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Review Article

METHOTREXATE TRANSDERMAL PATCH: A NOVEL APPROACH FOR SUSTAINED DRUG DELIVERY IN RHEUMATOID ARTHRITIS

Akash S*, Asha Jyothi P

KLR Pharmacy College, Paloncha, Bhadradri Kothagudem, Telangana- 507511, India.

ABSTRACT

A transdermal patch containing methotrexate for the treatment of rheumatoid arthritis (RA) is developed and evaluated in this study. Despite the fact that methotrexate is one of the cornerstones of RA therapy, it is usually administered frequently, which can lead to systemic side effects and poor patient compliance with the medication. In addition to minimizing gastrointestinal side effects and improving therapeutic outcomes, transdermal delivery systems offer an innovative approach to administering methotrexate. There were a variety of formulations used in the development of the methotrexate transdermal patch, including polymers and plasticizers, as well as permeation enhancers to enhance drug release and skin penetration. A comfortable wearing experience was ensured by the formulation's favorable mechanical properties, such as adequate tensile strength and flexibility. Methotrexate released controlled amounts in vitro, suggesting sustained therapeutic effect. The patch penetrated more effectively than conventional topical formulations when applied to human cadaver skin. A transdermal patch targeting methotrexate is an effective approach to treating rheumatoid arthritis with sustained delivery of methotrexate. Transdermal systems allow controlled and targeted drug release, which can reduce dosing frequency, improve patient compliance, and minimize side effects.

Keywords: Rheumatoid arthritis, Joint Inflammation, Methotrexate, Transdermal Patches.

INTRODUCTION

In Rheumatoid arthritis, joints are most frequently affected by a congestive, systemic autoimmune disease [1]. The disease is characterized by joint inflammation, cartilage and bone destruction, and decreased mobility, as well as teratogenicity. Chinese rheumatoid arthritis sufferers have fallen in recent years, but it remains a chronic illness that mostly affects the middle-aged and elderly [2]. The dosage form of RA treatments can sometimes cause adverse side effects, including side effects. In general, patients who take this medication orally experience nausea, vomiting, and diarrhea as a result of its use. It leaves the body very quickly after injection of methotrexate. It is also more challenging to self-administer medications for patients who adhere to their medication regimen. It is estimated that 30% of patients discontinue treatment due to the side effects of methotrexate (MTX). RA can be treated more effectively through the development of an appropriate drug delivery system, leading to a higher recovery rate. The reduced negative effects on the system, improved bioavailability, and greater compliance of transdermal drug delivery systems (TDDSs) makes them highly promising for treating RA [3].

There is no known cause for RA, but genetics, gender, and environment may have a significant impact. There are two subregions of the major histocompatibility complex (MHC) associated with RA susceptibility: HLA-A (B, C) and HLA-DR (DP, DQ). There are three times as many RA cases among females as among males, due to a lack of sex hormones. A woman's risk of developing RA and worsening its symptoms increases after

Corresponding Author

Akash S

Email : akashsrinivaspvg@gmail.com

menopause and postpartum when estrogen levels decrease. The use of female sex hormones as a treatment for RA has never been successful, but the involvement of male sex hormones in RA's pathogenesis cannot be denied [4]. It is possible to develop RA as a result of both microbial infections and environmental triggers. The intestinal microbiota plays a significant role in both preclinical and postclinical aspects of RA immune dysfunction. It is still unclear what triggers RA, but we do know that intestinal flora dysregulation leads to mucosal damage and proinflammatory metabolites.

Rheumatoid Arthritis Pathogenesis

There is inflammation in the joints associated with rheumatoid arthritis. A synovium that is irritated, macrophages, and plasma cells proliferate are characteristics of rheumatoid arthritis. The pro-inflammatory effects of cytokines, tumor necrosis factor, and interleukins can be attributed to their interaction as mediators. The ability to function normally, reduce pain, and reduce inflammation can be maintained [5].

Current rheumatoid arthritis treatments include NSAIDs, glucocorticoids, DMARDs, and biological antirheumatics. A glucocorticoid, however, prevents joint destruction, unlike an NSAID, which is an analgesic and anti-inflammatory. As a result of clinical trials, it has been demonstrated that DMARDs and biological antirheumatic drugs can prevent joint damage in RA patients [6].

METHODOLOGY

Preformulation study:

Organoleptic Characteristics of Methotrexate

Physical examine was done to check Organoleptic Characteristics of Methotrexate like colour and odour

Solubility study

The standard procedure for solubilizing Methotrexate involves the use of a variety of solvents.

Standard calibration curve graph of Methotrexate

10 ml of ethanol was dissolved in 100 mg of methotrexate, and the volume was filled up with pH 7.4 phosphate buffer. 10ml of this solution was taken and made up into 100ml of pH 7.4 phosphate buffer. From this stock solution (100 µg/ml), a series of concentrations of 2, 4, 6, 8, 10, 12 µg/ml were prepared, and the samples were scanned with a UV-spectrophotometer. It was determined that the wavelength was 303 nm at its maximum. A UV spectrophotometer was used to measure absorbance at 303 nm, and the graph was plotted with concentration on the X axis and absorbance on the Y axis.

Formulation of transfersomes

Selection of method of preparation

It was determined that the method to be used for further studies had to be able to handle the maximum dosage level and the smallest particle sizes.

Ethanol Injection Method

Phospholipids, edge activator, and lipophilic drug are dissolved in ethanol and stirred magnetically until a clear solution is obtained, which is the organic phase. The phosphate buffer dissolves the water-soluble substances to form the aqueous phase. It is possible to incorporate hydrophilic drugs.

During the heating process, both solutions are heated to a temperature of 45-50°C. The ethanolic phospholipid solution is then injected dropwise into the aqueous solution with continuous stirring for the designated period of time, followed by the injection of the ethanolic phospholipid solution.

To remove ethanol, the resultant dispersion is transferred into a vacuum evaporator and then sonicated for particle size reduction in order to remove the ethanol.

Other methods

High-Pressure Homogenization Technique

Following ultrasonic shaking and simultaneous stirring of the phospholipids, edge activator and drug, they are uniformly dispersed in PBS or distilled water containing alcohol.

Intermittent ultrasonic shaking is then applied to the mixture. A high-pressure homogenizer is then used to homogenize the resulting mixture. A suitable storage environment is then provided for the transfersomes.

Centrifugation Process

A solution of phospholipids, edge activator and lipophilic drug is prepared by dissolving them in an organic solvent. The solvent is then removed by means of a rotary evaporator operating under reduced pressure and at the desired temperature, in order to remove the solvent. It is then vacuumed under high pressure in order to remove any remaining solvent traces. The lipid film, which has been deposited, is hydrated with the appropriate buffer solution by centrifuging it at room temperature for a short period of time. During this stage, it is possible to incorporate the hydrophilic drug into the formulation.

A swollen vesicle is the result when vesicles are exposed to room temperature. It is then necessary to further sonicate at room temperature the obtained multilamellar lipid vesicles to obtain the final product.

Characterization of Transpersonal Formulation

Particle Size and Polydispersity Index

Using a computerized zeta sizer instrument, we were able to measure the particle size and poly dispersity

index of the transferosome formulation at 25°C. A zeta sizer cell is used to measure the size of the particles in a transfersomal formulation.

Zeta Potential

The electrophoretic mobility of transfersomal dispersions, which is measured using a zeta sizer, was analyzed and the values obtained using the zeta sizer. There were no significant differences between the zeta potential values and the standard values. At a temperature of 25°C, the Zeta potential was measured using a Zeta sizer. The samples were kept in a polystyrene cuvette, and the zeta potential of the samples was determined by using a zeta dip cell. If the zeta potential of the formulations is >0 or <30 mv, it is assumed that the formulations are stable without any aggregation.

It was necessary to clean the zeta sizer capillary cell with 90% ethanol and distilled water before the analysis was performed. The measurement was conducted at a temperature of 25°C after a suitable dilution with distilled water was applied. There was a minimum of three repetitions of all measurements and the zeta potential was calculated by taking the mean ±SD.

Entrapment Efficiency

A centrifugation method was used to estimate the entrapment efficiency of transfersomal suspensions. The transfersome suspension has been taken and placed in a centrifuge tube where it is centrifuged at 15,000 rpm for 45 minutes in order to extract the transfersomes. The supernatant was collected and diluted with phosphate buffer 7.4 to obtain a concentrated solution. Using a UV spectrophotometer at 303 nanometers, the solution was analyzed using UV spectrophotometer. As a percentage of the drug that is entrapped, entrapment efficiency is determined.

$$\% \text{ Entrapment efficiency } \frac{\text{Total drug} - \text{unentrapped}}{\text{Total Drug}} \text{X } 100$$

Drug Content

A clear solution was obtained by sonicating the transfersomal formulation with methanol for 20 minutes and filtering to determine the drug content. An UV-based 303-nm analysis was performed on the filtrate to determine its drug content.

Invitro Drug Release Studies

A dialysis membrane was used to determine the in vitro release of Methotrexate bearing transfersomes. Approximately 8 mg of transferosome suspension is filled in dialysis membranes, which are then sealed with clamps and immersed in a beaker containing 200 ml of phosphate buffer (pH 7.4). The mixture is maintained at 32o±2oC and is constantly stirred at 100 rotations per minute.

A 2.5 ml sample was removed from the receptor compartment at predetermined intervals (1,2,3,4,5,6,7,8,9,10,11,12,24 hours) and immediately replaced with fresh buffer to maintain sink conditions throughout the experiment. Analyzing samples with a UV spectrophotometer at 303 nm was carried out. As a result of the results, the time on the X-axis and the cumulative % of drug released on the Y-axis were plotted on the graph.

Formulation of Patch

Preparation of Transdermal Patch

Solvent evaporation method is used to create transdermal patches. An ethanol solution of hydroxy propyl methyl cellulose was stirred at a magnetic stirrer until it reached a semisolid consistency. This solvent is continuously stirred while the drug is suspended in transfersomal suspension. As a plasticizer, glycerol should be added drop by drop as a plasticizer to the mixture. In the next step, it will be poured into the petridish, which will then be covered with the inverted funnel and left to dry for 24 hrs for it to become a patch. The patches were removed after 24 hours by using a sharp knife by inserting it along the edge of the patch and storing them for future investigations.

Evaluation of transdermal patch

Physical appearance

The patches that have been prepared were visually inspected to determine their colour, clarity, flexibility, and smoothness.

Thickness and Weight Variation

Using micrometer screw gauze, three different points of the patches were measured in order to determine their thickness. It was determined that the average weight of three patches was obtained by weighing them individually and calculating their average weight.

Folding Endurance

There were several times where the patch was folded in the same place until it broke. A film's folding endurance is determined by how many times it can be folded at the same place without breaking.

Percentage Moisture Content

It was decided to weigh each prepared patch individually before storing them in a dessicator containing fused calcium chloride at room temperature for a 24-hour period. A new weighing was performed after 24 hours, and the moisture content of each patch was determined by determining its percentage.

$$\% \textit{ Moisture content} = \frac{\text{Initial weight} - \text{ Final weight}}{\text{Initial weight}} \times 100$$

Percentage Moisture Uptake

The weighed patches were stored at room temperature for 24 hours in a desiccator containing saturated solution of potassium chloride in order to maintain an RH of 84% throughout this period of time. We reweighed the patches after 24 hours, and the percentage of moisture that was absorbed by the patches was determined after 24 hours.

$$\%\ Moisture\ content = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Drug Content

After 24 hours the UV spectrophotometer was used to measure the absorbance of a small circular patch of skin that was cut out and immersed in 100ml of PBS pH 7.4 for 24 hours.

Flatness

The flatness test was conducted by measuring the uniformity of the flatness by using a circular patch that was prepared and had been divided into three longitudinal strips for the purpose of measuring the flatness.

Invitro Drug Release Study of Patch

An in vitro drug release study of the patch is performed using an open-ended tube to measure the release of the drug. There is a pair of ties attached to either end of the tube, which will be tied to the dialysis membrane, and the prepared transdermal patch will be placed over the membrane. With the help of a magnetic bead, the receiver medium is filled with the freshly prepared phosphate buffer pH7.4 and stirred to ensure that it is uniform with a magnetic bead to ensure uniformity. A temperature of 32°C should be maintained in the medium.

The sink condition is maintained by withdrawing 2.5 ml of medium at a predetermined rate and adding equal volumes of fresh phosphate buffer. After evaluating the samples, the absorbance is determined by using a UV spectrophotometer in order to calculate the cumulative percentage of drug release from the patch [7 - 13].

Table 1: Particle size and Polydispersity index

Formulation code	Particle Size (nm)	Polydispersity Index
F7	236.6	0.599

Table 2. Zeta Potential

Formulation Code	Zeta potential
F7	-14.1

Table 3: Formulation of Patch

S. No	Ingredients	Quantity
1	HPMC	300mg
2	PEG-400	150mg
3	Ethanol	Q.S
4	Glycerol	0.5 ml
5	Drug loaded transferosomes	3.5 ml

Table 4: Thickness of Patch

S. No	Patch Area	Thickness
1	1	0.5 mm
2	2	0.4mm
3	3	0.5mm

Table 5: Drug content.

DRUG CONTENT		
Actual yield	**Theoretical yield**	**Drug content**
7.21	8	90.12%

Figure 1: Pathogenesis and pathways of rheumatoid arthritis.

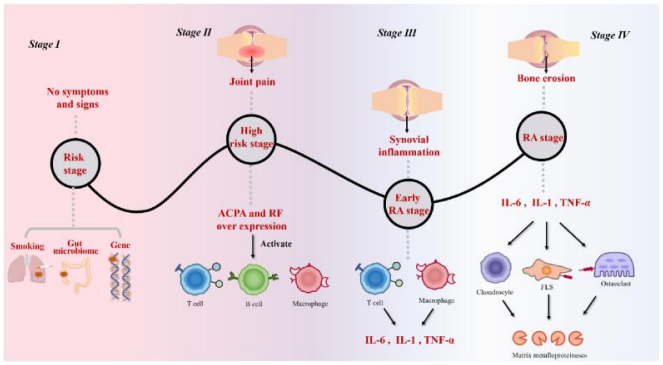


Figure 2: Methotrexate transdermal patch

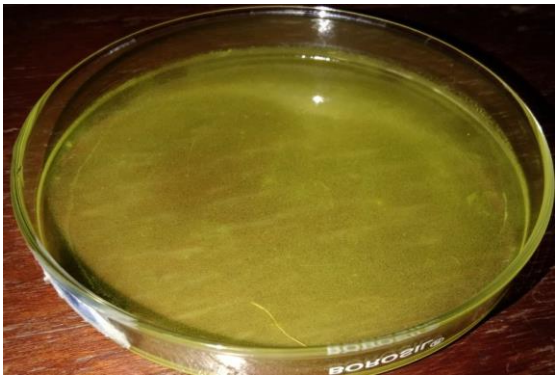


Figure 3: Calibration curve of Methotrexate

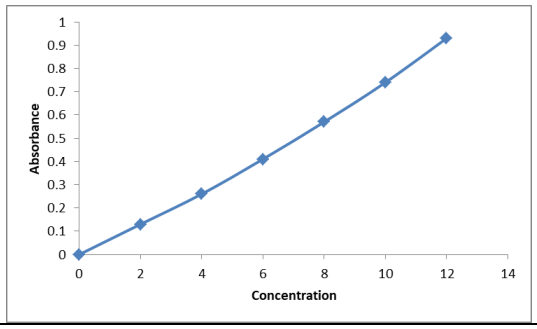


Figure 4: Entrapment Efficiency

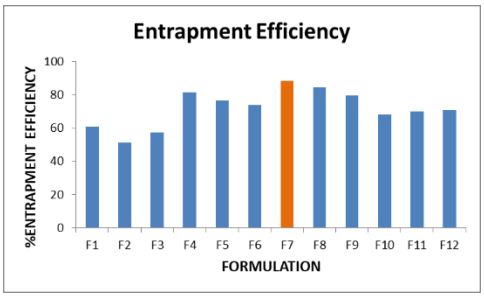


Figure 5: Drug content (%)

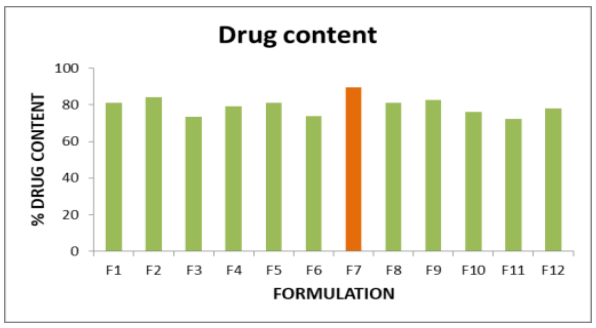


Figure 6: In vitro Drug release of Transferosome

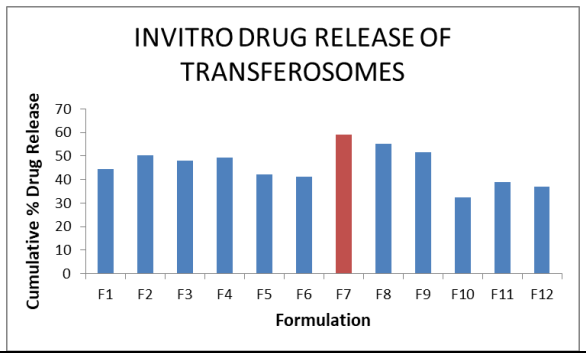
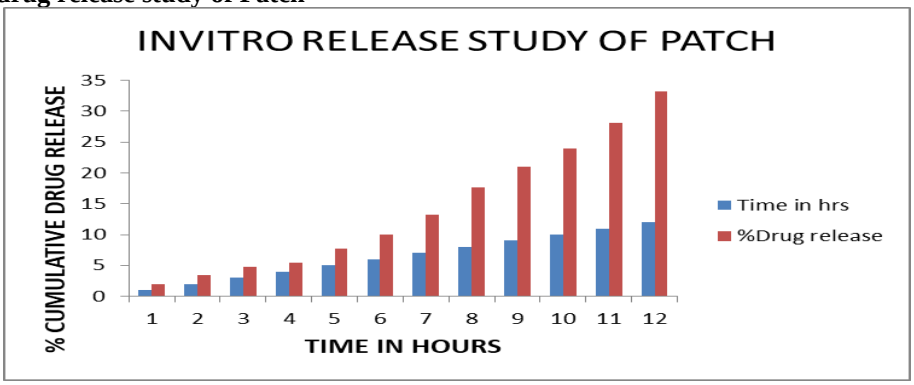


Figure 7: In vitro drug release study of Patch



RESULTS AND DISCUSSION

Determination of Wavelength

The λ max concentration of Methotrexate was determined by scanning a solution containing the concentration (10 µg/ml) of the drug in phosphate buffer pH 7.4 with a UV spectrophotometer in the wavelength range 200-800 nm and obtaining the maximum value of the drug.

Preparation of Calibration Curve

A calibration curve for Methotrexate was prepared by accurately weighing 100mg of pure drug, dissolving it in ethanol for 15ml, and making up to 100ml with phosphate buffer pH 7.4, stock- A. 10ml of stock- A were taken and made into 100ml with stock- B's phosphate buffer pH 7.4.

A regression value of 0.9995 was determined based on these dilutions using phosphate buffer pH 7.4 and 2, 4, 6, 8, 10, 12 µg/ml were made accordingly. Calibration values are shown in the image is shown in figure 3.

Solubility Studies

Methotrexate was actually studied in a variety of solvents to determine its solubility, and it was found that the solubility of this drug was much better in ethanol than it was in any other solvent.

Particle Size and Polydispersity Index

A computerized zeta sizer instrument was used to measure the particle size and the polydispersity index of transferosomes using dynamic light scattering technique by utilizing a computerized zeta sizer instrument. It is filling the transfersomal formulation in the zeta sizer call and measuring the size of the transfersomal formulation.

There is a range of particle sizes within the nanometric range of the formulation. In the optimized batch, the average particle size of the particles was found to be 236.6 nm and the PDI was found to be 0.599. According to table 1, the results were as follows.

Zeta Potential

Zeta sizer was used to analyze the lectrophoretic mobility (zeta potential) of transfersomal dispersions. Values within the standard range of zeta potential. Surfactants are used as edge activators, while soya lecithin is used as phospholipids to create neutral charge on the particles. A vesicular formulation's zeta potential indicates its good stability. Formulations with zeta potentials over or above 30mv are assumed to be stable without aggregation. A higher charge on the surface produced a repulsive force between the vesicles, allowing them to be stable and devoid of aggregation. The values obtained here indicate that the formulation does not cause aggregation due to the use of phospholipids and surfactants. As a result, formulation (F7) appeared to be more stable than other formulations with a -14.1 mV voltage. In table 2, we can see the results.

Entrapment Efficiency

Entrapment efficiency of a formulation is a good indicator of the characteristics of its drug, phospholipids, and surfactants. It is identified in refrigerator centrifuge-prepared formulations of transfersomes. Transfersomes were centrifuged for 1 hour at 15000 rpm. Separated supernatants containing unentrapped drug were measured by UV spectrophotometer using the 303 nm λmax wavelength. All batches had an entrapped efficiency ranging from 51.02% to 88.32%. Among all the entrapment methods, F7 showed the highest efficiency. The results, while the graphical representation of them can be seen in figure 4 which gives an overview of the results.

Drug Content

The drug-loaded transfersomes were mixed with methanol and sonicated for 20 minutes to obtain a clear solution and spectrophotometrically determined at 303 nm. There is a greater amount of drug in F7 since it has 89.56±0.74. F7 is suitable for further studies, therefore it contains more drugs. There are some results that are shown in a graphical representation is shown in (figure 5).

Selection of Best Formulation

Good entrapment efficiency and stability are also desirable for a compound to penetrate the skin well. Based on these considerations, we were able to determine that the formulation F7 is the best formulation. It has a drug content of 89.56±0.74 and 88.32%±0.74 of entrapment efficiency and this is best suited for characterization studies.

In Vitro Drug Release Study

Dialysis membranes were used to study the release of transfersomal formulations. The structure and properties of lipids have been found to be important determinants of drug release. A maximum of 59.21%±1.25 of drug was released after 24 hours, indicating sustainable release. There is a possibility that the sustained release is due to the high entrapment of lipophilic drugs in the lipid matrix. The best formulation, formulation F7, has been selected for further study because it has achieved the best drug release. The results that were obtained as a result of the study. As you may have seen in figure 6, the image is shown in a more detailed way.

Formulation of Patch

An optimized formulation of F7 was used to prepare the transfersomal patch. Based on the formula

provided by the drug manufacturer, the amount of drug that was required to be loaded into the patch was calculated. A good consistency was provided by HPMC and ethanol, while the plasticizer activity was provided by Glycerol. Packaging and wrapping of the formulation patch were done using butter paper and aluminum foil. An explanation of the ratios is given in table 3.

Patch Characterization

Physical Appearance

- Light yellowish in colour
- Smoothness
- Flexible

Thickness Uniformity

By measuring the patch using a calliper at three different areas, the thickness of the patch was calculated, and from this, it can be concluded that the patch has better uniformity when it comes to its thickness. The results showed that the average thickness of the sample was 0.46 mm±0.05. Based on the table 4, these results were confirmed.

Folding Endurance

In terms of folding endurance, the number of folds to break the patch or develop visible cracks was measured by the number of folds to break the patch. This test holds the importance of checking if the patch is able to resist folding in response to the folding force. There is a correlation between brittleness and less folding endurance. This value is determined by the number of times the patch has been folded at the same place without breaking. Therefore, by measuring the formulated patch by folding, it can be concluded that it is able to withstand folding 76 times without the patch breaking or showing visible signs of cracking or damage to its surface.

Folding endurance = 71±13

Percentage Moisture Loss

As a result of placing the container in the desiccator, the loss of moisture was calculated using the following formula:

$$\%\ Moisture\ content = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

A percentage of 5.74% moisture loss was found to be the result of the test.

Percentage Moisture Uptake

As a result of placing the item in the desiccator, moisture absorption was calculated using the following formula:

$$\%\ Moisture\ content = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Based on the tests conducted, it was found that the percentage of moisture uptake or moisture absorption was 4.29%. Due to the low moisture content of the patch, it remains stable and is able to become completely dry and brittle over time. A protective layer is applied to it to prevent the growth of microorganisms.

Drug Content

The amount of the drug present in the patch was calculated by soaking the patch overnight in a buffer, and then measuring it UV spectrophotometrically at 303 nm after 24 hours. Based on calculations, it was calculated that the formulated patch had a drug content of 90.12%. Table 5 shows the results of the experiment.

Flatness

There were three different areas of the prepared patch that were cut into longitudinal strips and uniformity was measured in each of those areas. During this process, the prepared patch is ensured to have a considerable degree of flatness uniformity.

***In Vitro* Drug Release Study**

A calculation was made to determine the amount of drug that could be loaded into a small circular patch to provide the ideal dosage. After preparing such a patch, a small circular patch was cut down and the area of the patch was used for a release study using a dialysis membrane after which the patch was prepared. Spectrometric measurements were performed on the samples after they were collected and measured. The results of these experiments were used to plot a graph by taking the time on the x-axis and the cumulative drug release on the y-axis. There was an increase in drug release from the patch after 12 hours, which was found to be 33.15%±0.09. This shows that the patch has a sustained effect, which in turn will reduce the need for frequent administration of the patch as it provides a sustained effect. A representation of the image is shown in figure 7.

CONCLUSION

Using ethanol injection method, active methotrexate transdermal patches were produced using various excipients in this novel approach of producing active methotrexate transdermal patches.While transdermal systems can be a promising route of delivery for new age drugs, conventional as well as new dosage forms are equally important for other drugs in terms of increasing their therapeutic efficacy despite their potential in transdermal systems. These parameters aim to make TDDS patented innovations more patient-friendly and ensure site-specific drug delivery. More importantly, the *in vivo* performance of the dosage form determines the drug's therapeutic efficacy.

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