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### Research

## Formulation And Evaluation Of Torasemide Transfersomes Loaded Transdermal Patch

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|                                                                                                                                                       | <b>Abstract</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Published on: 20 Jun 2024                                                                                                                                                                                                               | <p>The objective of present investigation has been focused on formulation and characterization of Torasemide loaded transfersomal transdermal patch as an alternative delivery method for localized drug action to the targeted site. Torasemide was encapsulated into transfersome vesicle using different edge activators and characterized for particle size, vesicle morphology by scanning electron microscopy, entrapment efficiency and drug release. Later, the optimized F4 transfersome formulations were selected for patch preparation by solvent casting method. Transfersomal vesicles were found to be nanometric range [below 400 nm] with spherical structure. F4 formulation showed maximum drug release of 97.25 % and entrapment efficiency of 96.75 %. Based on the in vitro permeation studies, Torasemide loaded transfersomal patch was found to have greater drug permeation of Torasemide. The results obtained revealed that Torasemide in all the formulations was successfully entrapped with good uniformity and followed Peppas release kinetics model. This research work suggested that Torasemide loaded transfersomal transdermal patch can be a novel alternative approach to oral therapy in the treatment of high blood pressure and swelling due to fluid buildup.</p> |
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| 2024  All rights reserved.<br><br><a href="https://creativecommons.org/licenses/by/4.0/">Creative Commons Attribution 4.0 International License.</a> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                                                                                                                                                                                                                         | <b>Keywords:</b> Transdermal patches, Torasemide and transfersome.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

## INTRODUCTION

### Novel drug delivery system<sup>1</sup>

For many decades treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms including tablets, capsules, pills, suppositories, creams, ointments, liquids, aerosols and injectables, as drug delivery systems are the primary pharmaceutical products commonly seen in the market, even though these drug delivery system ensure a prompt release of drug, it is necessary to take this type of drug several times a day to achieve as well as to maintain the drug concentration with in the therapeutically effective range needed for the treatment. This results in significant fluctuations in drug level.

In the past two and a half decades several advancements have been made. They have resulted in the development of new techniques for drug delivery. These techniques are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and targeting the delivery of drug to a cell or tissue. Recently pulsatile drug delivery system is gaining importance. These advancements have led to the development of several novel drug delivery systems that could revolutionise the method of medication and provides a number of therapeutic benefits.

### Novel drug delivery system can be broadly divided into two classes<sup>2</sup>

#### Sustained Release Drug Delivery System

Sustained release drug delivery system is described as a pharmaceutical dosage form formulated to retard the release of a therapeutic agent such that its appearance in the systemic circulation is delayed and/or prolonged and its plasma profile is sustained in duration. The onset of its pharmacological action is often delayed and the duration of its therapeutic effect is sustained (e.g. coated granules).<sup>3</sup>

#### Controlled Release Drug Delivery System

Controlled release drug delivery system has a meaning that goes beyond the scope of sustained drug release. It implies a predictability and reproducibility in the drug release kinetics. The release of drug ingredients from a controlled release drug delivery system proceeds at a rate profile that is not only predictable kinetically but also reproducible from one unit to another.<sup>4</sup>

## MATERIALS AND METHODS

Toraseamide Provided by SURA LABS, Dilsukhnagar, Hyderabad. Soya lecithin Purchased from Loba Chemie, Mumbai. Tween 80 Purchased from SD Fine Chem Ltd., Mumbai. Span 80 Purchased from Merck Limited, Mumbai (India). Methanol Purchased from Himedia, Mumbai. Chloroform Purchased from SD Fine-Chem Limited, Mumbai. Eudragit L 100 Purchased from SD Fine-Chem Limited, Mumbai. Ethylcellulose Purchased from Loba Chemie Pvt Ltd. (Mumbai, India). Propylene glycol Purchased from SD Fine-Chem Limited, Mumbai. Dimethyl sulfoxide Purchased from Merck Limited, Mumbai (India).<sup>5</sup>

## Methodology

**Table 1: Formulation composition of Toraseamide Transfersomes**

| Ingredients (mg)         | F1  | F2  | F3  | F4  | F5  | F6  | F7  | F8  |
|--------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Toraseamide              | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  |
| Soya lecithin : Tween 80 | 1:1 | 1:2 | 1:3 | 1:4 | -   | -   | -   | -   |
| Soya lecithin : Span 80  | -   | -   | -   | -   | 1:1 | 1:2 | 1:3 | 1:4 |
| Methanol : Chloroform    | 1:2 | 1:2 | 1:2 | 1:2 | 1:2 | 1:2 | 1:2 | 1:2 |

## RESULTS AND DISCUSSION

Initially the drug was tested by UV to know their significant absorption maximum which can be used for the diffusion study of the drug.

### Analysis of drug

#### UV scan

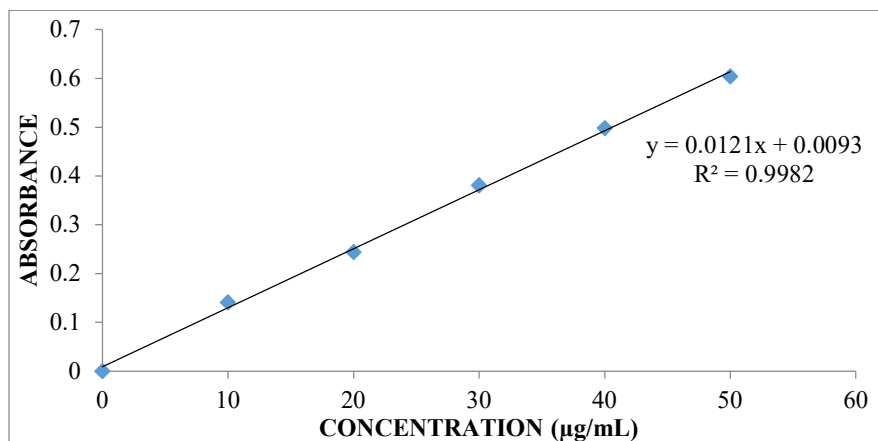
The lambda max of Toraseamide was found to be 289 nm.

### Construction of calibration curve

**Table 2: Standard graph of Toraseamide**

| Concentration<br>(µg/ml) | Absorbance<br>(at 289 nm) |
|--------------------------|---------------------------|
| 0                        | 0                         |
| 10                       | 0.141                     |
| 20                       | 0.244                     |
| 30                       | 0.381                     |

|    |       |
|----|-------|
| 40 | 0.498 |
| 50 | 0.604 |



**Fig 1: Standard calibration curve of Torasemide**

Standard graph of Torasemide was plotted as per the procedure in experimental method and its linearity is shown in Table and Fig. The standard graph of Torasemide showed good linearity with  $R^2$  of 0.998, which indicates that it obeys “Beer- Lamberts” law.

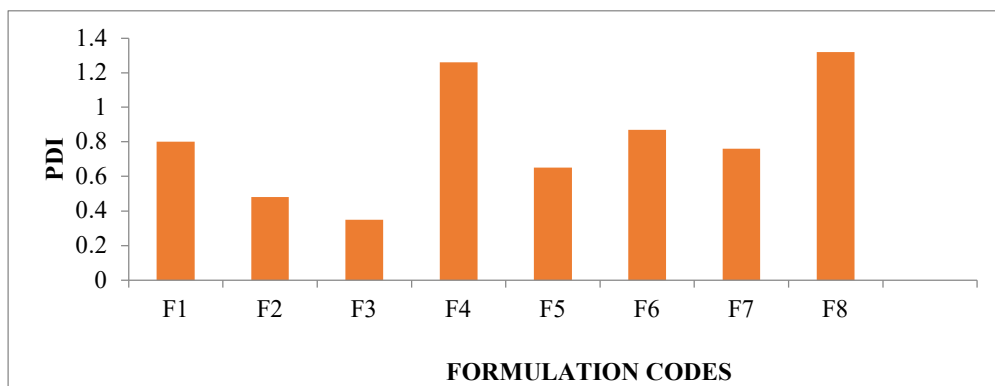
#### Characterization of Transfersomes

**Table 3: Percentage yield, Drug Content, Entrapment Efficiency of all Transfersomes formulations**

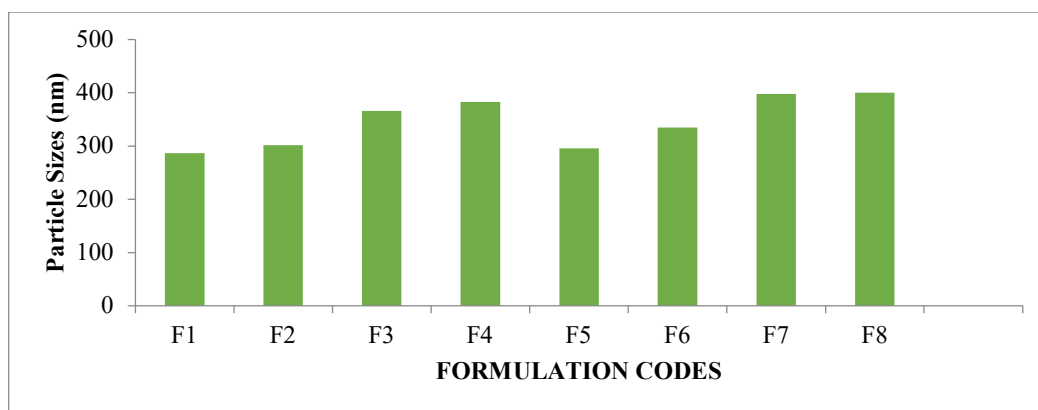
| Formulation | PDI  | Particle Sizes | Zeta Potential   | Entrapment Efficiency |
|-------------|------|----------------|------------------|-----------------------|
| F1          | 0.80 | 286.12         | $0.565 \pm 0.37$ | 80.14                 |
| F2          | 0.48 | 301.64         | $0.660 \pm 0.38$ | 92.36                 |
| F3          | 0.35 | 365.92         | $0.672 \pm 0.37$ | 95.67                 |
| F4          | 1.26 | 382.76         | $0.678 \pm 0.32$ | 96.75                 |
| F5          | 0.65 | 295.17         | $0.563 \pm 0.34$ | 75.92                 |
| F6          | 0.87 | 334.87         | $0.573 \pm 0.35$ | 86.24                 |
| F7          | 0.76 | 397.34         | $0.591 \pm 0.41$ | 90.47                 |
| F8          | 1.32 | 400.51         | $0.605 \pm 0.27$ | 92.64                 |

The particle size of transfersome was found to be in the range of 286.12 to 382.76 nm with Soya lecithin: Tween 80 whereas for Soya lecithin: Span 80 it was in the range of 295.17 to 400.51 nm. Entrapment efficiency of Torasemide loaded transfersome formulations was found to be in range of 75.92 % to 96.75 % as shown in Table. The concentration and type of edge activator used has a very crucial effect on entrapment efficiency.

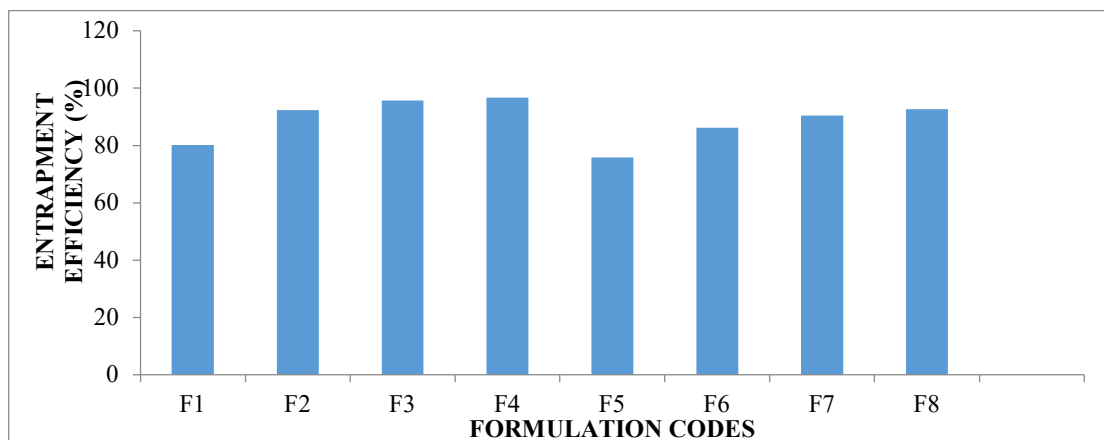
Zeta potential results reveal that Soya lecithin: Tween 80 transfersomes possess negative charge at pH 6.8 indicating that a weak electrostatic repulsive force exists in niosomal bilayer. Also, the inclusion Tween 80 transfersomes found to have increased the zeta potential. Particles with zetapotential close to zero have been found less phagocyttable in comparison with charged particles. The nature and density of charge on the surface of transfersomes influence the extent of biodistribution as well as interaction and uptake of transfersomes by target cells. F4 formulation highest zeta potential and it had good stability. Vesicle morphology Transfersome with Soya lecithin: Tween 80 was selected as optimal carrier owing to smaller particle size, good entrapment efficiency and maximum elasticity and hence used for SEM analysis. Shape and surface morphology of transfersome formulation was studied using scanning electron microscopy at various magnifications as shown in fig. 5. The drug loaded transfersome formulation was found to be smooth, spherical in shape with sharp boundaries having internal aqueous space.



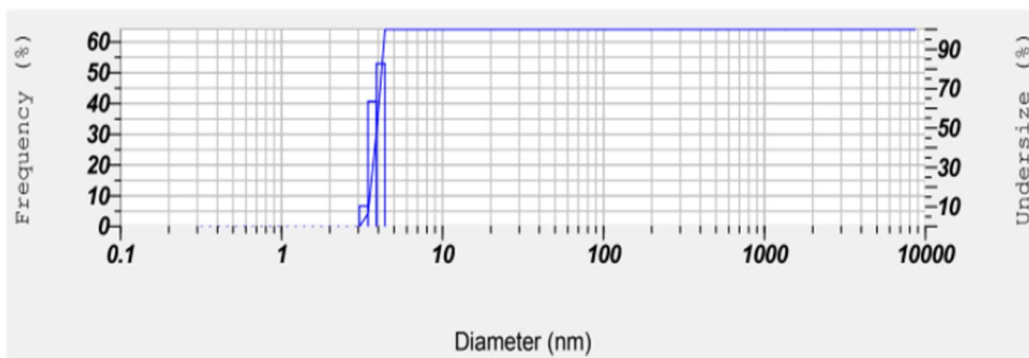
**Fig 2: PDI of all Transfersomes formulations**



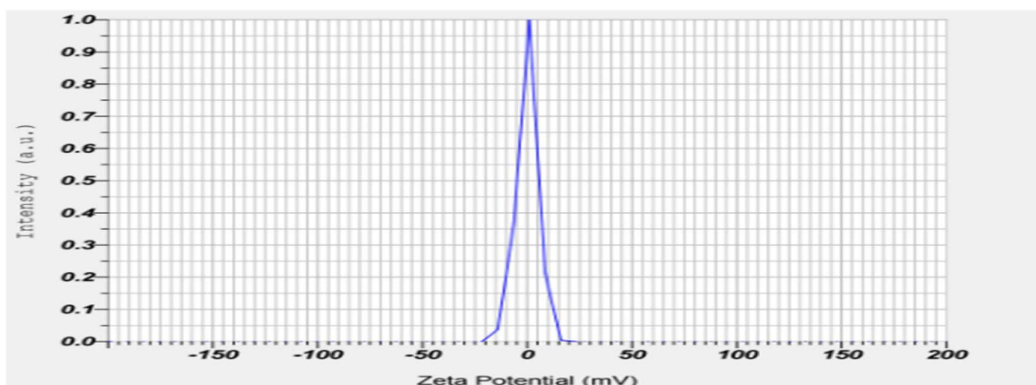
**Fig 3: Particle Sizes of all Transfersomes formulations**



**Fig 4: Entrapment efficiency of all Transfersomes formulations**



**Fig 5: Particle size of F4 Formulation**



**Fig 6: Zeta potential of F4 Formulation**

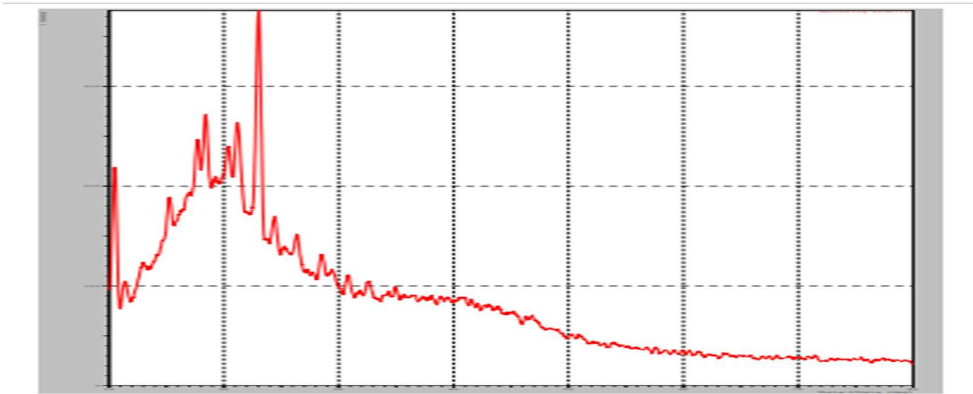
## SEM



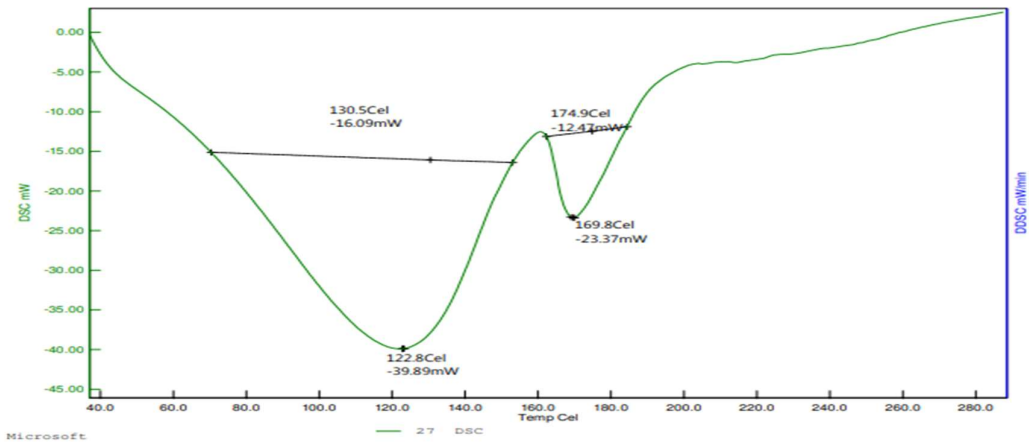
**Fig 7: Torasemide Transfersomes optimised formulation (F4)**

SEM studies showed that the Torasemide- loaded Transfersomes had a spherical shape with a smooth surface as shown in Figure.

The transdermal patch was prepared using solvent casting method from the optimised Transfersomes formulation F4 formulation. The prepared optimised Transfersomes F4 formulation was loaded into the patch formulation. The formulations of Transfersomes transdermal patches are shown in methodology table.



Graph 1: XRD graph of optimised formulations

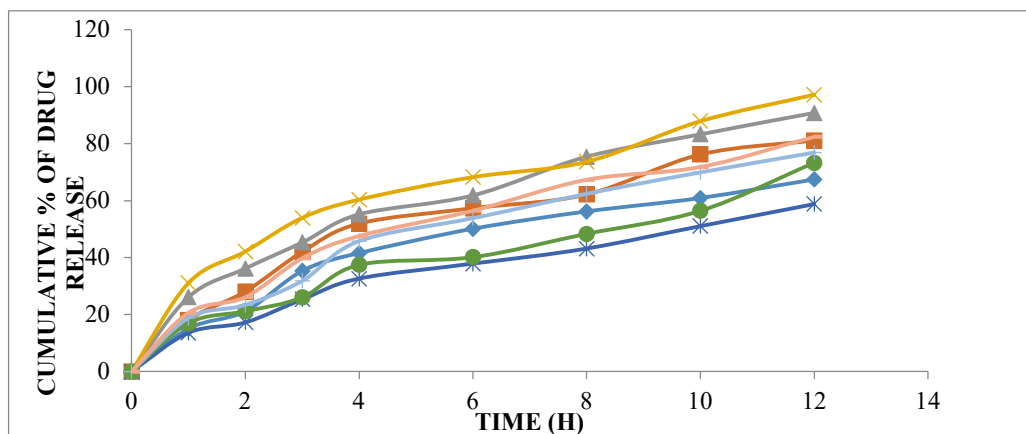


Graph 2: DSC graph of pure drug

% Drug release

Table 4: In vitro dissolution studies of F1-F8 Transfersomes formulations in percentage

| Time (hour) | F1    | F2    | F3    | F4    | F5    | F6    | F7    | F8    |
|-------------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0           | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
| 1           | 15.07 | 18.04 | 26.13 | 31.08 | 13.61 | 16.98 | 18.80 | 20.61 |
| 2           | 21.14 | 28.11 | 36.20 | 42.14 | 17.25 | 21.16 | 23.35 | 26.15 |
| 3           | 35.36 | 42.00 | 45.33 | 53.93 | 25.32 | 26.05 | 31.86 | 39.77 |
| 4           | 41.55 | 51.95 | 55.16 | 60.32 | 32.64 | 37.43 | 45.81 | 47.53 |
| 6           | 50.09 | 57.36 | 61.86 | 68.26 | 37.86 | 40.17 | 53.82 | 56.48 |
| 8           | 56.17 | 62.14 | 75.36 | 73.66 | 43.17 | 48.35 | 62.37 | 67.27 |
| 10          | 60.93 | 76.23 | 83.24 | 87.92 | 51.05 | 56.42 | 69.96 | 71.79 |
| 12          | 67.49 | 81.11 | 90.79 | 97.25 | 58.82 | 73.29 | 76.87 | 82.42 |



**Fig 8: In vitro dissolution studies of F1-F8 Transfersomes formulations**

In vitro studies of selected formulations were carried out in PBS pH 6.8 and results were shown in Figure. It was clearly observed from the data as shown in Figure that in vitro drug release of Transfersomes containing Tween 80 of different quantities was sharply increased up to 12 hr. Maximum drug release that is, 97.25%, was reported in case of Transfersomes containing 1:4 ratio Transfersomes as shown in Figure. F4 formulation considered as optimised formulation (97.25%).

#### Evaluation of Patch

The formulations F4TP1 to F4TP4 were varying in thickness when compared to other formulations which is due to the variation in the polymer concentration. Which shows the increase in polymer concentration increases the thickness of patch. For all other formulations it was found to be in between  $0.050 \pm 0.003$  to  $0.061 \pm 0.003$  mm. Folding endurance from formulations F4TP1 to F4TP4 was found to be in between  $74 \pm 1.57$  to  $87 \pm 1.69$  which can withstand the folding of the skin. All formulations showed % drug content from 96.26 to 98.20.

**Table 5: Evaluation of Transfersomal transdermal patches**

| Formulation Code | Thickness (mm)    | Folding endurance | Flatness (%) | Appearance                                | % Drug Content   |
|------------------|-------------------|-------------------|--------------|-------------------------------------------|------------------|
| F4TP1            | $0.050 \pm 0.003$ | $85 \pm 1.69$     | 98           | Transparent, Smooth, uniform and flexible | $96.14 \pm 0.96$ |
| F4TP2            | $0.053 \pm 0.005$ | $76 \pm 0.35$     | 100          | Transparent, Smooth, uniform and flexible | $95.89 \pm 1.75$ |
| F4TP3            | $0.057 \pm 0.001$ | $82 \pm 0.62$     | 98           | Transparent, Smooth, uniform and flexible | $97.11 \pm 2.32$ |
| F4TP4            | $0.061 \pm 0.003$ | $87 \pm 1.57$     | 99           | Transparent, Smooth, uniform and flexible | $98.01 \pm 0.21$ |

**Table 6: Evaluation of Transfersomal transdermal patches**

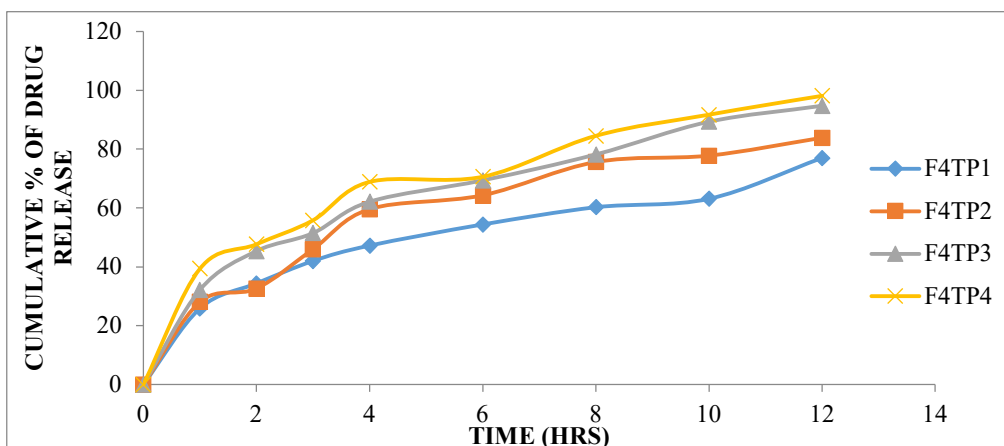
| Formulation Code | Weight Variation (gm) | %Moisture Content |
|------------------|-----------------------|-------------------|
| F4TP1            | $2.54 \pm 0.2$        | $9.36 \pm 0.06$   |
| F4TP2            | $2.61 \pm 2.5$        | $8.98 \pm 0.04$   |
| F4TP3            | $3.58 \pm 0.1$        | $9.15 \pm 0.11$   |
| F4TP4            | $2.69 \pm 2.3$        | $8.01 \pm 0.03$   |

#### In vitro diffusion study

All the formulation in vitro diffusion study was carried out by using Franz type diffusion cell under specific condition such as temp maintained at  $37 \pm 0.5^\circ\text{C}$ . The diffusion was carried out for 12 hr and 5 ml sample was withdrawn at an interval of every 1 hr.

**Table 7: In vitro drug permeation of Torasemide containing different concentrations**

| Time(hr) | F4TP1 | F4TP2 | F4TP3 | F4TP4 |
|----------|-------|-------|-------|-------|
| 0        | 0     | 0     | 0     | 0     |
| 1        | 25.86 | 28.09 | 32.16 | 39.42 |
| 2        | 34.34 | 32.60 | 45.34 | 47.71 |
| 3        | 41.99 | 45.98 | 51.49 | 55.86 |
| 4        | 47.20 | 59.59 | 62.14 | 68.90 |
| 6        | 54.39 | 64.32 | 69.39 | 70.61 |
| 8        | 60.26 | 75.64 | 78.20 | 84.55 |
| 10       | 63.15 | 77.81 | 89.31 | 91.74 |
| 12       | 77.01 | 83.83 | 94.78 | 98.17 |

**Fig 9: In vitro drug permeated studies of Transfersomal transdermal patch formulations**

The in vitro release studies of various formulations of Torasemide loaded Transfersomal transdermal patch was also done for 12 h in phosphate buffer pH 6.8. A constant temperature and pressure conditions were maintained throughout the experiment. The in vitro drug release studies of Torasemide Transfersomal transdermal patches at different time intervals are shown in fig. The drug release was found to be F4NT4 formulation with the highest release of 98.17% thus regarded as the optimised formulation. The release was initially found to be burst followed by the gradual sustained increase in free drug concentration.

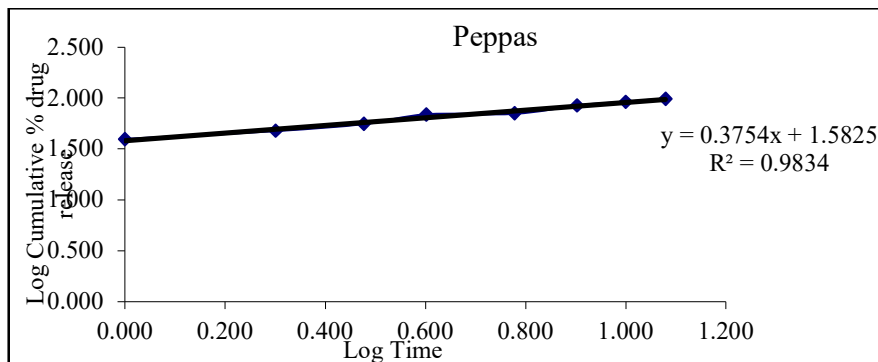
#### Release Kinetics

To analyze the drug release mechanism the in vitro release was fitted into various release equations and kinetic models first order, zero order, Higuchi and Korsmeyer-peppas. The release kinetics of optimized formulation is shown in Table and in following Figures.

**Table 8: Release kinetics of optimised formulation**

| CUMULATIVE<br>(%) RELEASE Q | TIME (T) | ROOT (T) | LOG(%)<br>RELEASE | LOG (T) | LOG (%)<br>REMAIN | RELEASE RATE<br>(CUMULATIVE<br>% RELEASE / t) | 1/CUM%<br>RELEASE | PEPPAS log<br>Q/100 | % Drug<br>Remaining | Q01/3 | Qt1/3 | Q01/3-Qt1/3 |
|-----------------------------|----------|----------|-------------------|---------|-------------------|-----------------------------------------------|-------------------|---------------------|---------------------|-------|-------|-------------|
| 0                           | 0        | 0        |                   |         | 2.000             |                                               |                   |                     | 100                 | 4.642 | 4.642 | 0.000       |
| 39.42                       | 1        | 1.000    | 1.596             | 0.000   | 1.782             | 39.420                                        | 0.0254            | -0.404              | 60.58               | 4.642 | 3.927 | 0.714       |
| 47.71                       | 2        | 1.414    | 1.679             | 0.301   | 1.718             | 23.855                                        | 0.0210            | -0.321              | 52.29               | 4.642 | 3.739 | 0.902       |
| 55.86                       | 3        | 1.732    | 1.747             | 0.477   | 1.645             | 18.620                                        | 0.0179            | -0.253              | 44.14               | 4.642 | 3.534 | 1.108       |

|       |    |       |       |       |       |        |        |        |       |       |       |       |
|-------|----|-------|-------|-------|-------|--------|--------|--------|-------|-------|-------|-------|
| 68.9  | 4  | 2.000 | 1.838 | 0.602 | 1.493 | 17.225 | 0.0145 | -0.162 | 31.1  | 4.642 | 3.145 | 1.497 |
| 70.61 | 6  | 2.449 | 1.849 | 0.778 | 1.468 | 11.768 | 0.0142 | -0.151 | 29.39 | 4.642 | 3.086 | 1.556 |
| 84.55 | 8  | 2.828 | 1.927 | 0.903 | 1.189 | 10.569 | 0.0118 | -0.073 | 15.45 | 4.642 | 2.491 | 2.151 |
| 91.74 | 10 | 3.162 | 1.963 | 1.000 | 0.917 | 9.174  | 0.0109 | -0.037 | 8.26  | 4.642 | 2.021 | 2.620 |
| 98.17 | 12 | 3.464 | 1.992 | 1.079 | 0.262 | 8.181  | 0.0102 | -0.008 | 1.83  | 4.642 | 1.223 | 3.418 |

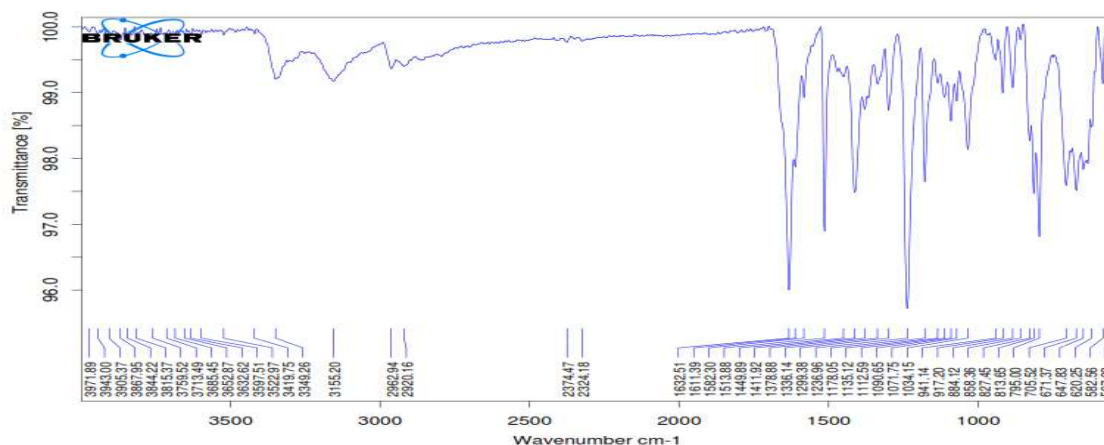


**Fig 10: Peppas release kinetics**

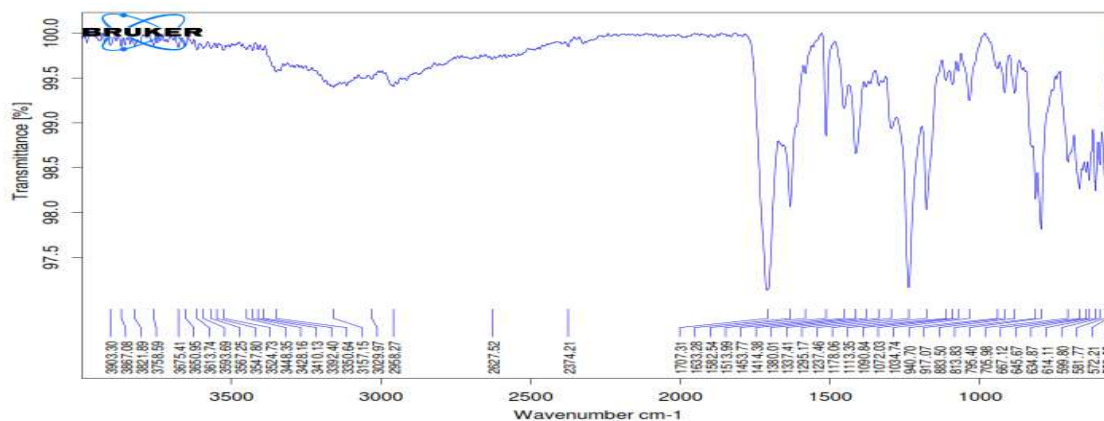
The prepared optimised Transfersomal transdermal patch was subjected to the drug release kinetics and release mechanism. The formulation were studied by fitting the drug release time profile with the various equations such as Zero order, First order, Higuchi and Korsmeyer pappas. The data revealed a better fit to the Peppas release kinetics.

#### FT-IR

FTIR spectra of the drugs and the optimised formulation were recorded. The FTIR spectra of pure Torasemide drug, optimised formulation shown in the below figures respectively. Drugs are also present in the physical mixture, which indicates that there is no interaction between drug and the polymers, which confirms the stability of the drug. There was no disappearance of any characteristics peak in the FTIR spectrum of drug and the polymers used. This shows that there is no chemical interaction between the drug and the polymers used. The presence of peaks at the expected range confirms that the materials taken for the study are genuine and there were no possible interactions.



**Graph 3: FT-IR graph of Torasemide**



Graph 4: FT-IR graph of optimised formulations

## CONCLUSION

The objective of the present study was to develop transdermal matrix patch of Torasemide and assess its feasibility for transdermal application. Torasemide is a diuretic medication used in the treatment of high blood pressure and swelling due to fluid buildup. Low dose maintenance therapy of Torasemide has the capability to reduce potential side effects and improved patient compliance which are more common with conventional drug delivery. Torasemide loaded Transfersomes were prepared using a surfactant (Tween 80 and span 80). showed the best entrapment efficiency and percent drug released within 12 h. F4 showed better entrapment efficiency as of 96.75%, minimal mean vesicular diameter as of 382.76  $\mu\text{m}$ , and zeta potential as of  $0.678 \pm 0.32\text{mV}$ . Maximum drug release, that is, 97.25%, was reported in the F3 formulation. F4 formulation considered as optimised formulation. Among the formulations prepared, Torasemide loaded Transfersomes (F4) and Torasemide loaded Transfersomal transdermal patch (F4TP4) was fixed to be optimised formulations, which had maximum drug release of 97.17%. The optimised Transfersomal transdermal patch (F4NT4) revealed a better fitted to first order release kinetics. Thus formulated Torasemide loaded Transfersomal transdermal patch represents to be an efficient and stable vesicular carrier for the transdermal delivery of an diuretic drug like Torasemide.

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