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THE FORMULATION AND CHARACTERIZATION OF LIPOSOMES OF THIOLCHICOSIDE & EFFECT ON AGAINST INFLAMMATION

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Abstract:

Thiocolchicoside, an FDA-approved drug, serves as an anti-inflammatory and muscle relaxant for orthopedic, traumatic, and rheumatologic disorders. Despite the efficacy of conventional formulations like capsules, these are linked to rapid drug release and frequent dosing, along with an unpalatable bitter taste, rendering them unsuitable for tablet forms. Controlled release oral formulations of thiocolchicoside can mitigate these issues by prolonging drug release, reducing dosing frequency, enhancing patient compliance, and improving bioavailability. Liposome encapsulation, leveraging their biodegradable and biocompatible properties, presents a viable solution for these challenges. This study utilized the thin film hydration method to fabricate thiocolchicoside liposomes using soya lecithin, cholesterol, and organic solvents, chosen for their cost-effectiveness and availability. Evaluations included visual appeal, yield percentage, particle size, zeta potential, micromeritic properties, drug content, encapsulation efficiency, in vitro drug release, release kinetics, and stability.

Keywords: Inflammation, Liposome, Drug delivery system, Stearic acid, Soya.

1. Introduction:

Generally speaking, inflammation happens when pathogenic microbes like bacteria, viruses, or fungi enter the body, settle in certain tissues, or move through the bloodstream. Other mechanisms that might cause inflammation include tissue damage, malignancy, ischemia, degeneration, & cell death. The genesis of inflammation is mostly attributed to both the

innate and adaptive immune responses. The primary defensive mechanism against invasive microbes and cancerous cells is the innate immune system, which is composed of a variety of cells such as mast cells, dendritic cells, and macrophages. More specialized cells called B and T cells work with the adaptive immune system to produce certain receptors and antibodies that destroy cancer cells and invasive infections (Chaplin, 2010).

Liposomes are spherical fat (lipid) based nanocarriers used in targeted drug delivery encapsulated with bioactive compounds. Particles in liposomes that range in size around 50 to 500 μ and are made up of one or more lipid bilayers, that contain API in dissolved or dispersed form and phospholipid layer as external layer for stabilization.

Liposomes have attained attention these days for its stability, biocompatibility, ease of synthesis, higher bioavailability, higher drug loading efficiency, controlled drug release and for its effectiveness in protecting the drug or active moiety from hydrolysis. So far, the Liposomes have been used for several drug candidates that include anti-inflammatory, anesthetics, anti-bacterial and anti-cancer agents for its controlled drug release property.

1.1. Mechanism of drug release:

Three main processes underlie the action of liposomes: diffusion, degradation, and swelling.

- **Diffusion:** Diffusion is the method by which the medicine is released via the pores in the polymer matrix that surrounds the core substance. When a polymer layer develops over the core material, the drug / active component is released in a regulated manner.
- **Degradation:** In some cases, the polymer and API are mixed together uniformly to form matrix system. Through the polymeric layer, the medication diffuses into the surrounding environment and is released from the matrix system. The drug releases from the matrix system at a slower rate when it takes longer for it to move from the inner core to the outside world.
- **Swelling:** Normally dry, the controlled release systems become wet when they come into touch with bodily fluids or liquids, the polymers present in coating material swells and enables the core material or drug to diffuse through the swollen polymeric material that form pores on its layer during swelling. This allows the release of the drug from the swollen material to external environment.

2. Material & Method:

Table 1. List of Materials used in the Study

Sr. No.	NAME	SOURCE
1.	Thiocolchicoside	Catasta Pharmaceuticals
2.	SoyLecithin	India's S.D.Fine Chemicals Ltd.
3.	Cholesterol	India's S.D.Fine Chemicals Ltd.
4.	Chloroform	India's S.D.Fine Chemicals Ltd.
5.	Methanol	India's S.D.Fine Chemicals Ltd.
6.	Ethanol	India's S.D.Fine Chemicals Ltd.
7.	Phosphate Buffer	India's S.D.Fine Chemicals Ltd.
8.	Sodium Chloride	India's S.D.Fine Chemicals Ltd.
9.	Citric acid	India's S.D.Fine Chemicals Ltd.
10.	Disodium hydrogen phosphate	India's S.D.Fine Chemicals Ltd.

11.	Hydrochloric acid	India's S.D.Fine Chemicals Ltd.
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Table No. 2 Equipments used in the Study.

Sr. No.	EQUIPMENT	MANUFACTURER
1.	Melting point apparatus	Spruce Enterprise, India
2.	U.V spectrophotometer	Shimadzu 2700
3.	FTIR	BrukerAlpha-E
4.	Hot Air Oven	Hindustan apparatus Mfg. Company
5.	Water-Bath	Micro Teknik, Ambala
6.	Sonicator	PCI Analysis(P) Ltd Mumbai.
7.	Dissolution Apparatus	Electrolab, India
8.	Centrifugator	Accumax India

2.1.1. Preformulation Studies

A major part of formulation of new formulation is pre-formulation. These studies concentrate on the characteristics of the medication that may have an impact on its effectiveness and the creation of an effective dosage form. By using different analytical methods like IR spectroscopy, UV spectroscopy, melting point, etc., the drug sample that was obtained was identified.

2.2. Physical Appearance

The active material Thiocolchicoside purchased from supplier was observed for its physical appearance.

2.3. Melting Point

The capillary fusion technique was used to determine the melting point. Thiocolchicoside, in a minute amount, was fed into a capillary from a melting point device. The drug starts to melt and the temperature was noted on which the solid drug turned into liquid (Patel et al., 2013).

2.4. FTIR

An FTIR spectrum for pure drug was obtained by adding 1-2 mg of drug sample in the suitable holder in FTIR chamber. Fourier-transform infrared spectrum was measured in the scanning range of 400 to 4000 cm^{-1} (Kulkarni et al., 2015).

- Partition Coefficient**

The 10 mg of Clopidogrel bisulphate was weighed and taken in the stoppered vials already containing 10 mL of n-octanol and water. The vials were shaken for 24 h or until the two phases separated and collected in separating funnel which was analyzed by spectrophotometric method. The drug concentration in water divided by the drug concentration in n-octanol is the partition coefficient (P.C.) (Patel et al., 2013).

2.5. Uv-Visible Spectroscopy Preparation of Calibration Curve Of Thiocolchicoside

A 100 ml standard flask was used to dilute a precisely measured quantity of medication (100 mg) with methanol until it reached the mark. After diluting the sample by a factor of 100, it was scanned in the ultraviolet (UV) range of 200 to 400 nm. At 248 nm, the absorbance was found to be at its peak. Transferring 0.2, 0.4, 0.6, 0.8, 1, and 1.4 ml from the solution to a 10 ml standard flask was the next step. Using a UV spectrophotometer, the absorbance was

measured at 248 nm. The range of medication concentrations from 2 to 14µg/ml was used to study the linearity Dixit et al. (2022).

2.6. Solubility Studies.

The solubility of the medicine was tested by dissolving 10 mg of the drug in various solvents and shaking the mixture for 30 minutes in a water bath shaker. The solvents used were water, alcohol (methanol, ethanol), 0.1N hydrochloric acid, ABS (pH 4.5), PBS (pH 6.8), and pH 7.4. Visual examination was used to examine the solubility. For quantitative solubility, 10 mg Thiocolchicoside was taken in test tube. 10 mL of different solvents were added and closed tightly and shaken for 24hrs. The drug was determined at λ_{max} 248 nm Patel et al. (2013).

3. Drug Excipient Compatibility Studies

3.1. FTIR Spectrometric Studies: Fourier Transform Infrared

Soy Lecithin and Cholesterol (an excipient) were analyzed in an FTIR spectra by placing 1-2 mg of the sample in the appropriate holder inside the FTIR chamber. The Fourier-transform infrared spectra were recorded within the 400–4000 cm⁻¹ scanning range. Similar study was conducted for Thiocolchicoside and excipient combination (Y. Wang et al., 2022)

3.2. Preparation Of Liposomes

Liposomes loaded with Thiocolchicoside were prepared using Thin Film Hydration method. Thiocolchicoside, Soy lecithin and cholesterol in varied concentrations were dissolved in mixture of Chloroform and methanol in the ratio of 9:1. This solution was taken in a round bottom flask and kept on a rota evaporator at 60°C for 15 minutes on 90 rpm. This evaporated the chloroform leaving behind a thin layer of lipids. This thin layer of lipids was also dried further for 15 minutes to obtain dry residue. The organic solvent was completely dried by vacuum overnight to obtain film. This film was then hydrated with phosphate buffer 7.4 by vortexing for 10 minutes and then hydrated for 60 minutes at 70°C on 90rpm. The formed liposomal suspension is centrifuged by ultra-centrifugation at 3000 rpm for half an hour to obtain liposomes (Ramasamy Ramamurthy & Roy, 2018)(Rabe et al., 2018).

3.3. Characterization Of Liposomes

- **Appearance**

The prepared Liposomes were visually checked for its color and surface texture (Roy et al. 2018).

- **Percentage Yield**

State that the yield of the formulations was calculated by dividing the formulation's end weight by the drug & an excipient begin weights. state that the yield of the formulations was calculated by dividing the formulation's end weight by the drug & the excipient begin weight.(Trivedi et al., 2009).

- **Particle Size**

Using the dynamic light scattering method, we were able to measure the size of the nanoparticles. recommended diluting all samples ten times with distilled water prior to testing for optimal signal strength.

- **Zeta Potential**

Zeta potential was measured employing Zetasizer. (Lankalapalli et al., 2015) presented the findings of diluting the nanoparticles with distilled water and putting them in a zeta cell.

3.4. Micromeritic Properties

- Angle of repose:** The angle of repose was calculated by dividing the pile height (as a function of pile radius) by the pile radius created by liposomes. The value less than 25 indicates excellent flow properties of formulated liposomes.
- Bulk density:** The mass of solid lipid nanoparticles (M) and their bulk volume (V_b) in

a measuring cylinder were divided to get the bulk density (ρ_b). The unit for bulk density is gm/cm^3 .

- c. **Tapped Density:** The tap density (ρ_t) is determined by dividing the mass (M) of liposomes by their tap volume (V_t), that is obtained via a tap density device. Tapped density is measured in gm/cm^3 .
- d. **Compressibility index:** A Carr's index or under 10 percent indicates an exceptional flow of liposomes. The Carr's index (I) is measured using the formula:

$$I = [(V_t - V_b) / V_t] \times 100$$
- e. **Hausner ratio:** The tapped density (ρ_t) and bulk density (ρ_b) of the liposomes that were created are divided to get the Hausner ratio. The Hausner's ratio must be between 1-1.1 for excellent flow of liposomes.

3.5. Drug Content

A 100 ml volumetric flask was used to hold the ground, weighed, and SLN formulations, each of which contained 100 mg. Before being filled to the mark with PBS (pH 7.4), the flask was shaken. The drug content was determined by analyzing the absorbance of the diluted solution at 248 nm using UV spectroscopy after the solution was further diluted to a concentration of $10\mu\text{g/ml}$ (Shivhare et al., 2009).

- **Encapsulation Efficiency**

We used the following formula to determine the liposome encapsulating efficiency:

$$\text{Encapsulation Efficiency} = \frac{\text{Estimated \% Drug Content}}{\text{Theoretical \% Drug Content}} \times 100$$

3.7. In Vitro Drug Release Studies

USP Dissolution Testing Apparatus II was used to find out how quickly the produced formulations released their contents. A phosphate buffer solution with a pH of 7.4 was used in the dissolving tests, which was carried out at a temperature of $37 \pm 0.5^\circ\text{C}$ and a speed of 50 rpm. Using a UV spectrophotometer, we measured the percentage of drug release after withdrawing 5 ml of sample every 2 minutes for up to 20 minutes and replacing it with new medium to keep the sink condition constant (Roy et al., 2018).

4. Result:

4.1. Preformulation studies:

Thiocolchicoside is white crystalline powder form with no order. And its melt on 192°C temperature.

4.2. UV Analysis:

The drug (0.01% w/v) in methanol was scanned between 200–400 nm and absorption maxima was determined spectrophotometrically. It exhibited absorption maxima at 248 nm. The method estimated the drug concentration in methanol followed the Beer's Lambert law in the concentration ranges ($2.0\text{--}14\mu\text{g/ml}$) at 248nm (**Figure 1,2**). The regression equation for Thiocolchicoside, as represented by the standard curve in the graph, is $y = 0.064x - 0.007$, and the R^2 value is 0.981, which, indicates good linearity. The estimation procedure was found to be fairly reproducible, convenient, inexpensive and sensitive.

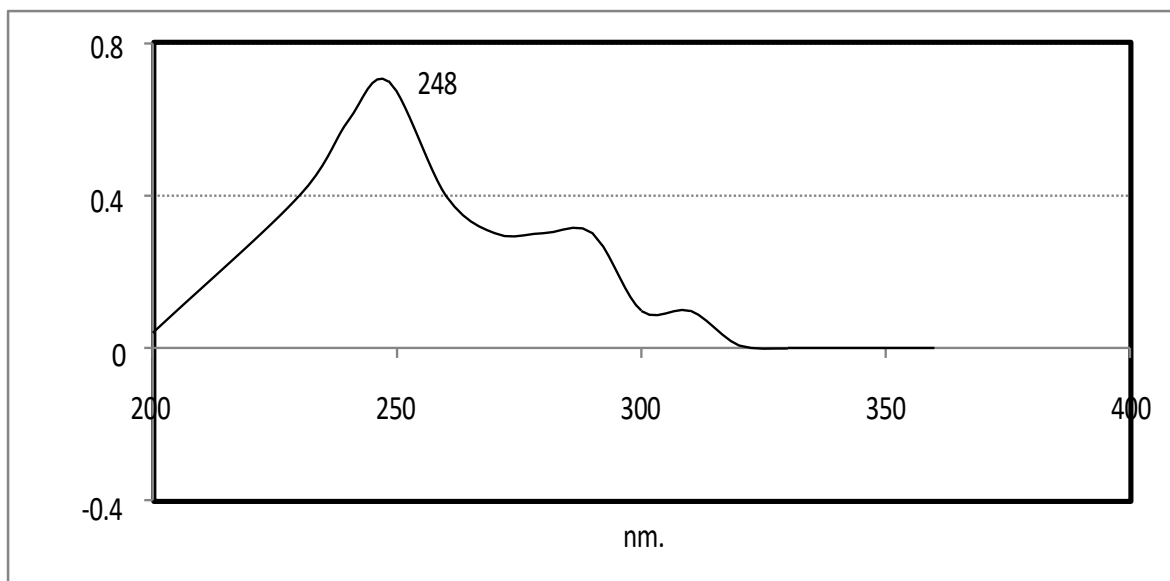


Figure 1 UV graph of Thiocolchicoside

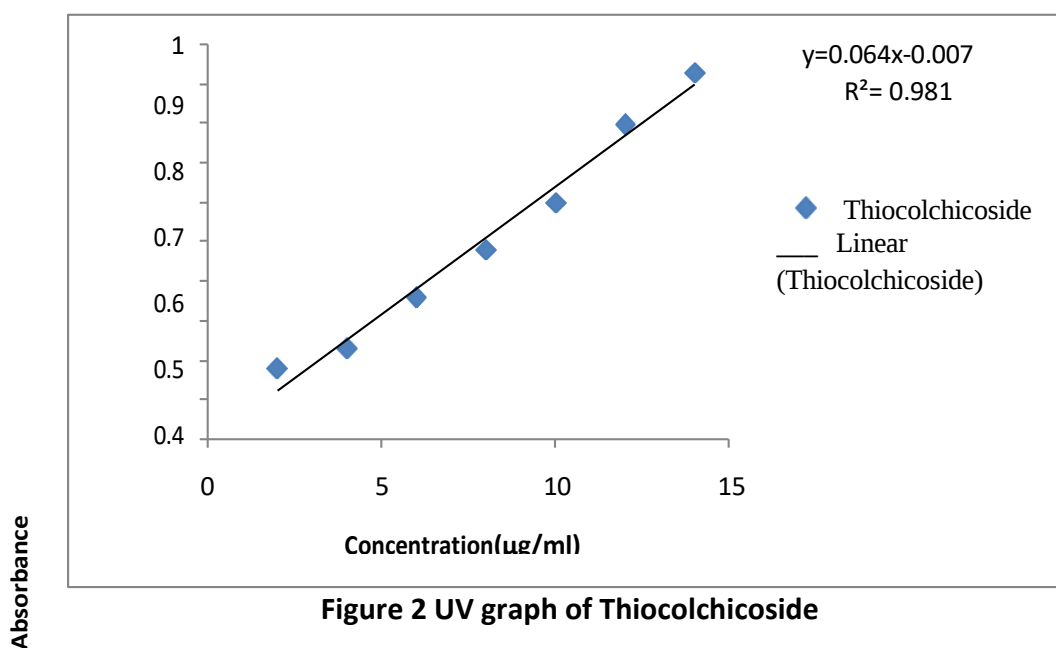


Figure 2 UV graph of Thiocolchicoside

4.3. Solubility Study:

The solubility was checked in different mediums for Thiocolchicoside which was found to be soluble in water. The amount of the drug dissolved was analyzed spectrophotometrically using UV Visibles pectrophotometer and the solubility(mg) was represented in **Figure 2**. The solubility of the Thiocolchicoside was found to be in the following order:

pH 6.8 Phosphate buffer < pH 7.4 Phosphate buffer < pH 4.5 Acetate Buffer < Ethanol < Water < 0.1N HCl < Methanol

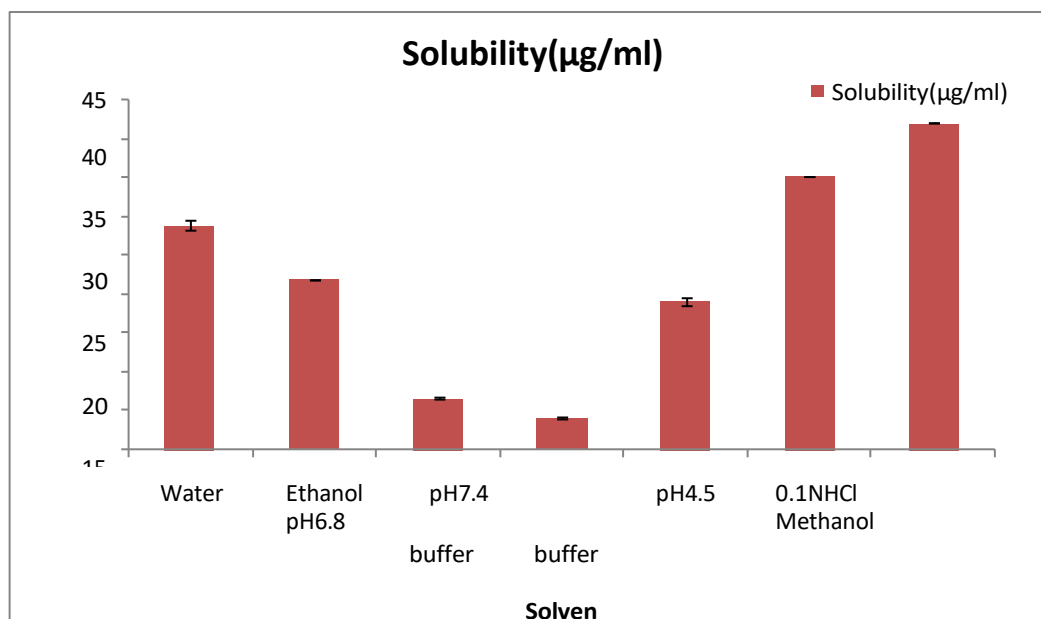


Figure 3 Solubility of Thiocolchicoside in various solvents

4.4. Drug excipient compatibility studies:

The FTIR of excipient and pure drug was done and the FTIR of drug and excipient mixture was done (Figure 4,5) and few additional IR peaks were observed. Although, this didn't have any interference with the major peaks of drug and can be further used for fabrication.

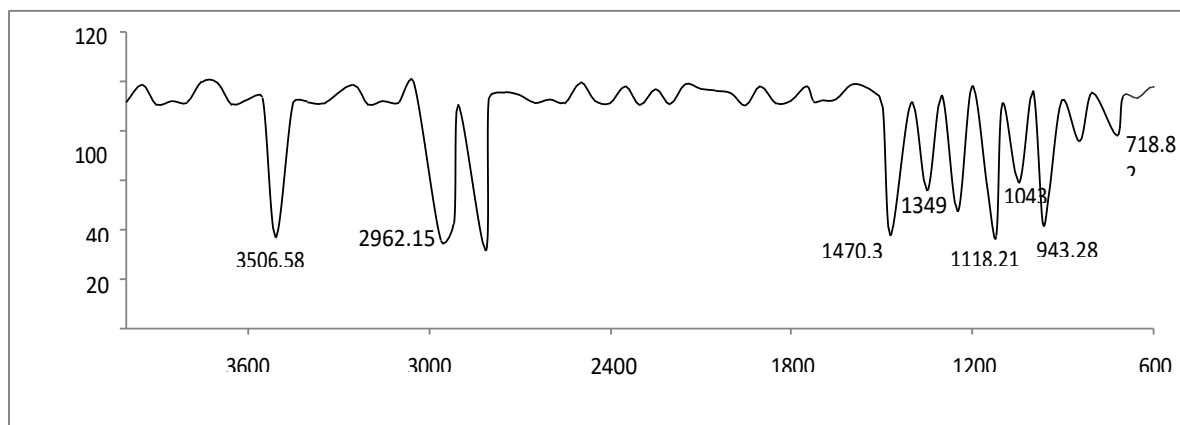


Figure 4 FTIR spectra of Stearic acid

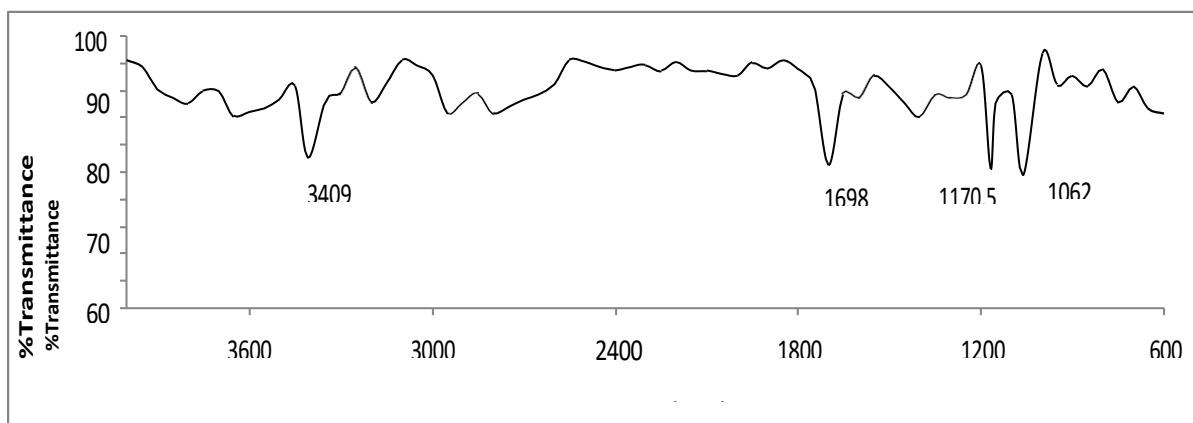


Figure 5 FTIR spectra of Soy Lecithin

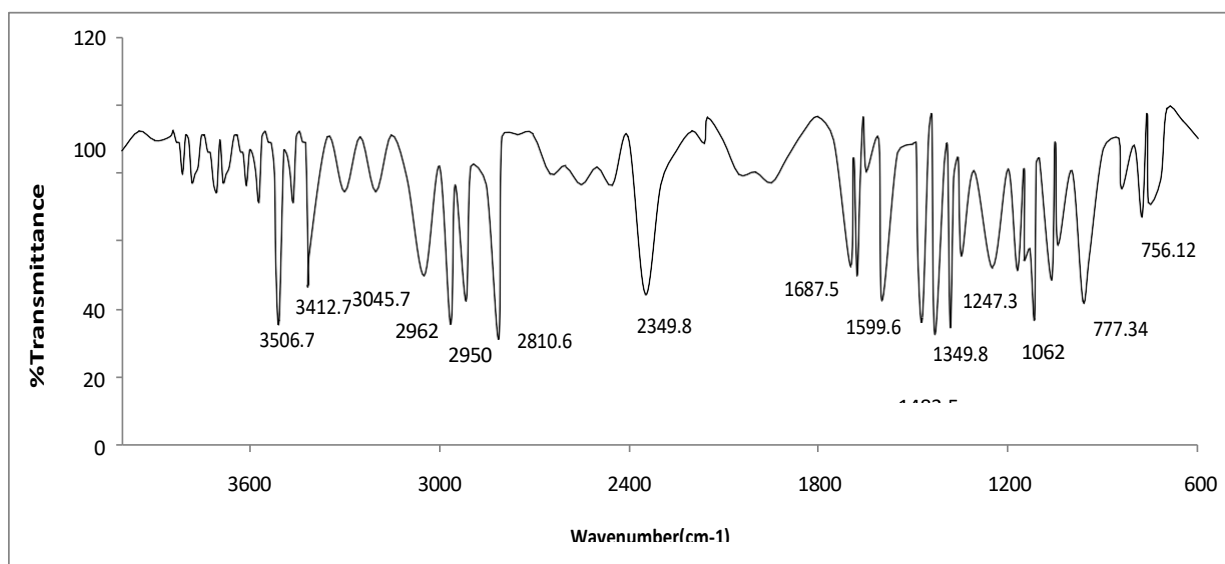


Figure 6 FTIR spectra of drug and excipient mixture

4.5. Preparation of Liposomes

The liposomes were prepared by thin film hydration method using varied concentrations of stearic acid and Soy Lecithin. This method was chosen because this method has ease of fabrication, is straightforward and repeatable.

4.6. Characterization of Liposomes

- **Appearance**

The prepared liposomes were visually checked for its color and surface texture. The results are depicted in Table 3 and illustrated stable, white liposomes with slightly smooth texture

- **Percentage Yield**

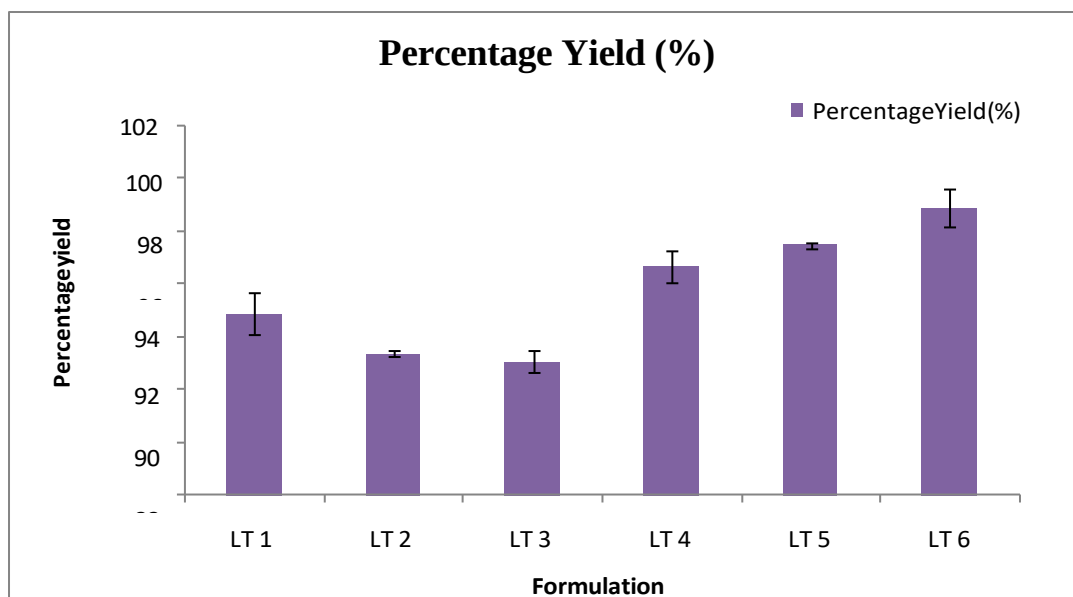


Figure 7 Percentage Yield (%)

- **Particle size**

The dynamic light scattering method, which is shown in Table 3, was used to measure the liposomes' particle size. When soy lecithin content was raised, liposome size of particles increased. Particle sizes for each formulation ranged from 2042.1 μm to 2158.4 μm .

Table no 3 Particle size of Thiocolchicoside Liposomes (LT 1 - LT 6)

S.No.	Formulation	Particlename(μm)
1	LT1	2042.1 $\pm$ 0.2
2	LT2	2046.6 $\pm$ 0.4
3	LT3	2045.9 $\pm$ 0.5
4	LT4	2157.2 $\pm$ 0.8
5	LT5	2158.4 $\pm$ 0.66
6	LT6	2157.9 $\pm$ 0.47

5. Zeta Potential

The zeta potential of Liposomes was determined using Zetasizer and the results were found in the range of -43.5 to -47.2 mV. The zeta potential of all formulations comprising from LT 1 to LT 6 are depicted in **Figure 8**.

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -43.5	Peak 1: -43.5	100	3.15
Zeta Deviation (mV): 5.25	Peak 2: 0.00	0.00	0.00
Conductivity (mS/cm): 0.147	Peak 3: 0.00	0.00	0.00

Result quality **Good**

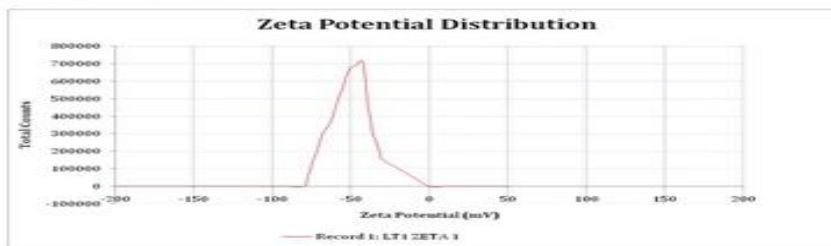


Figure 8 Zeta Potential of ThiocolchicosideLT1

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -44.1	Peak 1: -44.1	100	3.74
Zeta Deviation (mV): 4.01	Peak 2: 0.00	0.00	0.00
Conductivity (mS/cm): 0.174	Peak 3: 0.00	0.00	0.00

Result quality **Good**

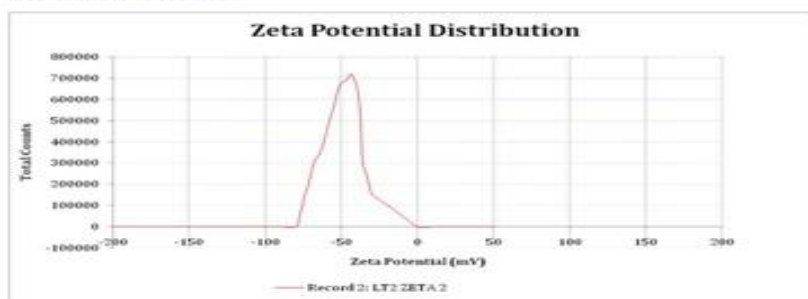


Figure 9 Zeta Potential of Thiocolchicoside LT 2

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -45.2	Peak 1: -45.2	100	4.1
Zeta Deviation (mV): 2.71	Peak 2: 0.00	0.00	0.00
Conductivity (mS/cm): 0.147	Peak 3: 0.00	0.00	0.00

Result quality **Good**

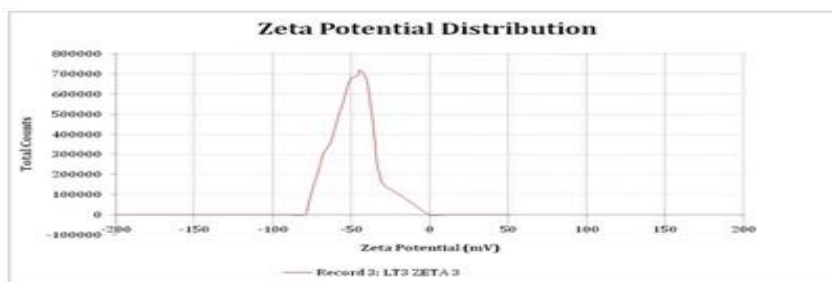


Figure 10 Zeta Potential of Thiocolchicoside LT 3

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -46.5	Peak 1: -46.5	100	3.78
Zeta Deviation (mV): 4.12	Peak 2: 0.00	0.00	0.00
Conductivity (mS/cm): 0.181	Peak 3: 0.00	0.00	0.00

Result quality **Good**

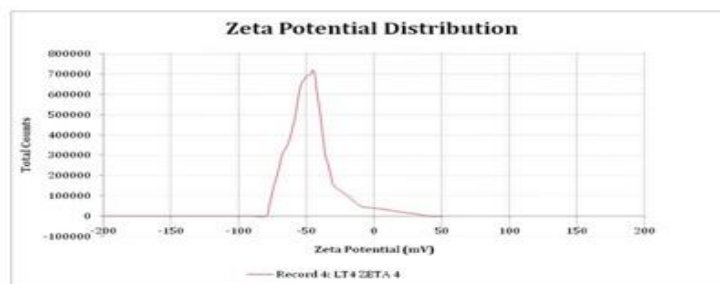


Figure 11 Zeta Potential of ThiocolchicosideLT4

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -47.1	Peak 1: -47.1	100	2.73
Zeta Deviation (mV): 2.67	Peak 2: 0.00	0.00	0.00
Conductivity (mS/cm): 0.17	Peak 3: 0.00	0.00	0.00

Result quality **Good**

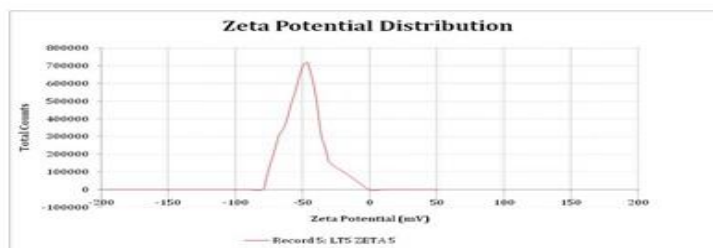


Figure 12 Zeta Potential of ThiocolchicosideLT5

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -47.2	Peak 1: -47.2	100	3.64
Zeta Deviation (mV): 3.74	Peak 2: 0.00	0.00	0.00
Conductivity (mS/cm): 0.163	Peak 3: 0.00	0.00	0.00

Result quality **Good**

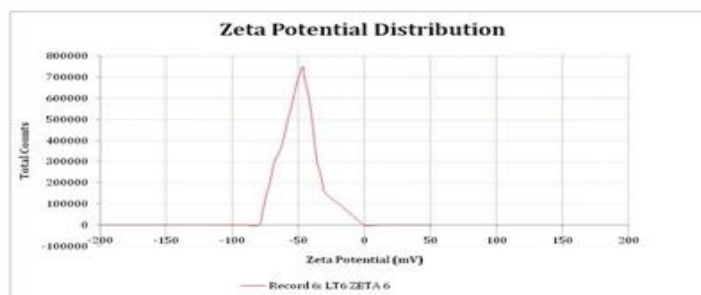


Figure 13 Zeta Potential of ThiocolchicosideLT6

6. Encapsulation Efficiency

The Encapsulation efficiency (E.E.) of liposomes was calculated and was found to be in the range of 89.6 to 93.7%. The formulation LT 6 showed highest drug encapsulation efficiency

Table no 4 Encapsulation Efficiency

Formulation	Drug Encapsulation Efficiency (%)
LT 1	89.6 $\pm$ 0.5
LT 2	89.9 $\pm$ 0.1
LT 3	90.1 $\pm$ 0.5
LT 4	92.7 $\pm$ 0.1
LT 5	93.1 $\pm$ 0.4
LT 6	93.7 $\pm$ 0.8

• *In vitro* Drug Release Studies

The release rate of drug loaded Liposomes was determined using USP dissolution testing apparatus II (Electro lab, India). The *in vitro* release data are the graphical representation is shown in Figure 14. The cumulative percentage drug release from Liposomes prepared with higher concentration of Soy lecithin showed slower drug release when compared to lower amount of phospholipid. Higher concentration of soy lecithin leads to slower drug release and the rate of drug release is found to be in the order LT 6 < LT 5 < LT 4 < LT 3 < LT 2 < LT 1.

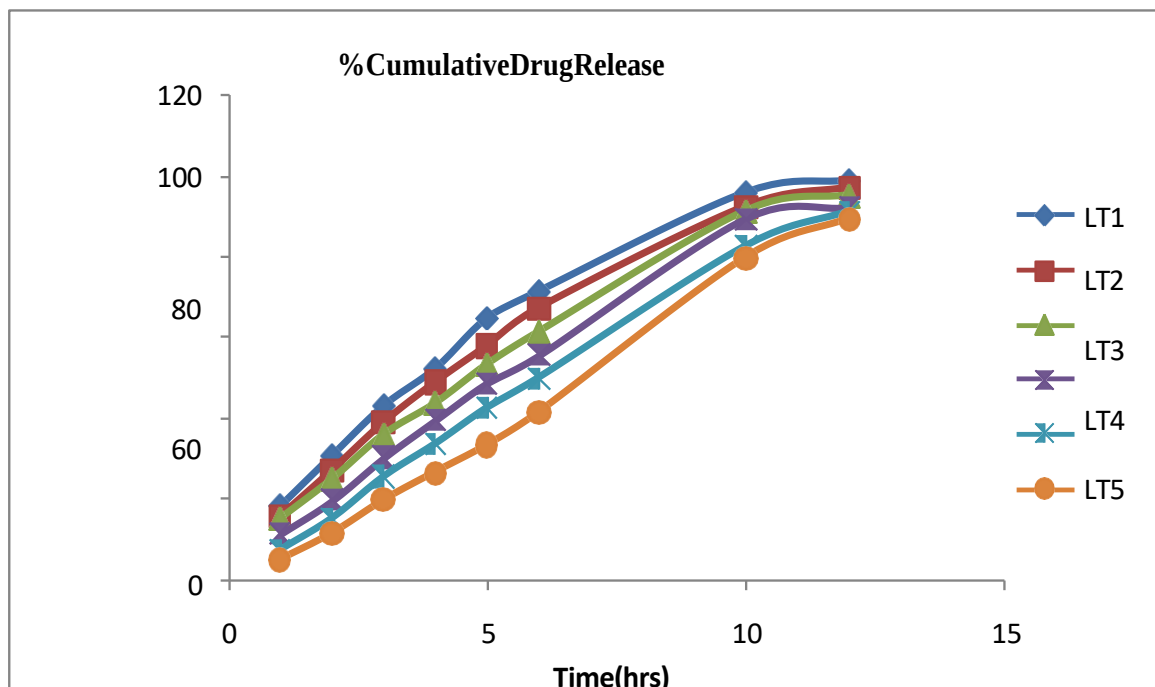


Figure No. 14 % Cumulative Drug Release

6. Conclusion:

Thiocolchicoside, an FDA-approved anti-inflammatory drug, is used in treatment of orthopaedic, traumatic, and rheumatologic disorders. Conventional oral formulations are effective but have rapid drug release and high dosing frequencies. To improve drug release, controlled release oral formulations are needed. Liposomes, a biodegradable and biocompatible material, have gained attention for their low toxicity and low cost. A study used a thin film hydration method to create Thiocolchicoside liposomes using soya lecithin, cholesterol, and organic solvent. The liposomes showed a range of drug content and encapsulation effectiveness, with the most effective formulation being LT 6. Stability tests and drug release kinetics were conducted on LT6, and the Korsmeyer Peppas release kinetics were found to be most appropriate for drug-loaded liposomes. Liposomes are crucial for maintaining medication release, decreasing dosage frequency, and improving patient compliance.

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