efficient encapsulation of both hydrophilic and lipophilic molecules, offering sustained drug release, high drug-loading capacity, and improved stability.[2]The incorporation of turmeric extract into cubosomal systems presents a promising approach to enhance the solubility, stability, and bioavailability of curcuminoids while preserving their therapeutic efficacy.[3]Turmeric rhizomes are rich in bioactive polyphenolic compounds, collectively known as curcuminoids, with curcumin being the principal constituent.[4] Curcumin exhibits a broad range of pharmacological properties, including anti-inflammatory, antioxidant, antimicrobial, anticancer, and wound-healing activities. Despite its promising therapeutic potential, the clinical translation of curcumin and turmeric extracts is severely limited by poor aqueous solubility, low chemical stability, rapid metabolism, and poor oral bioavailability.[5] The present study aims to formulate and evaluate turmeric extract-loaded cubosomes using suitable lipid and stabilizing agents.

II. EXPERIMENTAL WORK

Materials

Curcumin extract were collected from the Tirupati. Glyceryl monostearate and Poloxamer 407 were obtained from Synpharma Research Labs, Hyderabad. Other chemicalsand the reagents used were of analytical grade.

Methodology

Fourier transform infrared spectroscopy:

Fourier transform IR spectra were obtained on Shimadzu FT-IR spectrometer. Samples were prepared in KBr disks (2mg sample in 200mg KBr). The scanning range was 450-4000 cm^{-1} and the resolution was 4 cm^{-1}.[6]

Extraction process[7]

Soxhlet Extraction

1. Use dried, powdered Turmeric powder (30–50 g).
2. Load the powder into a thimble and place it in the Soxhlet extractor.
3. Use ethanol (95%) as the extracting solvent.
4. Allow extraction to proceed for 6–8 hours or until the solvent in the siphon becomes colorless.
5. Collect the extract and evaporate the solvent using a rotary evaporator.
6. Dry and store the extract in an amber vial under refrigeration (4–8°C).

Formulation development

Bottom up Method

Table-1: Formulation development

Ingredients	F1(1:1)	F2(1:2)	F3(1:3)	F4(1:4)	F5(1:5)	F6(1:6)
Turmeric extract (mg)	10	10	10	10	10	10
Glyceryl monostearate (mg)	50	50	50	50	50	50
Poloxamer 407 (mg)	10	20	30	40	50	60
Ethanol (ml)	10	10	10	10	10	10
Water	q.s	q.s	q.s	q.s	q.s	q.s

Method of Preparation:[8]

Bottom up Method

The cubosomal formulation comprised Glyceryl Mono Stearate (GMS) and Poloxamer 407. The process involved the gentle melting of GMS at 70°C in a water bath, followed by dropwise injection into preheated Poloxamer 407 solutions at 70°C. Simultaneously, 50mg of the drug was introduced, and distilled water was gradually added to achieve a final volume of 50mL. Mechanical stirring at 1500 rpm continued for 45 minutes, after which the solutions were sonicated for 15 minutes at a maximum power of 120 W. The resulting cubosome dispersions exhibited a milky white appearance following a 48-hour equilibration period subsequent to cooling to ambient temperature.

Evaluation of Cubosomes:

Particle size: The cubosomessuspension (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 100–300 cubosomeswere noted using calibrated stage and ocular micrometers (Elico Instruments, Hyderabad).[9]

SEM analysis: The shape, surface characteristics, and size of the cubosomeswere observed by scanning electron microscopy. [10]

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.[11]

Entrapment efficiency: To 0.2 g of cubosomes, weighed in a glass tube, 10 ml phosphate buffer pH 7.4 were added. The aqueous suspension was then sonicated. Cubosomes containing Turmeric extract were separated from unentrapped drug by centrifugation at 9000rpm for 45 min at 4◦C.[50] The supernatant was recovered and assayed spectrophotometrically using UVspectrophotometer. [12]

The encapsulation percentage of drug (EP) was calculated by the following equation:

$EP = [(C_t - C_r)/ C_t] * 100$

where,

Ct, concentration of total Turmeric extract,

Cr, concentration of free Turmeric extract.

***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 Turmeric extract 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.[13]

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.[14]

III. RESULTS AND DISCUSSION

DRUG - EXCIPIENT COMPATIBILITY STUDIES (FT-IR):

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

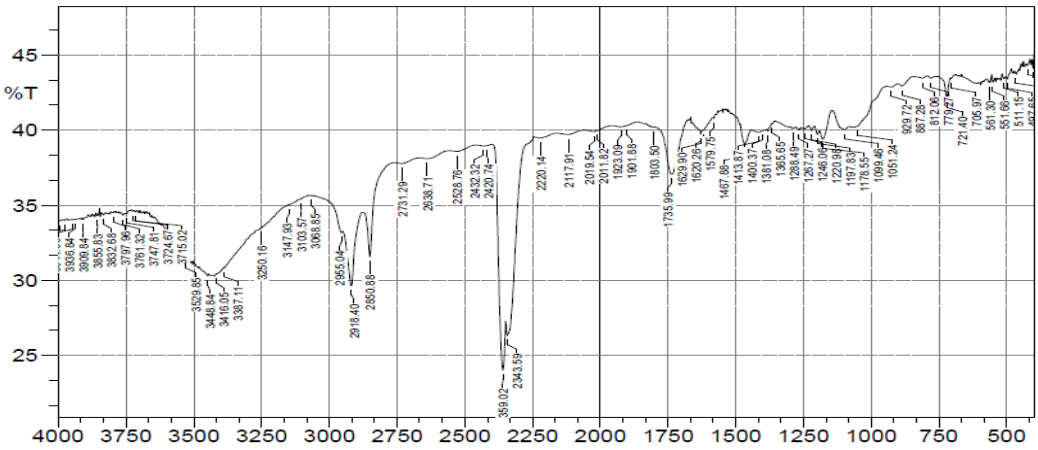

Fig-1: FTIR Spectra of Turmeric extract

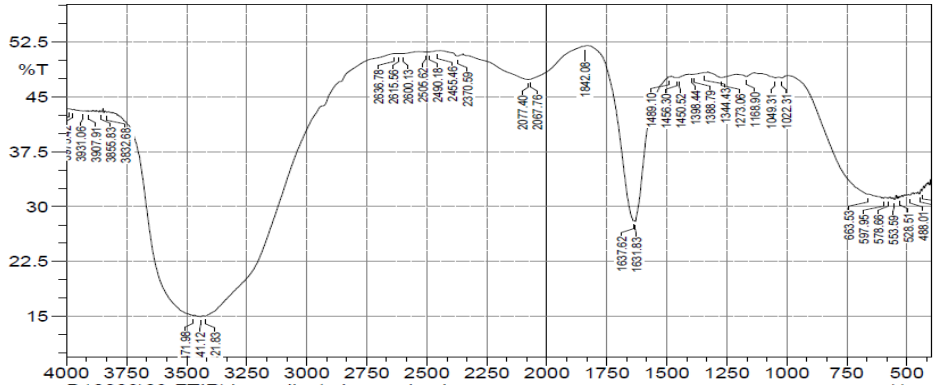

Fig-2: FTIR Spectra of Optimized formulation

EVALUATION PARAMETERS:

Entrapment Efficiency Table-2: Drug entrapment efficiency of all formulation

F.no	Drug entrapment efficiency
F1	82.39
F2	81.54
F3	79.8
F4	84.58
F5	86.89
F6	85.31

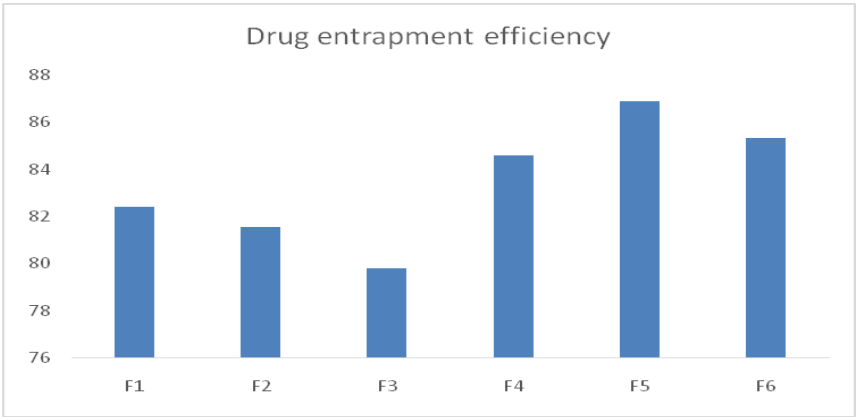

FIG-3: DRUG ENTRAPMENT EFFICIENCY OF ALL FORMULATION

Determination of Vesicle morphology and Size

ThemorphologicalcharacteristicsofformulatedCubosomeswerecarriedbyusingScanningelectron microscopy (SEM). A small drop of Cubosomes was placed between two rivets fixedon a gold-plated copper sample holder. The whole system was slushed under vacuum in liquid nitrogen. The sample was heated to -85^{0}C 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^{0}c 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.

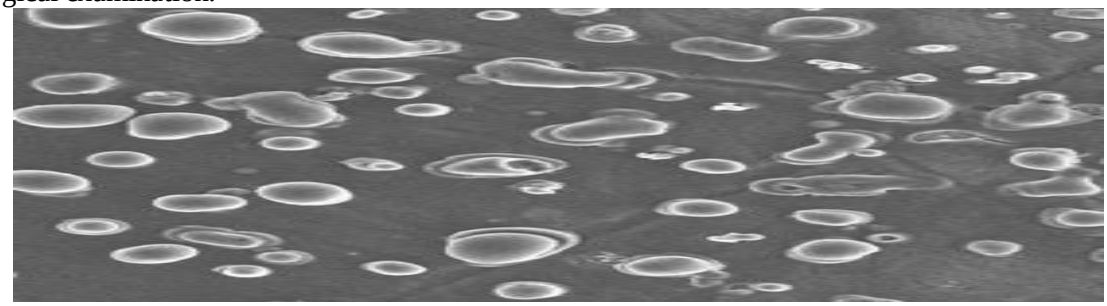

Fig-4: SEM Analysis of optimized formulation

Zeta potential:

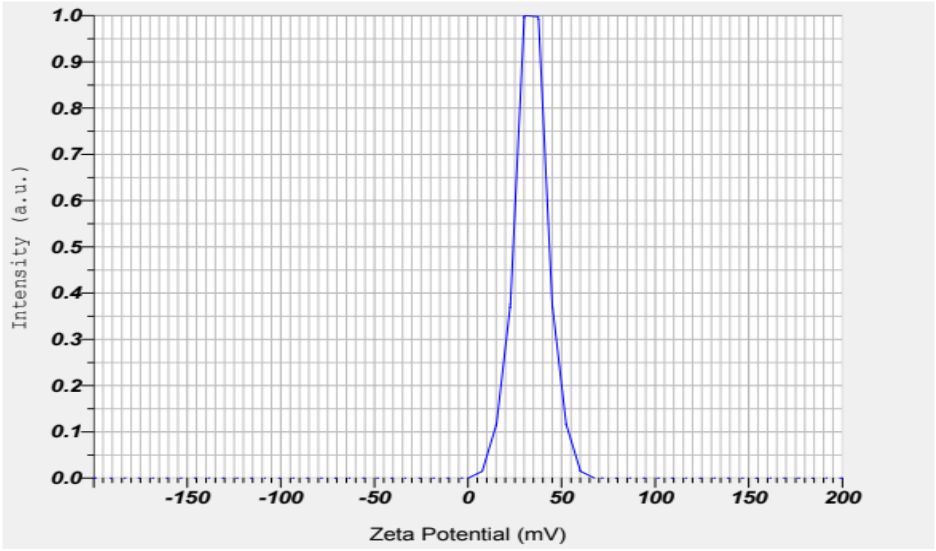

Fig-5: Zeta potential of optimized formulation

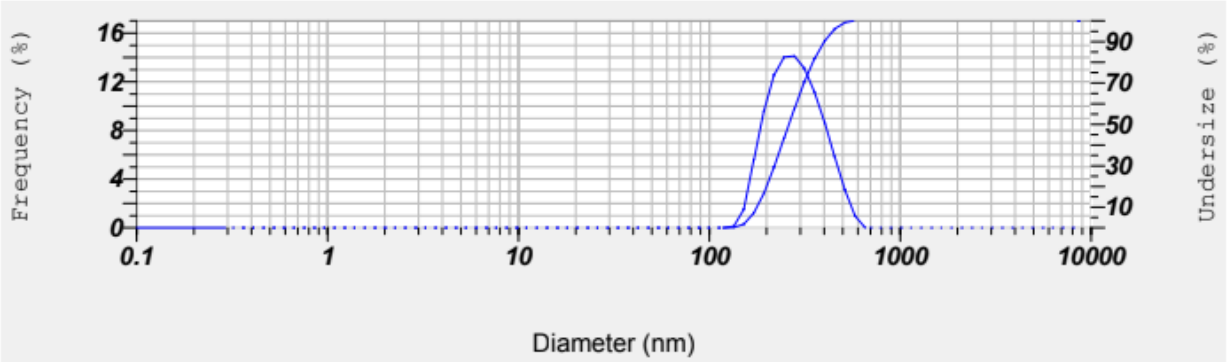

Fig-6: Particle size of optimized formulation

TABLE-3: EVALUATION STUDIES OF PARTICLE SIZE AND ZETA POTENTIAL CUBOSOMES

F. No	Particle size (nm)	ZETA POTENTIAL
F1	281	-30
F2	279	-29
F3	285	-37
F4	278	-32
F5	295	-27
F6	276	-26

In vitro drug release studies:

Table-4: Cumulative percentage drug release from various formulation of Cubosomes

Time(hr)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	25.16	26.37	27.18	28.10	29.10	26.37
2	31.67	35.67	34.63	35.39	34.69	35.83
3	45.19	48.10	49.82	48.13	47.91	45.18
4	56.37	55.96	55.93	55.27	56.37	58.15
5	68.50	67.65	68.46	63.69	65.18	67.22
6	75.79	77.80	76.37	77.53	78.55	76.18
7	82.37	83.36	85.10	83.19	85.10	83.19
8	94.68	95.50	96.89	95.27	97.15	95.20

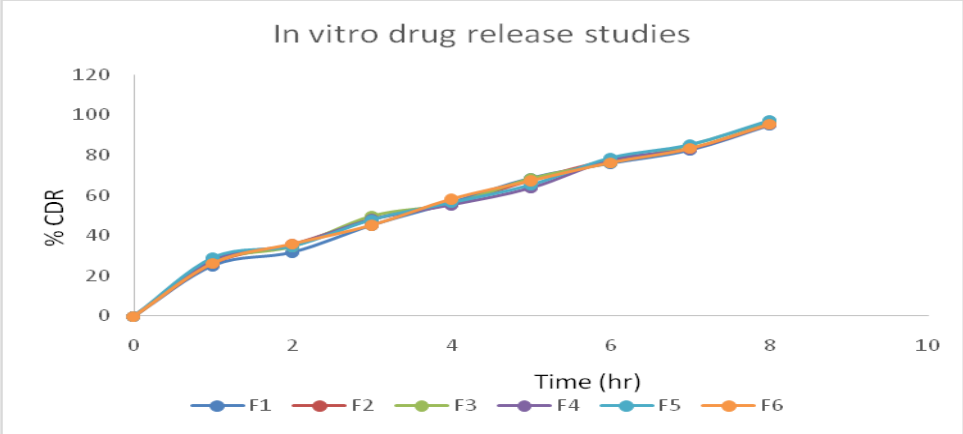

Fig-7: In vitro drug release for (F1- F6) formulations

An initial burst release is usually observed in the first 1–2 hours due to turmeric adsorbed on the surface of cubosomes or loosely entrapped in outer layers.This phase provides a quick onset of action.The following hours show a slower, sustained release, controlled byDiffusion through lipid bilayers and Swelling and restructuring of the cubic phase. The optimized formulation of F5 showed higher percentage of drug release is97.15 for 8hrs.

Stability studies

There was no significant change in physical and chemical properties of the cubosomesof formulation F-5 for 3 months. Parameters quantified at various time intervals were shown.

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

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-5	25^0C/60%RH % Release	97.15	96.82	95.67	94.68	Not less than 85 %
F-5	30^0C/75% RH % Release	97.15	96.18	95.53	94.42	Not less than 85 %
F-5	40^0C/75% RH % Release	97.15	96.10	95.25	94.28	Not less than 85 %

CONCLUSION

Curcumin-loaded cubosomes represent a promising nanocarrier system for enhancing the solubility, stability, and bioavailability of curcuminCubosomes, formed from biocompatible lipids like glyceryl monostearate (GMS) and stabilized by surfactants such as Poloxamer 407, offer a unique continuous cubic phase structure. This allows for the effective encapsulation of hydrophobic drugs like curcumin. The bottom up preparation method, involving homogenization and sonication, enables the production of stable cubosomal dispersions with Nanoscale particle size and controlled drug release profiles. Our results suggest that cubosome formulation is an ideal candidate for many Curcumin extract cubosomes required in various applications. In vitro study revealed that cubosomes formulations F5 (1:5) containing 50 mg of Poloxamer 407 shown better releases than other dispersion. In conclusion cubosomes are promising control release vehicle for the effective drug delivery of Curcumin extract.

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