# DEVELOPMENT AND OPTIMIZATION OF SOY LECITHIN-BASED TRANSFERSOMES TO ENHANCED TRANSDERMAL DELIVERY OF ETORICOXIB FOR RHEUMATOID ARTHRITIS

**Mr. BABU LAL PATEL[1]*,** Dr. RITIKA DADHICH[1], Mr. TEJA RAM[1]

*1 – Assistant Professor, Jai Narain Vyas University, Jodhpur- 342001*

This research aims to develop a soy lecithin-based transfersome for the transdermal delivery of Etoricoxib, intended to enhance pain management and provide therapeutic benefits for rheumatoid arthritis (RA). The study began with a preformulation assessment of Etoricoxib, which included identifying physical characteristics (such as appearance, melting point, and IR spectra), solubility profile, and determining λ-max to guide formulation procedures. Formulation optimization involved assessing the effects of different process variables, including varying amounts of lecithin and surfactant. Transfersomes were prepared using a modified hand-shaking method, with Tween 80 as the surfactant in multiple concentrations. Entrapment efficiency depended on the ratio of lecithin to Tween 80, reaching a peak of 72.34±0.022 in formulation F5. Vesicle size, which also influenced entrapment efficiency, averaged 9.69 μm in the F5 formulation. Drug permeation from the transfersomal gel was similarly influenced by the lecithin-to-Tween 80 ratio. Formulation F5, exhibiting high entrapment efficiency, demonstrated superior drug permeation. This study suggests that a PC : Tween 80 ratio of 90:10 (mmol) in transfersomes could be a promising strategy for enhancing the permeability and effectiveness of Etoricoxib over time. The values of the release exponent for both formulations were relatively low, indicating a Fickian diffusion mechanism for the release of the drug. Overall, these results highlight the different kinetic behaviors of the two formulations, with the hydrogel showing a more consistent release pattern as described by the Korsmeyer-Peppas model.

**Keywords:** Etoricoxib, Transfersomes, Surfactants, Transdermal.

## INTRODUCTION

A novel vesicular drug carrier system called transfersomes, which is composed of phospholipids, surfactant, and water for enhanced transdermal delivery. Transfersomes, a novel class of modified liposomes, are variously described as deformable, highly deformable, elastic or ultra-flexible liposomes or vesicles, which were first introduced in the early 1990s. Transfersomes are advantageous as phospholipids vesicles for transdermal drug delivery. Because of their self-optimized and ultra-flexible membrane properties, they are able to deliver the drug reproducible either into or through the skin, depending on the choice of administration or application, with high efficiency.[1-6] Etoricoxib is a non-steroidal anti-inflammatory drug that exhibits anti-inflammatory, analgesic and antipyretic activities. Etoricoxib is a selective COX-2 inhibitor, a potent widely prescribed anti-inflammatory and analgesic drug belongs to Class II under 'BCS' and exhibit low and variable oral bioavailability due to its poor aqueous solubility. As such oral absorption of etoricoxib is dissolution rate limited and it requires enhancement in solubility and dissolution rate for increasing its oral bioavailability. Transdermal delivery of etoricoxib is a better suited altered to overcome the problem associated with its oral delivery. The major problem with transdermal drug delivery is the permeation characteristics of the stratum corneum, which limits, drug transport, making the rout of administration frequently in sufficient for medical use: Transfersome are promising nano carriers for non invensive transdermal delivery.

Therefore, the present research work has been understood with the aim to develop a transdermal formulation of etoricoxib, by encapsulation it into transfersomes which would overcome the side effect associated with oral etoricoxib therapy.

## MATERIALS AND METHODS

Etoricoxib was obtained as a gift from MorepenPvt. Ltd, Himachal Pardesh. Soya lecithin was purchased from HiMedia laboratories Pvt. Ltd., Nasik. Tween 80 was purchased from Loba Chemie Pvt. Ltd., Mumbai.De-ionized water and other chemical and solvents were of analytical grade/IP/equivalent grade were procured from laboratory.

**Preparation of transfersome**

Transfersomes were prepared using a modified hand-shaking and lipid film hydration technique. Drug, lecithin (PC), and edge activator were dissolved in a chloroform (2:1) mixture. (Table N0. -1). The organic solvent was evaporated through hand-shaking at 43°C, forming a thin lipid film on the flask wall, which was left overnight for complete solvent removal. This film was hydrated with pH 7.4 phosphate buffer by gently shaking for 15 minutes at the same temperature, and then the suspension was further hydrated for up to 1 hour at 2-8°C.[1,6,7]

**Table 1: Compositions of transfersome of etoricoxib**

| **Formulation code** / **Ingredient** | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** | **F7** | **F8** | **F9** |
|---|---|---|---|---|---|---|---|---|---|
| **Etoricoxib (mg)** | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| **Soya lecithin (mg)** | 85 | 85 | 85 | 90 | 90 | 90 | 95 | 95 | 95 |
| **Tween 80 (mg)** | 5 | 10 | 15 | 5 | 10 | 15 | 5 | 10 | 15 |
| **Chloroform: Methanol(ml) (2:1)** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

**Analytical and Evaluation Studies of Etoricoxib Transfersome Gel**

UV-visible analysis of Etoricoxib was conducted using an Elico double-beam UV-visible spectrophotometer (1800 model) with quartz cells to assess drug concentration. Etoricoxib's solubility was tested using the shake flask method in methanol and a pH 7.4 phosphate buffer by shaking for 6 hours, allowing it to stand for 24 hours, filtering, and analyzing with UV

spectrophotometry. A physical mixture of Etoricoxib and soy lecithin (1:1) was prepared, with IR spectra recorded initially and after 14 days at 37°C to confirm compatibility.

**Entrapment Efficiency (EE %)**

The prepared 10 ml transfersomes was placed in centrifugation tube and centrifuged at 9000 rpm for 40 minute. The supernatant (1ml) was withdrawn and diluted with PBS (pH 7.4) up to 10ml. The un-entrapped Etoricoxib was determined by UV spectrophotometer at 283nm. Entrapment efficiency is expressed as the percent of drug trapped.[11]

***In-vitro* diffusion study**

In this study, 1 ml of the formulated transfersome was applied to an egg membrane, which was then placed between the donor and receptor chambers of a diffusion cell. The receptor chamber was filled with a pH 7.4 phosphate buffer solution to facilitate drug solubility and stirred at 200 rpm. The entire setup was kept at a stable temperature of 37±0.5°C. Drug release was monitored by taking 0.5 ml samples at intervals of 30, 60, 120, 240, and 360 minutes, each time replacing the withdrawn volume with fresh buffer solution. After proper dilution, the samples were analyzed using a Shimadzu UV 1800 spectrophotometer at a λ max of 283 nm, with pH 7.4 phosphate buffer as the blank.

**Optimization of formulation:**

The optimization of the transfersome formulation for Etoricoxib involved evaluating key process variables, primarily focusing on the lecithin-to-surfactant ratios (85:5, 85:10, 85:15, 90:5, 90:10, 90:15, 95:5, 95:10, 95:15) to maximize entrapment efficiency and in-vitro drug release. During formulation, other variables were kept constant to ensure a controlled analysis. For the gel preparation, 1 g of Carbopol 934 was dispersed in 88 g of distilled water, stirred at 800 rpm for one hour, with propylene glycol added as a stabilizer and triethanolamine to adjust pH to 5.5, forming a clear gel. Finally, the optimized transfersome batch containing Etoricoxib was incorporated into the 1% Carbopol gel, ensuring a 2% Etoricoxib concentration (2 mg per gram of gel).[13,14]

**Evaluation of formulations[15]**

The evaluation of the Etoricoxib transfersome gel formulations involved multiple quality assessments. Physical properties, such as color, texture, and homogeneity, were visually inspected. The pH was measured by dissolving 1 g of the gel in 100 ml distilled water and testing

after 2 hours. Rheological properties were analyzed with a Brookfield viscometer to determine viscosity at various speeds. Drug content was assessed by analyzing 1 g of the gel with a UV spectrophotometer at 283 nm to measure Etoricoxib concentration. Extrudability was tested by placing 1 kg on an aluminum collapsible tube, and the percentage extruded was calculated. Spreadability was evaluated by placing gel between two glass plates under a 1 kg weight for uniform distribution, then applying a 150 g pull to measure the time required for the top plate to move 10 cm, with shorter times indicating better spreadability.

## Result and discussion

### Identification of etoricoxib by IR spectroscope

Transmittance peaks exhibited in the recorded IR spectrum (Fig. No. – 1,2) of etoricoxib were compared with peaks exhibited in the reported spectrum of etoricoxib and were found to be similar as presented in Table 2.

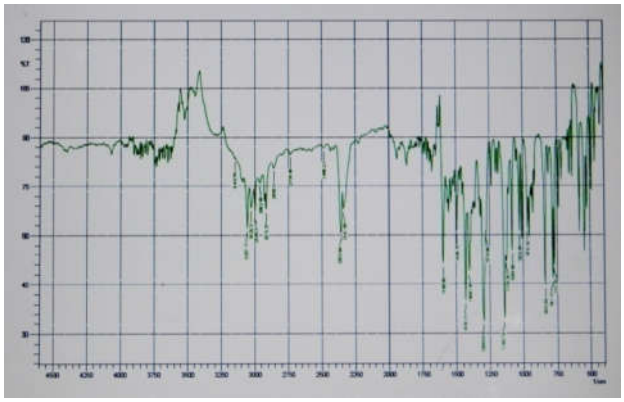


**Figure 1: Reported IR spectrum of Etoricoxib**

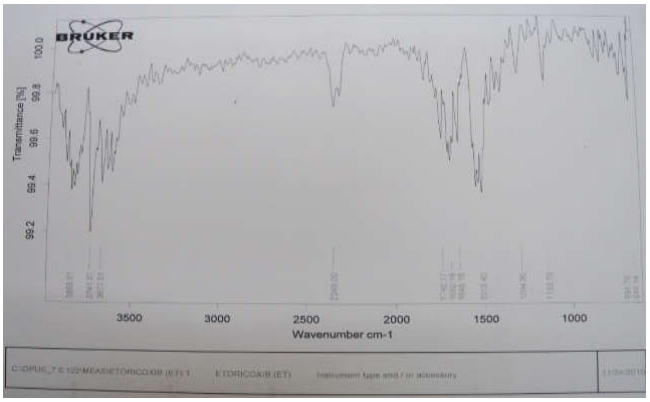



**Figure 2: Recorded IR spectrum of Etoricoxib**

**Table 2: Comparison between the reported and recorded IR spectrum of etoricoxib**

| Peak No. | Wave number ($cm^{-1}$) | Absorption frequency band ($cm^{-1}$) | Characterisation functional group/ vibrations |
|---|---|---|---|
| 1 | 3741.87 | 3680-3760 | C=O Stretching |
| 2 | 1740.77 | 1680-1760 | C=O Stretching (aldehyde) |
| 3 | 1692.19 | 1680-1760 | C=O Stretching |

| | | | |
|---|---|---|---|
| 4 | 1645.15 | 1500-1650 | N-H Bending |
| 5 | 1515.40 | 1500-1600 | -C=C Stretching |
| 6 | 1294.80 | 1000-1300 | C=O Stretching (alcohol) |
| 7 | 1139.79 | 1000-1260 | C=S Stretching |

**Analytical studies by UV- spectrophotometry**

The λmax of Etoricoxib was determined to be 283 nm, with a linear response observed in accordance with Beer's law over a concentration range of 2-20 mg/ml. The slope and intercept of the standard curve were 0.044, and the correlation coefficient ($R^2$) was 0.997. The absorbance data and concentration ranges are detailed in Table 3, and the standard curve is illustrated in Figure 3.

**Figure 3 : Calibration curve for etoricoxib in PBS 7.4 pH at 283nm**

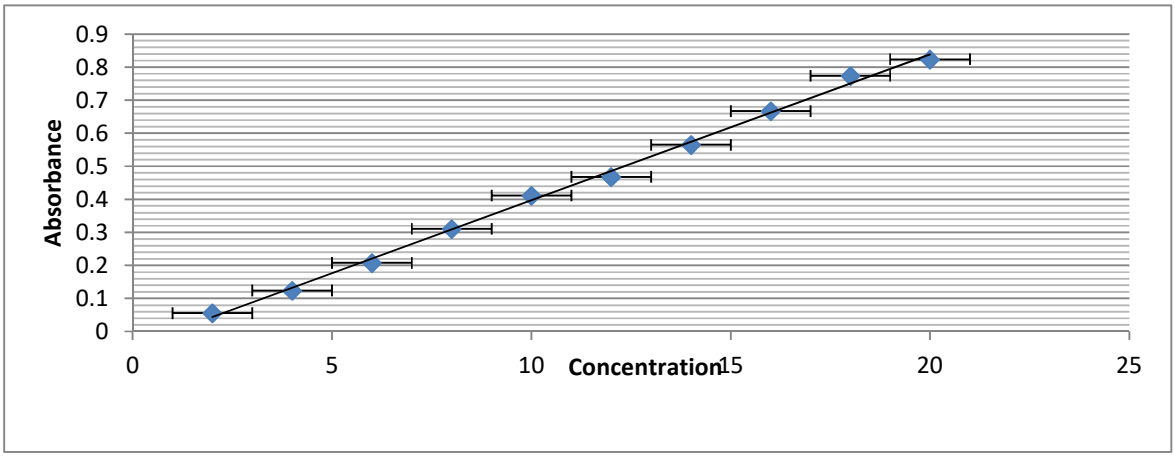



**Table 3: Calibration curve data of etoricoxib**

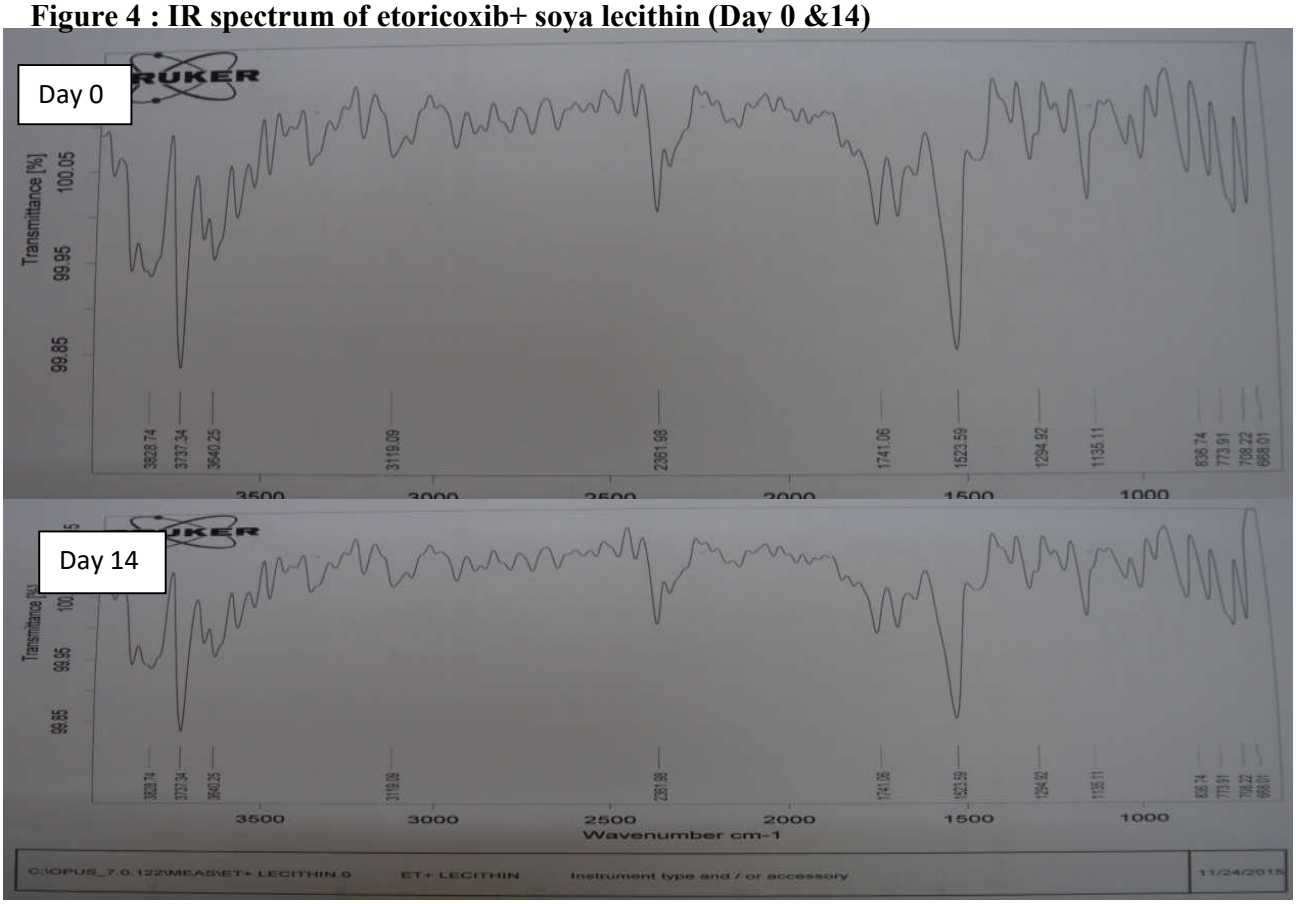
| Concentration (µg/ml) | Absorbance | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | Average |
| 2 | 0.054 | 0.055 | 0.057 | 0.056±0.001 |
| 4 | 0.124 | 0.126 | 0.125 | 0.124±0.002 |
| 6 | 0.204 | 0.209 | 0.211 | 0.208±0.003 |
| 8 | 0.311 | 0.311 | 0.311 | 0.311±0.000 |
| 10 | 0.413 | 0.413 | 0.410 | 0.412±0.001 |
| 12 | 0.468 | 0.464 | 0.471 | 0.468±0.002 |
| 14 | 0.566 | 0.565 | 0.565 | 0.565±0.001 |
| 16 | 0.667 | 0.669 | 0.665 | 0.667±0.001 |

| | | | | |
|---|---|---|---|---|
| 18 | 0.771 | 0.775 | 0.776 | 0.774±0.002 |
| 20 | 0.824 | 0.824 | 0.824 | 0.824±0.000 |

**Drug-excipient compatibility study**

The analysis of the physical mixture revealed that there were no missing or new peaks, indicating no chemical incompatibility. Additionally, there were no signs of liquefaction, discoloration, or gas formation, confirming the absence of physical incompatibility. (Fig. No. – 4 ).

**Figure 4 : IR spectrum of etoricoxib+ soya lecithin (Day 0 &14)**




**Formulation and Vesicle size and % Entrapment efficiency of etoricoxib transfersome:**

Etoricoxib transfersomes were formulated using varying concentrations of soy lecithin and Tween 80 in ratios from 85:5 to 95:15 through the thin film hydration technique with emulsification and hand shaking. Vesicle size and entrapment efficiency were directly influenced by the concentrations of lecithin and Tween 80(Fig. No. -5). The vesicle size across formulations ranged from 5.61 to 10.13 µm, with larger vesicles formed at higher lecithin concentrations. The

entrapment efficiency increased with Tween 80 concentrations from 5% to 10%, reaching optimal results in formulation F5 (lecithin : Tween 80 at 90:10), but decreased with further surfactant increase to 15% due to micelle rigidity. Higher lecithin concentrations also enhanced entrapment efficiency.(Fig. No. -6,7,8)

**Figure 5: Photograph of prepared transfersome by optical microscopy (F5)**

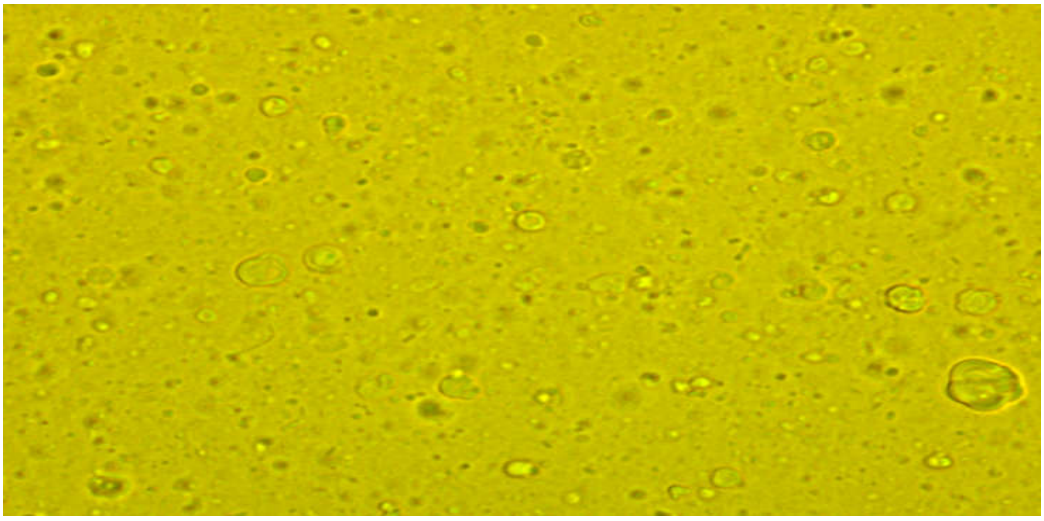


**Figure 6 : % Entrapment efficiency determination of various transfersome formulations**

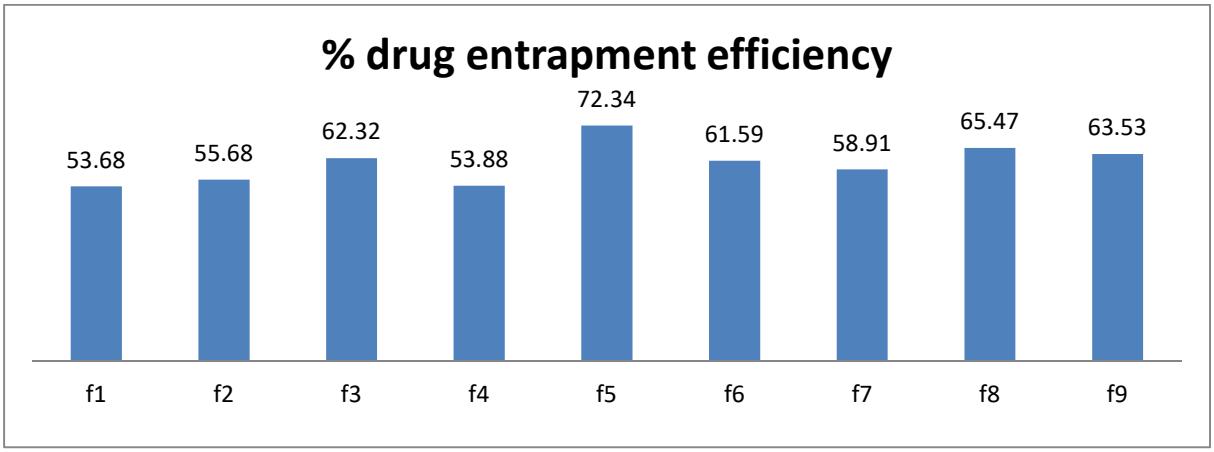



**Figure 7: Average effect of different concentration of tween 80 on %Entrapment efficiency**

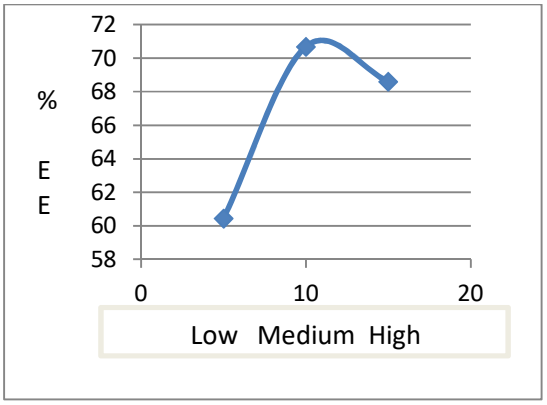
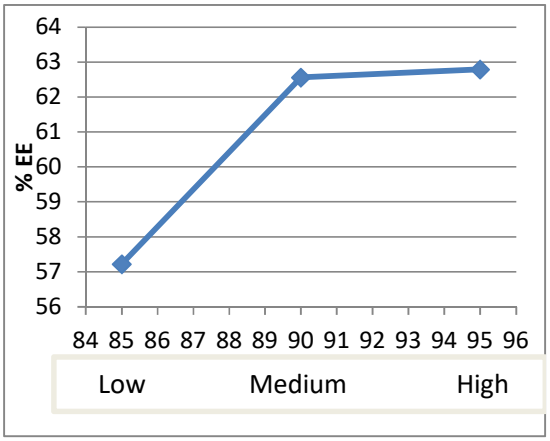



**Figure 8: Average effect of different concentration of soya lecithin on %Entrapment efficiency**




***In-vitro* diffusion studies:**

In-vitro diffusion studies assessed drug release from transfersomes with varying ratios of phosphatidylcholine to Tween 80. Drug release increased with Tween 80 concentrations from 5% to 10%, peaking in formulation F5 (lecithin : Tween 80 at 90:10), but decreased with further increases in surfactant and lecithin levels.(Fig. No.-9) The enhanced release at optimal Tween 80 levels (10%) likely results from improved drug partitioning in the phospholipid bilayer. Lower surfactant levels led to less drug release due to the more ordered, less leaky lipid membrane, while higher concentrations reduced release by forming rigid micelles that disrupted vesicle structure.(Fig. No. – 10,11)

**Figure 9: *In-vitro* Diffusion Profile of Various Formulations**

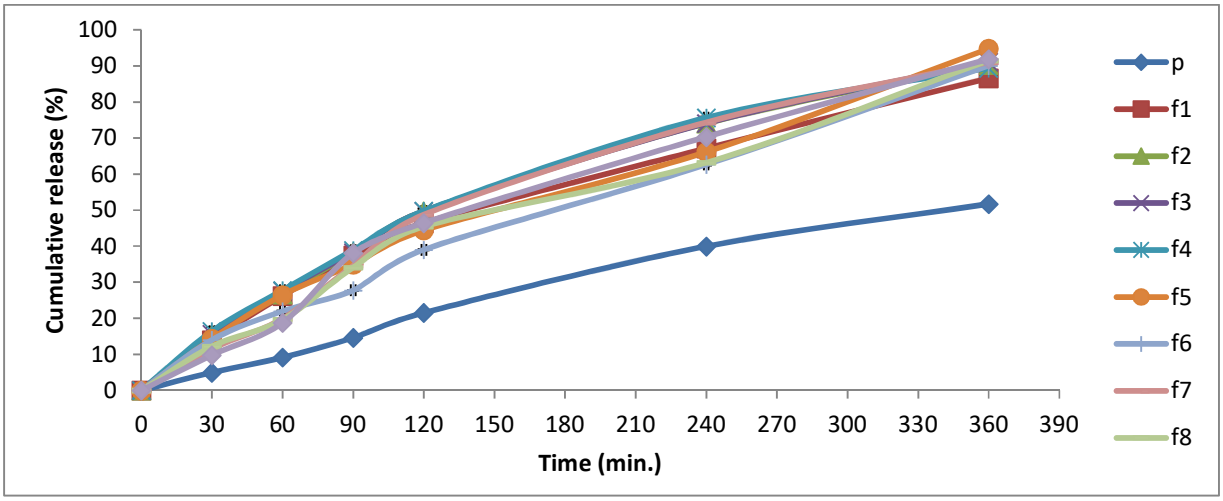



**Figure 10: Average effect of concentration of tween 80 on release of etoricoxib from prepared transfersome at 6 hours**

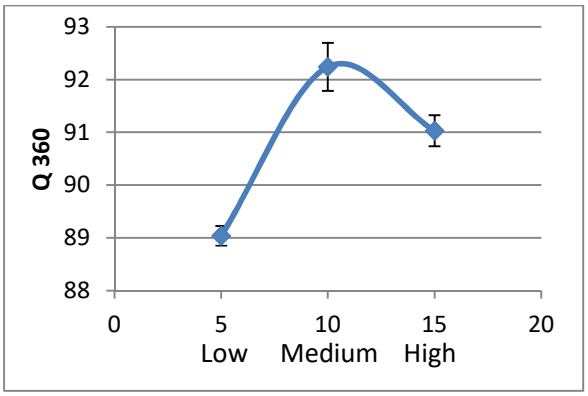
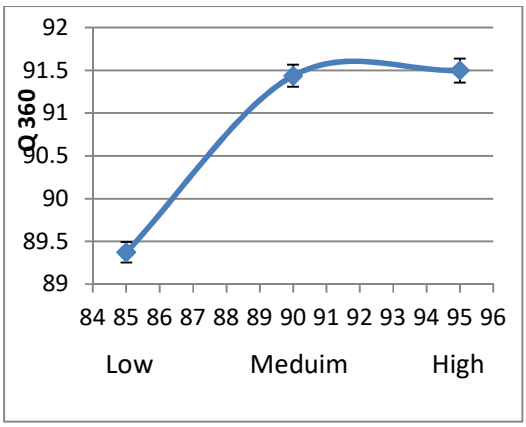



**Figure 11 : Average effect of concentration of soya lecithin on release of etoricoxib from prepared transfersome at 6 hours**



**Stability studies of the optimized batch as per ICH guidelines:**

The Etoricoxib transfersome gel formulations were characterized by their colorlessness, homogeneity, good texture, and clarity. A comparison between the transfersome gel and hydrogel formulations revealed several key parameters: the pH of the transfersome gel was 6.9, slightly higher than the hydrogel's pH of 6.6; the drug content was measured at 84.29% for the transfersome gel, outperforming the hydrogel's 81.62%; extrudability was found to be 7.8% for the transfersome gel compared to 8.3% for the hydrogel; however, the spreadability of the transfersome gel was lower at 29.45% versus 48.9% for the hydrogel. Overall, these findings indicate that transfersomes are a promising delivery system for dermal applications.

**Rheological studies using Brookefield viscometer -**

The effect of rotational speed (rpm) on the viscosity of the etoricoxib transfersome gel was evaluated, revealing a general decrease in viscosity as the rpm increased. Table 6 presents the viscosity measurements of the transfersome gel (TFS gel) and hydrogel at various rpm levels. At 5 rpm, the viscosity of the TFS gel was 37,820 cps, significantly lower than the hydrogel's viscosity of 66,454 cps. As the rpm increased to 10, 20, 50, and 100, the viscosity of the TFS gel decreased to 23,828 cps, 15,032 cps, 7,545.5 cps, and 3,620.5 cps, respectively. In comparison, the hydrogel's viscosity also decreased but remained higher than that of the TFS gel, reaching 41,387 cps at 10 rpm, 13,110.5 cps at 20 rpm, 5,315 cps at 50 rpm, and 4,254 cps at 100 rpm (Fig No. -9). This data indicates a shear-thinning behavior, where the viscosity declines as the shear rate increases, due to the alignment of the gel's molecules in the direction of flow, which reduces the material's internal resistance and results in lower viscosity.

**Table 4: Vesicles size and Entrapment efficiency Determination for different transfersome formulation**

| Formulation code | Vesicles size (µm) | % Entrapment efficiency |
|---|---|---|
| F1 | 5.61 | 55.53±0.07 |
| F2 | 6.38 | 55.68±0.06 |
| F3 | 5.80 | 62.33±0.02 |
| F4 | 9.12 | 53.88±0.09 |
| F5 | 9.69 | 72.34±0.02 |
| F6 | 9.89 | 61.59±0.04 |

| | | |
|---|---|---|
| F7 | 9.51 | 58.91±0.01 |
| F8 | 9.92 | 65.47±0.01 |
| F9 | 10.12 | 63.53±0.04 |

***In-vitro* release study of topical hydrogel:**

The dissolution rate studies were conducted to evaluate how varying surfactant concentrations impact the release profile of etoricoxib transfersome formulations. In vitro release studies of the topical hydrogel and transfersome gel were carried out over six hours using modified Franz diffusion cells in a phosphate buffer (pH 7.4) maintained at 37±0.5°C, with stirring at 100 rpm under sink conditions. The diffusion release data, summarized in Table 7 and depicted in Figure 11, indicated that the 1% concentration of the gelling agent (Carbopol 934P) slightly affected the release rate. After six hours, the drug release from the hydrogel was 63.24%, while the transfersome gel exhibited a higher release rate of 78.87%. The in vitro diffusion studies showed that the transfersome gel delivered a significantly greater amount of drug compared to the hydrogel, highlighting its potential for improved therapeutic efficacy.(Fig. No. – 12). The diffusion data also showed incremental increases in drug release over time, with the transfersome gel consistently outperforming the hydrogel at all measured time points.

**Figure No. – 12 : Rheological studies using Brookefield viscometer**

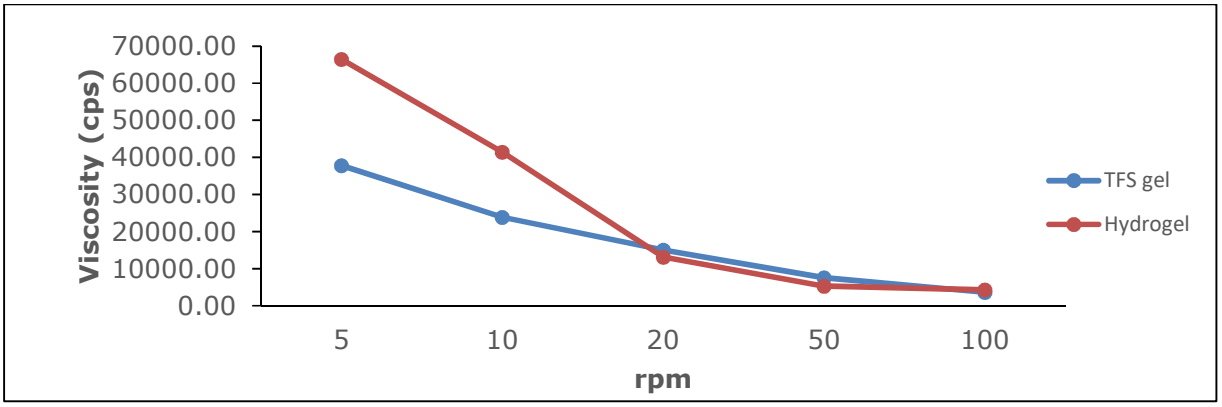
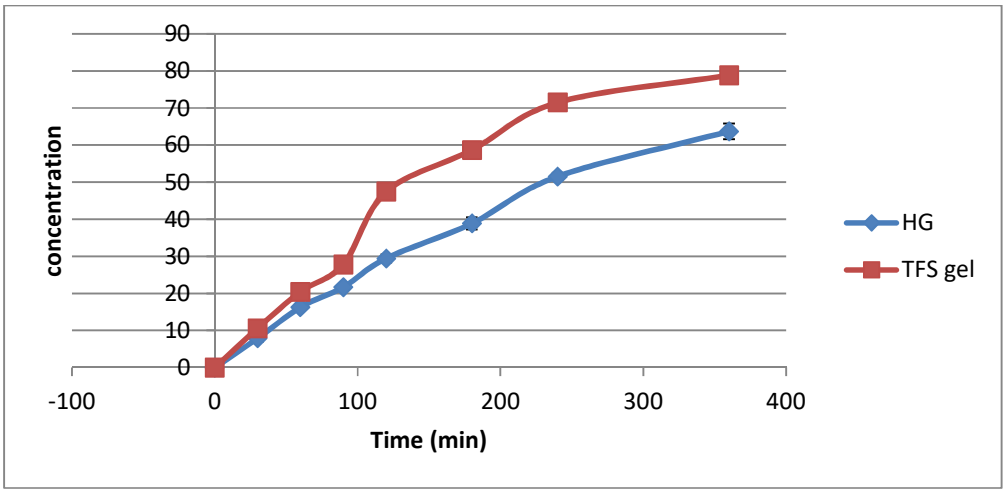


**Figure 13 : Graphical data of *in-vitro* release**




**Kinetic Analysis of Dissolution Data** :

The kinetic analysis of the dissolution data for hydrogel and transfersome gel (TFS gel) formulations aimed to determine their release kinetics through various models, including zero-order, first-order, Higuchi, Hixon-Crowell, and Korsmeyer-Peppas. The hydrogel formulation showed the best fit with the Korsmeyer-Peppas model, yielding a high correlation coefficient of 0.9995, which indicates a strong relationship between drug release rate and time. In contrast, the TFS gel exhibited the best fit with the Higuchi model, achieving a correlation coefficient of 0.9801. For the hydrogel, correlation coefficients were 0.9935 for zero-order, 0.9485 for first-order, and 0.9686 for Higuchi, while the TFS gel had coefficients of 0.9472 for zero-order, 0.8695 for first-order, and 0.9474 for Korsmeyer-Peppas. Both formulations had relatively low release exponent values, suggesting a Fickian diffusion mechanism for drug release. Overall, these findings illustrate distinct kinetic behaviors, with the hydrogel demonstrating a more consistent release pattern according to the Korsmeyer-Peppas model.

**Table 5: *In-vitro* diffusion studies data of transfersome formulations**

| **Time** | **p** | **f1** | **f2** | **f3** | **f4** | **f5** | **f6** | **f7** | **f8** | **f9** | **tp 6hr** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **30** | 4.95 | 13.97 | 15.3 | 15.64 | 16.43 | 14.35 | 14.01 | 11.51 | 12.19 | 9.81 | 8.33 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **60** | 9.14 | 26.17 | 26.66 | 27.57 | 27.8 | 26.46 | 21.96 | 19.92 | 19.62 | 18.75 | 16.66 |
| **90** | 14.59 | 37.42 | 38.37 | 38.33 | 38.93 | 34.92 | 27.76 | 34.62 | 34.09 | 38.1 | 25 |
| **120** | 21.52 | 46.47 | 49.73 | 49.73 | 49.77 | 44.43 | 39.01 | 48.75 | 45.45 | 46.47 | 33.33 |
| **240** | 39.96 | 67.15 | 74.09 | 74.09 | 75.64 | 66.13 | 62.65 | 74.35 | 63.18 | 70.33 | 66.66 |
| **360** | 51.71 | 86.62 | 90.37 | 91.13 | 89.43 | 94.81 | 90.07 | 91.06 | 91.55 | 91.89 | 100 |

**Table 6 : Viscosity of etoricoxib transfersome gel (TFS gel) and hydrogel at different rpm**

| RPM | TFS gel (cps) | Hydrogel (cps) |
|---|---|---|
| **5** | 37820.00±81 | 66454.00±47 |
| **10** | 23828.00±75 | 41387.00±56 |
| **20** | 15032.00±49 | 13110.5±81 |
| **50** | 7545.50±92 | 5315.00±69 |
| **100** | 3620.50±65 | 4254.00±99 |

**Table 7: *In-vitro* diffusion studies data of gel formulations**

| Time (min) | Hydrogel | Transfersome gel | Theoretical profile (8hr) |
|---|---|---|---|
| **0** | 0.00 | 0.00 | 0 |
| **30** | 7.92±0.91 | 10.62±0.62 | 8.33 |
| **60** | 16.28±0.65 | 20.46±0.23 | 16.66 |
| **90** | 21.73±0.24 | 27.85±0.51 | 25 |
| **120** | 29.46±1.09 | 47.46±0.95 | 33.33 |
| **180** | 38.89±1.62 | 58.67±0.69 | 66.66 |
| **240** | 51.53±0.99 | 71.50±1.21 | 75 |
| **360** | 63.74±2.01 | 78.87±1.69 | 100 |

**Conclusion**

Etoricoxib is a non-steroidal anti-inflammatory drug (NSAID) recognized for its anti-inflammatory, analgesic, and antipyretic effects. As a selective COX-2 inhibitor, it is commonly prescribed for pain relief but faces challenges due to its classification as Class II under the Biopharmaceutical Classification System (BCS), which is attributed to its low and variable oral bioavailability from poor aqueous solubility. Additionally, its effectiveness in topical applications is limited by low skin permeability. To overcome these issues, a vesicular drug delivery system called transfersomes was developed to enhance etoricoxib delivery through the skin. These transfersomes were formulated using phosphatidylcholine (PC) and Tween 80 in a 90:10 ratio (in mmol), successfully improving etoricoxib permeability over time. The study concluded that the transfersome-entrapped etoricoxib gel exhibited significantly better permeation than a plain drug gel, making the transfersomal formulation a more effective system for transdermal delivery of etoricoxib, especially given the need for higher drug permeability and substantial dosing.

# Reference


1. Jain N.K, Advances in Controlled and Novel Drug Delivery, CBS Publishers and Distributers First edition, New Delhi, 2001, 426-451.
2. Vyas SP, Khar RK, Controlled drug delivery system. Pitampura, Delhi, Vallabh prakashan, 2005, 417-426.
3. Kumar P, Sankar C, and Mishra B, Delivery of macromolecules through skin, The Indian Pharmacist, 2004, 7-17.
4. Misra AN, Transdermal Drug Delivery In: N.K. Jain (eds.) Controlled and Novel Drug Delivery, New Delhi, CBS Publishers, 61, 1997, 100-129.
5. Wearner RR, Myers MC, Taylor DA, Electron Probe Analysis of Human Skin: Determination of the Water Concentration Profile, Journal of Investigative Dermatology, 90, 1998, 218-24.
6. Modi CD, Bharadia PD, Transfersomes: New Dominants for Transdermal Drug Delivery, Am. J. Pharm Tech Res, 2 (3), 2012, 71-91.

7. Pandey S, Goyani M, Devmurari V, Fakir J, Transfersomes: A Novel Approach for Transdermal Drug Delivery, Der Pharmacia Letter, 1 (2), 2009, 143-150.
8. Chein YW, Chi SL. Transdermal Drug Delivery System with Enhanced skin Permeability, In: Ping IL and William RG (eds), Controlled Released Technology-Pharmaceutical Application, Washington DC: American Chemical Society, 1987, 81-299.
9. Heather AE, Benson: Transdermal Drug Delivery: Penetration Enhancement Techniques, Current Drug Delivery, 2, 2005, 23-33.
10. Raymond CR, Paul J, Sian CO, Handbook of Pharmaceutical Excipients, 5th edition, Pharmaceutical Press Publishers, 2006, 545-550.
11. Zia-You Fang, Song Yih Yu, and Pao Chu Wu., International Journal ofPharmaceutics. 2001, 215, 91-99.
12. Manosroi, A., Wongtrakul, P., Manosroi, J. et.al., Biointerfaces. 2003, 30, 129-138.
13. Devraj, G.N., Parakh, S.R. Devraj, R. et.al., J. colloid Interface Sci. 2002, 251, 360-365.
14. Dodov, M.G., Simonoska, M. Goracinova, K., Bulletin of the chemists andtechnologists of Macedonia. 2005, 24(1), 59-65.
15. Jalon, G.E. Ygartua, P., Santoyo, S., J Control Release. 2001, 75, 191-197.
16. Marzulli, F.N., Brown, D.WC., Maibach, H.I., Appl. Pharmacol. Supplement. 1969, 3, 76-83.
17. Kaza, R. P., Current Drug Discovery Technologies. 2006, 3,279-285.
18. Shakeel, F., Babota, S., Ahuja, A., AAPS PharmaSciTech. 2007, 8(4), article 104