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DEVELOP AND CHARACTERIZE CELECOXIB-LOADED LIPOSOMES FOR THE EFFECTIVE MANAGEMENT OF RHEUMATOID ARTHRITIS

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ABSTRACT

This study focuses on the formulation and characterization of Celecoxib-loaded liposomes aimed at enhancing the management of rheumatoid arthritis. Celecoxib, a selective COX-2 inhibitor, is poorly soluble in water, which limits its therapeutic efficacy. To address this, liposomes were prepared using phosphatidylcholine and cholesterol to encapsulate Celecoxib, optimizing parameters such as vesicle size and entrapment efficiency. The optimized formulation (F5) exhibited a vesicle size of 110.25 nm and an entrapment efficiency of 85.64%. Additionally, liposomal gel formulations were developed, showing desirable characteristics including appropriate pH, high drug content, and good spreadability. In vitro release studies demonstrated a controlled drug release profile, with 98% of Celecoxib released over 10 hours, indicating sustained therapeutic action. Regression analysis suggested the release mechanism follows a non-Fickian diffusion model. These results highlight the potential of Celecoxib-loaded liposomes as an effective drug delivery system, improving bioavailability and therapeutic outcomes in rheumatoid arthritis management. Further studies are warranted to evaluate the in vivo efficacy of this innovative formulation.

Keywords: Celecoxib, Liposomes, Rheumatoid Arthritis, Drug Delivery, Entrapment Efficiency, In Vitro Release, Bioavailability.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder characterized by synovial inflammation and joint destruction. Effective management of RA involves the use of anti-inflammatory drugs, with celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, being one of the widely prescribed non-steroidal anti-inflammatory drugs (NSAIDs). Celecoxib alleviates pain and inflammation associated with RA; however, it is limited by poor solubility and gastrointestinal side effects, which can compromise its therapeutic efficacy (Kreutz *et al.,* 2013; Rainsford, 2013).

Liposomes, lipid-based nanocarriers, have emerged as a promising strategy to enhance the bioavailability and therapeutic index of drugs like celecoxib. By encapsulating celecoxib within liposomes, it is possible to improve drug solubility, provide sustained release, and target delivery to inflamed tissues, thus minimizing systemic side effects (Torchilin, 2005; Sood *et al.,* 2018).

The development of celecoxib-loaded liposomes could address these limitations, making the treatment of RA more effective and safer. Previous studies have demonstrated that liposomal formulations can significantly enhance drug absorption and prolong its therapeutic action (Patel *et al.,* 2017).

This study aims to formulate and characterize celecoxib-loaded liposomes, focusing on their physicochemical properties, encapsulation efficiency, drug release profiles, and potential application in the management of rheumatoid arthritis.

MATERIALS AND METHODS

Materials

The formulation of Celecoxib-loaded liposomes utilized a variety of materials to ensure optimal properties. Celecoxib was provided as a gift sample by Bioplus Life Science, Bangalore. Phosphatidylcholine and cholesterol, both supplied by Himedia, served as the primary lipids for liposome construction. Tween-80, procured from Loba Chemie, Mumbai, acted as a surfactant to enhance stability. Solvents like methanol, ethanol, and chloroform were sourced from Qualigens Fine Chemicals, while disodium hydrogen phosphate and dipotassium hydrogen orthophosphate were obtained from S. D. Fine Chem. Ltd. Additionally, sodium hydroxide from Chempure Speciality Chemicals and hydrochloric acid from Thomas Baker were used for pH adjustments during the formulation process.

Methods

Formulation of Celecoxib loaded liposomes

Liposomes were prepared by the method described by Dheeraj *et al.,* (2016). In brief, Drug, PC, and cholesterol were dissolved in chloroform: methanol mixture (2:1) in a round bottom flask. The solvent was evaporated using rotoevaporator system for at least 1 h at 50mm Hg and 40°C. The remaining film was then resuspended in 25 mL of aqueous phase containing 0.25 ml Tween-80 in a homogenizer.

Characterization of liposomes

Vesicle size reduction

Liposomes were transferred to a beaker and placed on ice bath. The needle probe tip of probe sonicator was placed in the center of the beaker containing liposomal suspension. The sonicator was set to 40% amplitude, and the liposomes were exposed to ultrasonic irradiation at 37°C (15 min) (Laouini *et al.* 2012). The sonicated liposomes were stored in the refrigerator (4–8°C) for at least 6 h before the further use.

Vesicle size

Microscopic analysis was conducted to determine the average size of the prepared liposomes. The formulation was diluted with distilled water, and one drop of the diluted solution was placed on a glass slide, which was then covered with a coverslip. The prepared slide was examined under a trinocular microscope at 400x magnification. The diameters of more than 150 vesicles were randomly measured using a calibrated ocular micrometer and stage micrometer. The average diameter of the vesicles was calculated using the following formula:

$$Average\ Diameter = \frac{\Sigma n.d}{\Sigma\, n}$$

Where n = number of vesicles; d = diameter of the vesicles

Zeta potential

The size distribution and surface charge of the liposomes were analyzed using Dynamic Light Scattering (DLS) with a Malvern Zetamaster (ZEM 5002, Malvern, UK). The zeta potential was measured based on the electrophoretic mobility of the liposomes and calculated using the Helmholtz–Smoluchowsky equation (Miere *et al.,* 2020). For zeta potential measurements, a Zetasizer was employed with field strength of 20 V/cm

and a large bore measurement cell. The samples were diluted with 0.9% NaCl solution, adjusted to a conductivity of 50 µS/cm.

Entrapment efficiency

Celecoxib encapsulated within the liposomes was quantified after removing any unencapsulated drug (Bulbake *et al.,* 2017). The unencapsulated drug was separated from the liposomes by centrifuging the dispersion in a cooling centrifuge (Remi Equipments, Mumbai) at 18,000 rpm and 4°C for 45 minutes. This process yielded liposome pellets and a supernatant containing the free drug. The liposome pellets were then washed with phosphate buffer to further remove any unencapsulated drug, followed by centrifugation. The combined supernatant was analyzed for drug content after appropriate dilution with phosphate buffer. The absorbance was measured at 252 nm using a Labindia 3000+ spectrophotometer.

$$\%\ Entrapment\ Efficiency = \frac{Therotical\ drug\ content - Practical\ drug\ content}{Therotical\ drug\ content} \times 100$$

Preparation of gel base

Carbopol 934 (1-3% w/v) was accurately weighed and dispersed into 80 ml of double-distilled water in a beaker (Mezei *et al.,* 1993). The mixture was stirred continuously at 800 rpm for 1 hour. Following this, 10 ml of propylene glycol was added to the solution. The resulting slightly acidic solution was neutralized by gradually adding 0.05 N sodium hydroxide solution until the gel became transparent. The volume of the gel was adjusted to 100 ml and then sonicated for 10 minutes using a bath sonicator to remove air bubbles.

A gel was also prepared with the plain drug by adding 10 mg of the drug and dispersing it using the same procedure described above. For the formulation of the liposome-containing gel, liposomes corresponding to 0.75% w/w of the drug were incorporated into the gel base to achieve the desired drug concentration.

Characterization of liposomes containing gel

Measurement of viscosity

The viscosity of the topical gel containing liposomes was determined using a Brookfield viscometer (Pattini *et al.,* 2015). To obtain precise viscosity measurements, spindle number 63 was selected, as it is appropriate for measuring the viscosity of gel-like substances. The viscometer was operated at a rotational speed of 10 rpm, which is optimal for assessing the viscosity of the gel under these conditions. The measurements were conducted at room temperature to ensure consistency and accuracy in the results. This method provided insights into the gel's flow properties and stability, which are critical for ensuring its efficacy and ease of application.

pH measurements

The pH of the selected optimized formulations was determined using a digital pH meter. To ensure accurate pH readings, the pH meter was first calibrated using standard buffer solutions with pH values of 4.0, 7.0, and 9.2 (Elizondo *et al.,* 2011). Calibration was performed before each pH measurement to maintain precision.

Drug content

An accurately weighed amount of 100 mg of the topical liposome gel was transferred into a 10 ml volumetric flask. To extract the drug from the gel, 5 ml of methanol was added to

the flask. The mixture was then subjected to sonication for 10 minutes to ensure thorough dispersion and extraction of the drug. After sonication, the volume of the solution was adjusted to 10 ml with additional methanol, and the solution was mixed thoroughly to achieve homogeneity.

The resulting solution was filtered using Whatman filter paper No. 1 to remove any particulate matter or undissolved gel residues. Following filtration, 0.1 ml of the filtered solution was carefully measured and transferred into a separate 10 ml volumetric flask. The volume was then adjusted to 10 ml with methanol, resulting in a diluted solution suitable for analysis.

The diluted solution was analyzed using a UV-Visible spectrophotometer at the wavelength of maximum absorbance (λ_{max}) for the drug, which was determined to be 252 nm. The absorbance readings obtained from this analysis were used to calculate the drug content in the topical liposome-based gel (Liu *et al.,* 2011).

Extrudability study

Extrudability of the gel was assessed by measuring the amount of gel extruded from a collapsible tube under the application of a specific load. To perform this test, a collapsible tube filled with the gel was subjected to a series of increasing weights (Nogueira *et al.,* 2015). The test involved gradually applying these weights to simulate the pressure exerted during typical usage of the gel. As the load was applied, the gel began to extrude from the tube. The key measurement in this process was the weight at which the gel was expelled from the tube. A larger quantity of gel extruded under a given load indicates better extrudability.

This property is important as it reflects how easily the gel can be dispensed and applied, ensuring that the product can be used effectively by consumers. The results from this test provided insights into the performance of the gel in practical scenarios, highlighting its ease of use and effectiveness in application.

Spreadibility

To evaluate the spreadability, a specialized apparatus is used. This apparatus consists of a fixed wooden block with a stationary lower slide and a movable upper slide. The movable slide is connected to a weight pan (Briuglia *et al.,* 2015).

In the test procedure, 2-5 grams of the gel are placed between the two slides. Gradually, weight is added to the weight pan, increasing the pressure applied to the upper slide. The key measurement is the time required for the top slide to cover a distance of 6 centimeters under a specified load of 20 grams. The gel's spreadability is assessed by noting this time; a shorter time to spread indicates better spreadability. Good spreadability ensures that the gel can be evenly distributed over the skin with minimal effort, enhancing its effectiveness and user convenience. This measurement helps in evaluating how easily and uniformly the gel can be applied, which is essential for achieving optimal therapeutic outcomes.

$$Spreadibility\,(g.cm/\sec) = \frac{Weight\ tide\ to\ Upper\ Slide \times Lenth\ moved\ on\ the\ glass\ slide}{Time\ taken\ to\ slide}$$

***In vitro* drug diffusion study**

The in-vitro diffusion study was conducted using a Franz diffusion cell, with an egg membrane serving as the semi-permeable membrane for the diffusion process. The Franz diffusion cell features a receptor

compartment with an effective volume of approximately 60 mL and a permeation surface area of 3.14 square centimeters.

For the experiment, a 2 cm^2 patch of the formulation was accurately weighed and placed on one side of the membrane, facing the donor compartment. The receptor compartment was filled with phosphate buffer at pH 7.4, which served as the receptor medium (Yang *et al.,* 2017).

To maintain the temperature at 32 ± 0.5°C, the receptor compartment was surrounded by a water jacket. A thermostatic hot plate equipped with a magnetic stirrer provided the necessary heat, and the receptor fluid was continuously stirred using a Teflon-coated magnetic bead within the diffusion cell.

RESULTS AND DISCUSSION

The formulation and characterization of Celecoxib-loaded liposomes have shown promising results in enhancing the therapeutic efficacy for rheumatoid arthritis management.

Table 3 illustrates that the optimized formulation (F5) achieved a vesicle size of 110.25 nm and an entrapment efficiency of 85.64%. Smaller vesicle sizes are desirable for improving bioavailability and penetration in biological tissues, which is critical for effective drug delivery. The zeta potential of -45.65 mV further indicates good stability, as a negative charge helps prevent aggregation.

Table 4 presents the evaluation results for the liposomal gel formulations (LG1, LG2, LG3). All formulations displayed high drug content, indicating efficient encapsulation of Celecoxib. The pH values ranged from 6.75 to 6.85, which is suitable for topical application, minimizing irritation. The spreadability and viscosity values indicate that the gels would spread easily upon application, enhancing user compliance.

The *in-vitro* release data from Table 5 for formulation LG3 demonstrated a controlled release profile over time. The cumulative % drug release reached 97.85% by 10 hours, suggesting that the gel formulation provides sustained drug delivery, which is essential for managing chronic conditions like rheumatoid arthritis.

Table 6 regression analysis reveals that LG3 follows the Korsmeyer-Peppas model (R^2 = 0.9964), indicating a non-Fickian diffusion mechanism. This suggests that the drug release is influenced by both diffusion and erosion of the gel matrix, providing a balanced release profile. The results affirm the potential of Celecoxib-loaded liposomes and their gel formulations in effectively managing rheumatoid arthritis. Future studies could focus on in vivo assessments to confirm these findings and evaluate therapeutic efficacy in clinical settings.

Table 1: Formulation of Celecoxib liposomes by thin-film hydration technique

F. Code	Drug	Phosphatidylcholine (mg)	Cholesterol (mg)	Chloroform (ml)	Methanol (ml)	Tween-80 (ml)
F1	100	100	30	10	5	0.25
F2	100	100	50	10	5	0.25
F3	100	200	30	10	5	0.25
F4	100	200	50	10	5	0.25
F5	100	300	30	10	5	0.25
F6	100	300	50	10	5	0.25

Table 2: Evaluations of Celecoxib liposomes for vesicle size and entrapment efficiency

F. Code	F1	F2	F3	F4	F5	F6
Vesicle Size (nm)	185.56±0.15	168.85±0.22	176.65±0.32	155.85±0.89	110.25±0.88	174.45±0.74
Entrapment efficiency (%)	74.65±0.15	76.96±0.25	78.98±0.33	81.12±0.41	85.64±0.52	80.74±0.33

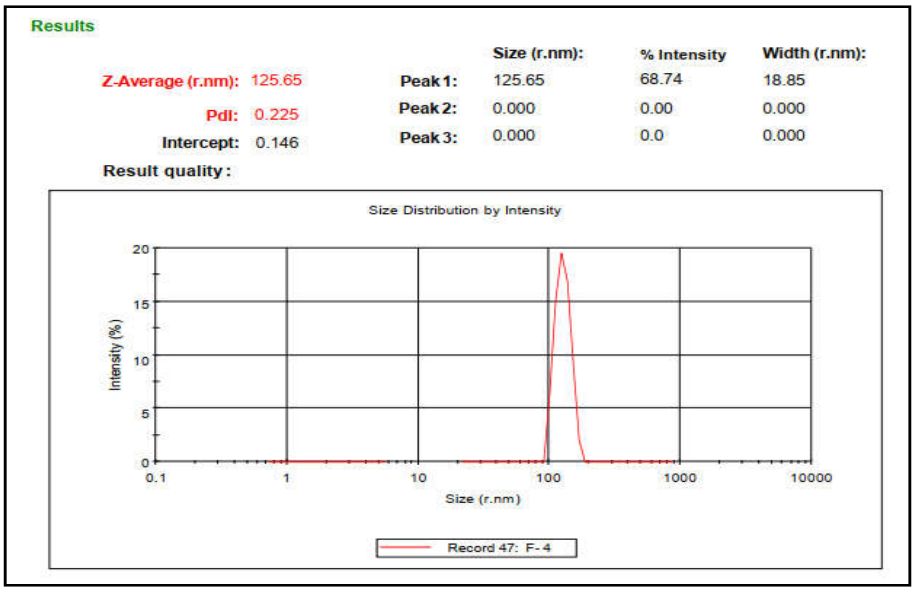

Figure 1: Vesicle Size of optimized liposomes formulation F5

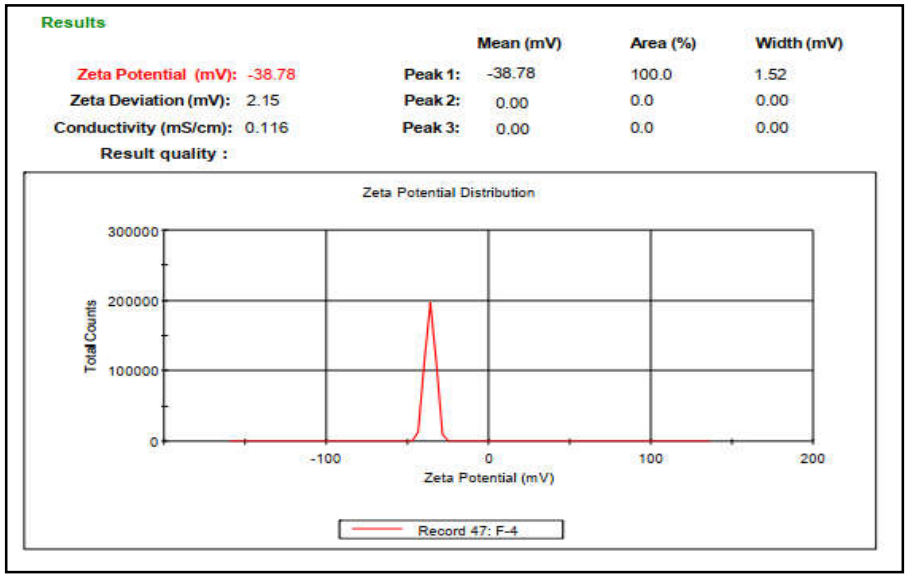

Figure 2: Zeta potential of optimized liposomes formulation F5

Table 3: Vesicle size and entrapment efficiency of optimized formulation

Formulation code	Parameters
Vesicle Size (nm)	110.25±0.88
Entrapment Efficiency (%)	85.64±0.52
Zeta potential (mV)	-45.65

Table 4: Results of liposomes gel formulations

Parameters	LG1	LG2	LG3
Drug content (%)	98.74±0.45	94.65±0.36	99.05±0.22
pH	6.85±0.32	6.80±0.45	6.75±0.15
Spreadability (Gm.cm/sec.)	11.45±0.85	10.32±0.18	9.98±0.36
Viscosity (cps)	3898±25	3565±35	3247±0.42

Table 5: *In-vitro* drug release data for LG3

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	33.65	1.527	66.35	1.822
1	1.000	0.000	45.85	1.661	54.15	1.734
2	1.414	0.301	56.98	1.756	43.02	1.634
4	2.000	0.602	70.32	1.847	29.68	1.472
6	2.449	0.778	81.16	1.909	18.84	1.275
8	2.828	0.903	92.32	1.965	7.68	0.885
10	3.162	1.000	97.85	1.991	2.15	0.332

*Average of three reading

Table 6: Regression analysis data of liposomes gel formulation

Batch	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas Equation
	R^2	R^2	R^2	R^2
LG3	0.9539	0.9454	0.9937	0.9964

CONCLUSION

The formulation and characterization of Celecoxib-loaded liposomes demonstrated significant potential for the effective management of rheumatoid arthritis. The optimized formulation (F5) achieved a small vesicle size of 110.25 nm and a high entrapment efficiency of 85.64%, indicating effective drug loading and stability. The liposomal gel formulations exhibited favorable properties, including suitable pH, high drug content, and adequate spreadability, which are essential for patient compliance. In vitro drug release studies revealed a controlled release profile, with nearly 98% of the drug released over 10 hours, suggesting sustained therapeutic effects. The regression analysis confirmed that the release mechanism aligns with a non-Fickian diffusion model, highlighting the formulation's capability to maintain drug levels over an extended period. These findings support the potential of Celecoxib-loaded liposomes as a viable delivery system, enhancing both bioavailability and therapeutic effectiveness in managing rheumatoid arthritis. Future investigations should focus on in vivo studies to validate these promising results and assess clinical applicability.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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