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Development and Evaluation of Curcumin-Loaded Liposomes for Anti-inflammatory Activity

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Abstract:

Curcumin, a primary bioactive polyphenolic compound found in turmeric has been widely recognised for its potent anti-inflammatory activity. However, its therapeutic use is limited due to poor solubility and poor bioavailability. This study aims to formulate curcumin-loaded liposomes by using thin film hydration method with lecithin and cholesterol. The formulated curcumin liposomes were then evaluated for particle size, drug content, entrapment efficiency, In-vitro drug release and anti-inflammatory activity. In-vitro anti-inflammatory activity was assessed by the protein denaturation inhibition method. The curcumin-loaded liposomes demonstrated satisfactory physicochemical properties and sustained release compared with plain curcumin. The study concludes that liposomal encapsulation can enhance the solubility and therapeutic performance of curcumin. Thus, curcumin-loaded liposomes may serve as a promising delivery system for improving its anti-inflammatory efficacy.

Keywords — Curcumin, Liposomes, Anti-inflammatory activity, thin-film hydration method, Drug delivery system, Entrapment efficiency, Sustained release, Liposomal encapsulation.

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I. INTRODUCTION

Inflammation is a complex biological response associated with harmful stimuli. although acute inflammation is a protective response, chronic inflammation indicates rheumatoid arthritis, cardiovascular disorders, cancer and neurodegenerative diseases (1). Conventional non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in clinical practice; however, their long-term use is associated with adverse effects such as gastrointestinal ulceration, renal toxicity and cardiovascular complications. This has resulted in a growing interest in safer alternatives with potent anti-inflammatory activity (2).

Curcumin is a natural polyphenol compound obtained from *Curcuma longa* (turmeric). It exhibits various pharmacological activities such as anti-inflammatory, anti-microbial, anti-oxidant and anti-cancer activity (3). The anti-inflammatory action of curcumin is mediated by inhibition of inflammation mediators such as cyclooxygenase-2 (COX-2), lipoxygenase (LOX), Tumour necrosis factor- α (TNF- α) and nuclear factor- κB (NF- κB) signalling pathway (4). Although curcumin has therapeutic potential, it suffers from low aqueous solubility, poor bioavailability and rapid metabolism which significantly limits its clinical application (5).

To overcome these limitations, various novel drug delivery systems have been explored to enhance the bioavailability, solubility and stability of curcumin. Liposomes are the most suitable drug delivery system to deliver curcumin due to their biocompatibility and biodegradability (6). Liposomes are bilayer spherical vesicles made of phospholipids that encloses the drug in its aqueous core which results in improved drug stability, provide sustained release of drugs and enhances

targeted drug delivery, minimizing the systemic toxicity (7). Encapsulation of curcumin into liposomal drug delivery system has been reported to significantly improve its pharmacokinetic profile and therapeutic efficacy. Liposomal curcumin enhances cellular uptake, protects the drug from degradation and prolongs circulation time, thereby improving its anti-inflammatory activity (8).

The aim and objective of this study is to formulate curcumin-loaded liposomes and to evaluate various physicochemical and pharmacological properties such as particle size, drug content, entrapment efficiency, drug release and anti-inflammatory activity. It also includes comparison of liposomal curcumin and plain curcumin.

II. METHODOLOGY

The curcumin-loaded-liposomes were prepared using thin-film hydration method, also known as solvent evaporation method. Accurately weighed quantities of lecithin (phospholipid) and cholesterol were transferred in a clean round bottom flask and dissolved in chloroform to obtain a clear lipid solution. Curcumin was dissolved separately in ethanol and gradually added to the lipid solution with continuous stirring under continuous magnetic stirring to ensure uniform mixing of the drug in the lipid solution.

The mixture was then subjected to rotary evaporation under reduced pressure and controlled temperature to evaporate the organic solvent. A thin and uniform lipid film was deposited on the inner wall of the round bottom flask. The flask is then kept under vacuum to ensure complete removal of residual solvents.

The dried lipid film was then hydrated by the addition of phosphate buffer saline (pH 7.4) at a temperature above the lipid transition temperature, resulting in swelling of the film and detachment of the lipid film. Sonication was performed subsequently to obtain uniform vesicle size. The prepared liposomes were stored under refrigerated conditions.

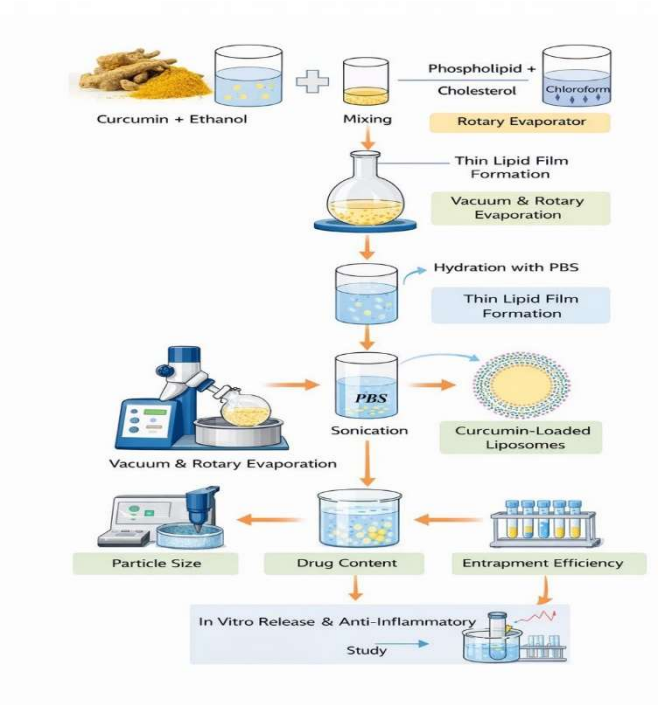


Fig. 1 Methodology for formulation and evaluation of curcumin-loaded liposomes

A. Evaluation of curcumin-loaded liposomes:

i. Physical appearance: the prepared liposomes were visually inspected for colour, homogeneity and presence of any particulate matter

ii. Particle size: the particle size of the liposomes was determined using optical microscopy/dynamic light scattering technique after suitable dilution with distilled water.

iii. Drug content: a known quantity of liposomal dispersion was disrupted using a suitable solvent to release the encapsulated curcumin. The solution was filtered and analysed using spectrophotometry at the appropriate wavelength to determine drug content.

iv. Entrapment efficiency: the entrapment efficiency of curcumin in liposomes was determined by separating free drug from the liposomal dispersion using centrifugation. The amount of unentrapped drug in the supernatant was analysed spectrophotometrically. Entrapment efficiency was calculated using the formula:

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

B. *In-Vitro drug release study:*

The in vitro release of curcumin from liposomes was studied using a dialysis membrane method. A known quantity of liposomal formulation was placed in a dialysis bag and immersed in phosphate buffer saline (pH 7.4) maintained at 37± 0.5°C with continuous stirring. Samples were withdrawn at predetermined time intervals and replaced with fresh buffer. The amount of curcumin released was analysed by spectrophotometer.

C. *In-Vitro anti-inflammatory activity:*

The anti-inflammatory activity of curcumin-loaded liposomes was evaluated using an in vitro protein denaturation inhibition method. The formulation was incubated with a protein solution under controlled conditions. The extent of protein denaturation was measured spectrophotometrically and compared with that of plain curcumin and control samples.

III. RESULTS

The prepared curcumin-loaded liposomes were evaluated for various physicochemical parameters including particle size, drug content, entrapment efficiency, in-vitro drug release and anti-inflammatory activity. The obtained results demonstrated satisfactory formulation characteristics and enhanced anti-inflammatory potential of liposomal systems.

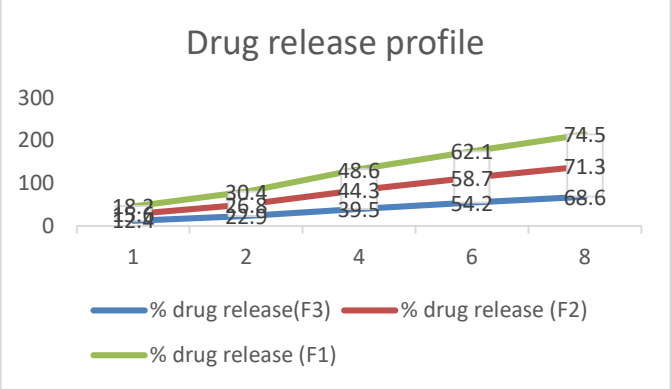
A. *Evaluation of curcumin-loaded liposomes:*

Formulation	Particle size	Drug content	Entrapment efficiency
F1	182 ± 4.6	91.2 ± 1.4	68.4 ± 2.3
F2	176 ± 3.9	93.6 ± 1.1	72.8 ± 1.9
F3	168 ± 5.1	95.1 ± 0.9	78.6 ± 2.1

The prepared curcumin-loaded liposomes showed satisfactory physicochemical properties. Among all formulations, F3 exhibited the smallest particle size (168 ± 5.1 nm), highest drug content (95.1 ± 0.9%), and maximum entrapment efficiency (78.6 ± 2.1%), indicating better vesicle formation and efficient drug incorporation.

Physical appearance: the curcumin-loaded liposomes were homogeneous and yellowish in colour with no visible particulate matter.

B. *In-Vitro drug release study:*



The in-vitro drug release study demonstrated a sustained release pattern for all formulations over a period of 8 hours. Among the formulations, F3 showed the most controlled release profile with 68.6% drug release at the end of 8 hours, indicating improved sustained-release behaviour compared to F1 and F2. The gradual release of curcumin from the liposomal system suggests effective drug encapsulation and prolonged drug release characteristics.

C. *In-Vitro anti-inflammatory activity:*

Sample	% inhibition of protein denaturation
Control	8.4 ± 0.7
Plain curcumin	52.6 ± 1.8
Curcumin-loaded liposomes	71.9 ± 2.1

The curcumin-loaded liposomes showed significantly higher inhibition of protein denaturation (71.9 ± 2.1%) compared to plain curcumin (52.6 ± 1.8%), indicating enhanced anti-inflammatory activity. This indicates that liposomal curcumin shows better anti-inflammatory activity due to targeted drug delivery.

IV. DISCUSSION

Among all formulations, F3 showed the best performance with optimum particle size and maximum entrapment efficiency, possibly due to the ideal lipid–cholesterol ratio which enhanced vesicle formation and drug incorporation. The liposomal formulations exhibited sustained drug release, which may be attributed to the phospholipid bilayer acting as a barrier and slowing the release of Curcumin.

Curcumin-loaded liposomes showed greater anti-inflammatory activity than plain curcumin, likely due to improved solubility, stability, and protection of the drug from degradation after encapsulation. The results are consistent with previously published studies reporting enhanced drug release and biological activity of liposomal curcumin formulations.

However, the study was limited to in vitro evaluation. Further in vivo and stability studies are required to confirm the therapeutic potential of the formulation.

V. CONCLUSION

Curcumin-loaded liposomes were successfully formulated by thin film hydration method. The prepared formulations showed satisfactory physical appearance, indicating uniform vesicle formation and good stability. The particle size of the liposomes was within the nanometre range, which is desirable for vesicular drug delivery system. All formulations exhibited high drug content and satisfactory entrapment was achieved. F3 showed optimal particle size and maximum entrapment efficiency.

The in-vitro drug release study demonstrated controlled and sustained release of the drug from the liposomal vesicle indicating the use of curcumin liposomes for prolonged release of drug. The in-vitro anti-inflammatory activity study revealed that curcumin-loaded liposomes exhibited higher inhibition of protein denaturation than plain curcumin.

Overall, the results indicate that liposomal encapsulation of curcumin improves its stability and anti-inflammatory activity, thereby supporting the potential of curcumin as an effective anti-inflammatory substance.

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