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Research

Development and Characterization of a Leflunomide-Loaded Liposomal Gel as a Novel Topical Drug Delivery System

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Abstract:
Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder characterized by persistent joint inflammation and progressive tissue damage. Leflunomide (LEF) is a disease-modifying antirheumatic drug (DMARD) used in the management of RA. Its active metabolite, teriflunomide, inhibits dihydroorotate dehydrogenase (DHODH), thereby interfering with pyrimidine synthesis and reducing lymphocyte proliferation. However, conventional systemic administration of LEF is associated with limitations such as prolonged half-life, slow elimination, and hepatotoxicity. Therefore, the present study aimed to develop a liposomal gel of leflunomide as a novel topical drug delivery system.
Preformulation studies were performed to evaluate the physical characteristics, melting point, and solubility of leflunomide. The drug was found to be a white to off-white crystalline powder with a melting point of 150–153°C and showed poor aqueous solubility. The maximum absorption wavelength of leflunomide in ethanol was determined to be 260 nm using UV-visible spectroscopy. Leflunomide-loaded liposomes were prepared using the ethanol injection method with soy lecithin and cholesterol as lipid components. The prepared liposomes were subsequently incorporated into a Carbopol-based gel. The developed liposomal gel was evaluated for viscosity, spreadability, pH, particle size, zeta potential, entrapment efficiency, and in-vitro drug release. The formulation exhibited a skin-compatible pH of 6.1 and demonstrated sustained drug release over 24 h, with approximately 85% drug release. The findings indicate that leflunomide-loaded liposomal gel may serve as a promising approach for topical drug delivery and sustained release of leflunomide.

Keywords: Leflunomide, Liposomes, Liposomal gel, Rheumatoid arthritis, Novel drug delivery system, Topical drug delivery, Sustained drug release.

1. INTRODUCTION

Liposomes are nanosized vesicular systems composed of one or more phospholipid bilayers (Yixuan Mou), Due to their ability to encapsulate both hydrophilic and lipophilic drugs, they have gained considerable attention as carriers for topical drug delivery. When applied to the skin, liposomes can improve drug deposition and facilitate its penetration through the skin layers, thereby enhancing the therapeutic effectiveness of the incorporated drug.(Pavelić et al., 2004). Liposomes possess a versatile structure that enables them to accommodate both hydrophilic and hydrophobic drugs within their aqueous core and lipid bilayer, respectively (Hua, 2014). Incorporating liposomes into a suitable gel matrix can further improve their applicability as a drug delivery system by enhancing formulation stability, retention at the site of application, and controlled drug release (Zhang et al., 2025).

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Following topical application, liposomes can facilitate drug transport across the skin through several pathways, including transcellular, intercellular, and follicular routes. These diverse penetration mechanisms have increased the potential of liposomal systems for topical delivery of drugs, genes, proteins, and peptides by helping to overcome the skin's natural barrier, improve site-specific delivery, and enhance therapeutic efficacy (Tabandeh & Mortazavi, 2013). Liposomes are versatile vesicular drug delivery systems whose physicochemical characteristics can be tailored by modifying parameters such as vesicle size, lipid composition, surface charge, and number of phospholipid bilayers (Singh & Rathod, 2014). Structurally, they consist of an aqueous internal compartment surrounded by one or more phospholipid bilayers, allowing them to accommodate a wide range of therapeutic molecules (Duman et al., 2014). When administered through the topical route, liposomal formulations can promote drug action at the skin site and, depending on their properties and the drug involved, may also contribute to systemic drug delivery. Consequently, considerable research has focused on developing advanced vesicular carriers that can improve skin penetration, enhance drug localization, regulate drug release, and minimize unwanted systemic exposure (Mazda et al., 1997). Compared with conventional topical formulations, liposomal systems may offer improved delivery of moisturizing and lipid-based substances to the skin because of their structural similarity to biological cell membranes. Their biocompatible and biodegradable characteristics make liposomes attractive carriers for therapeutic agents, while their ability to influence drug distribution and pharmacokinetic behaviour has supported their investigation in the management of several diseases, including rheumatoid arthritis (RA) (van den Hoven et al., 2011). In addition, several liposome-based drug delivery products have demonstrated encouraging outcomes in both preclinical and clinical studies, highlighting the potential of this approach for therapeutic applications (Prasad, O'Mary, & Cui, 2015).

Teriflunomide (TEF), the active metabolite of leflunomide (LEF), exerts its pharmacological action mainly by inhibiting dihydroorotate dehydrogenase (DHODH), an enzyme involved in the mitochondrial de novo synthesis of pyrimidines. This pathway is particularly important for rapidly proliferating lymphocytes, including T and B cells. By limiting pyrimidine availability, TEF suppresses lymphocyte proliferation and subsequently helps reduce the autoimmune inflammatory response associated with rheumatoid arthritis (RA) (Oh & O'Connor, 2014).

Despite its therapeutic potential, prolonged systemic administration of TEF/LEF has been associated with safety concerns, particularly hepatotoxicity, which can limit its long-term clinical use (Van den Hoven et al., 2011). Leflunomide is a synthetic disease-modifying antirheumatic drug (DMARD) belonging to the isoxazole class. It received approval from the U.S. Food and Drug Administration (FDA) in 1998 for the treatment of RA and was subsequently authorized in Europe in 1999. Following administration, leflunomide is converted to its pharmacologically active metabolite, teriflunomide (A77 1726), which produces its therapeutic effect through reversible inhibition of DHODH and consequent suppression of pyrimidine synthesis.

Dihydroorotate dehydrogenase (DHODH) is an essential enzyme in the de novo biosynthetic pathway of pyrimidines. Since activated T lymphocytes require increased pyrimidine production to support their proliferation and cell division, inhibition of DHODH by teriflunomide interferes with this metabolic pathway and consequently limits lymphocyte expansion (Dhiman & Garkhal, 2025). However, the clinical use of leflunomide (LEF) may be associated with several limitations, including its prolonged half-life, hepatotoxic potential, slow elimination from the body, and increased susceptibility to infections resulting from its immunomodulatory effects on lymphocyte proliferation. These limitations have encouraged researchers to explore alternative formulation and drug-delivery strategies that could improve the therapeutic profile of LEF while minimizing unwanted adverse effects. Accordingly, various approaches have been investigated to develop safer and more effective methods for delivering LEF in patients with rheumatoid arthritis (RA), including the use of novel drug delivery systems (Osiri et al., 2009).

Material and Methods

Leflunomide, tween 80, soya lecithin, Ethanol, Sodium chloride, Disodium hydrogen phosphate, potassium hydrogen phosphate, sodium Azide, Carbopol,

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Experimental Methodology

Pre-formulation Studies

Preformulation is a proactive process in which a new chemical entity is transformed into a safe, effective, and stable pharmaceutical formulation. Preformulation studies were conducted to establish a drug's identification, physiochemical factor, kinetics rate profile, physical features, and compatibility with excipients.

Organoleptic Properties

Visual observation was used to assess organoleptic qualities such as overall look. A small amount of the medication was placed on butter paper and observed in a well-litarea.

Melting point

The melting point of itraconazole was determined by using a melting point device from Labtronics. A metal block containing a heating mantle element is heated at the desired temperature by transferring electricity through a rheostat device. The chemical compound capillary is placed in one hole, and the thermometer is inserted in the other hole supplied by the block. With an eyepiece, the internal changes in the capillary tube may be observed via a glass window.

Solubility studies

The solubility of medication is determined through visual examination. Itraconazole was taken in excess and combined with 10 ml of different solutions. These liquids were forcefully mixed for a few minutes. After that, the solubility was determined.

Standard calibration curve of Leflunomide.

a) **Preparation of the stock solution of the Leflunomide (1000µg/ml)**

10 mg of API was accurately weighted and added into a 10 ml of the volumetric flask, the drug was then dissolved in Ethanol and the volume was make up to 10 ml thus the resulting concentration of the solution was 1000µg/ml.

b) **Preparation of the sub stock solution for Leflunomide (100µg/ml)**

Starting with the stock solution To make the final concentration of 1000ug/ml, 1 ml of the solution was put into a 10 ml volumetric flask, and volume was brought up to the 10ml mark using Methanol.

c) **Preparation of the dilutions from the sub stock.**

Dilution of 2µg/ml,4 µg/ml,6 µg/ml,8 µg/ml,10 µg/ml, was made from the substock by taking out the respective volumes in the 10 ml of the volumetric flask then the volume was make upto 10ml. the absorbance was these dilutions were the measured using U.V. visible spectrophotometer, the absorbance vs concentration data was plotted to construct the calibration curve.

Preparation Of Liposomes

The needed amount of soy lecithin, Cholesterol, and Leflunomide was dissolved in Ethanol (organic phase) to make liposomes. The solution was then injected into the aqueous phase, which consisted of 20 ml of normal saline and needed amount of Tween 80 in a 100ml beaker kept at 37°C with a constant rpm magnetic stirrer.

- This technique offers advantages of being a fast, one step-based process, and reproducible.
- Ethanol injection method is one of the preferred techniques used to produce small unilamellar liposome in a simple and rapid manner.

Figure no. 1: Preparation of Liposomal

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S.NO	INGREDIENTS	ITRACONAZOLE-LOADED LIPOSOMAL GEL
1.	Carbopol 940 P	2 gm
2.	Itraconazole loaded liposomes	20 ml
3.	Glycerine	10 ml
4.	Triethanolamine	500 ul
5.	Distilled water	50 ml

Table no. 1: Composition of gel formulation

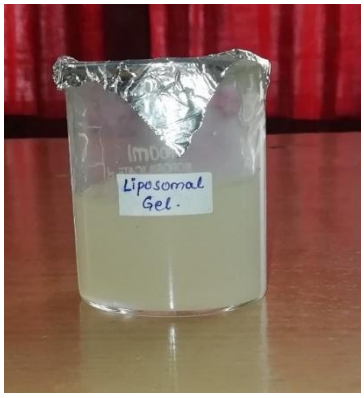

Figure no. 2: Leflunomide Loaded liposomal gel

S.No.	Test	Observation
1	Physical state	Solid (Powder)
2	Colour	White to Off-white
3	Odour	Odourless
4	Physical Appearance	Fine, White To Off-White Crystalline powder

RESULT AND ANALYSIS

This chapter presents the experimental findings and their interpretation. The results are accompanied by tables and figures for clarity and are discussed in relation to the objectives of the study and existing literature.

Pre-formulation studies:

i. **Organoleptic Properties:**

Table no. 2: Table of organoleptic Properties

ii. **Melting Point:**

Table no. 3: Table of Melting Point of Leflunomide.

S. No.	Drug	Observation
1	Leflunomide	150-153 ºC

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Solubility Study:

Table no. 4: Solubility of Leflunomide in Different Solvent

S. No.	Solvent	Observation
1	Water	Practically insoluble
2	Chloroform	Freely Soluble
3	Ethanol	Soluble
4	Methanol	Very soluble
5	Dimethyl sulfoxide	Freely soluble

UV-Visible Spectroscopy:

The maximum absorption (λmax) of Leflunomide in ethanol was found at 260 nm, consistent with literature values.

Table no. 5: Calibration Curve of Leflunomide

Concentration (µg/ml)	Absorbance
10	0.19
20	0.393
30	0.615
40	0.795
50	0.89

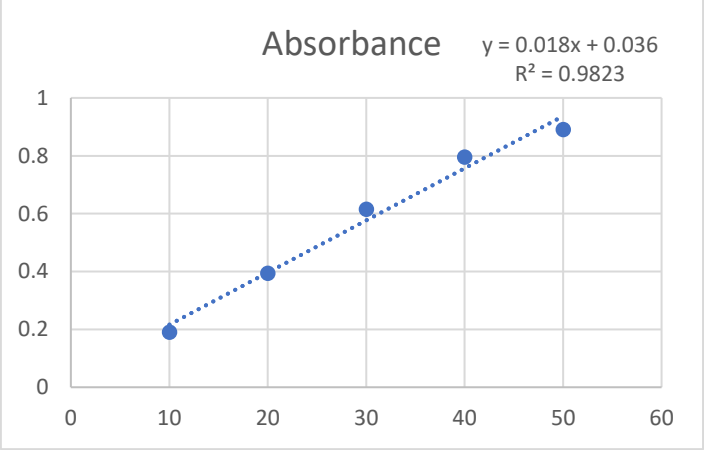

Figure no. 3: Calibration Curve of Leflunomide

Liposomes and Liposomal Gel Evaluation Parameter:

Viscosity:

Viscosity of the gel with Carbopol was optimized for stability, texture, and controlled drug release. The Viscosity of formulation is an essential parameter that was investigated, and the result is listed in Table No.

Spreadability:

Spreadability of the formulation was significantly influenced by the concentration of Excipient. Optimal levels of these components ensured easy application and uniform distribution of the gel, which is crucial for patient compliances and effective drug delivery.

pH Measurement:

pH of the formulations was closely affected by the amount of excipient used in the formulation. Adjusting these components helped maintain the pH within a skin-friendly range, promoting compatibility and reducing the risk of irritation. This balance also controlled to the overall stability and effectiveness of the gel formulation. pH of the gel was found to be 6.1.

Particle Size and Zeta Potential

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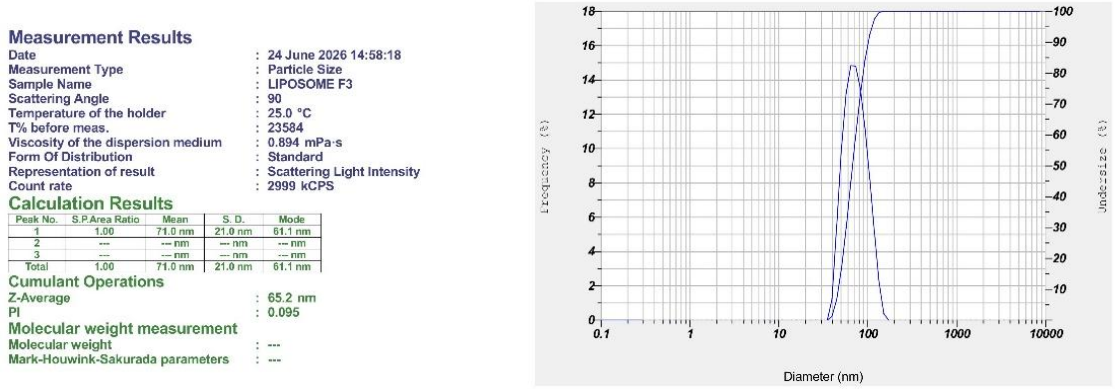
Particle size and the zeta potential was evaluated of the formulated formulation in order to detected their vesicle size and the zeta potential to detect their stability. Figure no. 12 shows the spectrum that shows the particle size and the zeta potential.

Figure no. 4: Particle size spectrum of formulation F2



In-vitro Drug Release Study

The *in-vitro* release profile showed sustained release over 24 hours. Approximately 85% of the drug was released, following zero-order kinetics.

Table no. 6: Particle size, Zeta potential, %Entrapment efficiency (% E.E.) and drug release *In-vitro* Drug Release Kinetics Study

S.No.	Formulation	Particle Size(nm)	Zeta Potential (mV)	Entrapment	Viscosity	DR(1 hr)	DR(2 hr)	DR(3 hr)	DR(5 hr)	DR(24 hr)
1	F1	106	-35.2	70%	7920	8.881	12.345	20.535	36.061	57.598
2	F2	65.2	-39.4	80%	6533	15.872	24.277	48.011	59.645	79.532
3	F3	99	-52.5	65%	3932	9.043	17.523	31.257	52.945	75.159

The *in-vitro* drug release profile of Luliconazole from the optimized PLO gel was analysed using five different kinetic models: zero-order, first-order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell. The purpose was to understand the mechanism and rate of drug release.

Table no.: shows the cumulative percentage drug release over 24 hours. The raw release data was further transformed and fitted into different kinetic models. The calculated parameters and coefficient of determination (R^2) for each model are presented in Table.

Table no. 7: Cumulative Drug Release(%) of Luliconazole over 24 hours with transformed data for kinetic modelling.

Time (h)	% Released	% Remaining	Log (% Remaining)	√Time	Log (Time)	Log (% Released)	Cube Root (% Remaining)
1	1.78	84.128	1.92597	1.0000	0.0000	1.20063	4.379
2	3.31	75.723	1.87923	1.414	0.30103	1.38519	4.231

3	15.15	51.989	1.71591	2.000	0.60206	1.68134	3.733
5	34.43	40.355	1.60590	2.449	0.77815	1.77557	3.431
24	86.47	20.468	1.31108	2.828	0.90309	1.90054	2.735

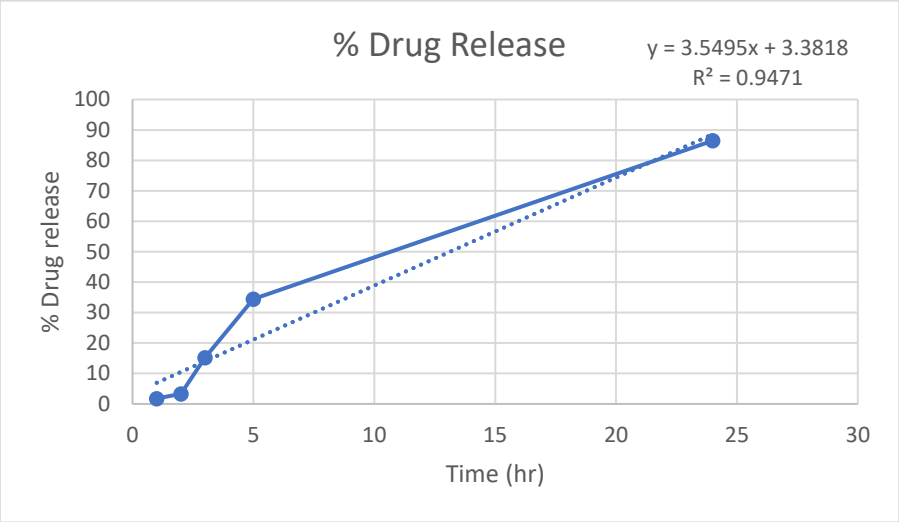


Figure no. 5: % Drug Release vs Time

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