Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

DEVELOPMENT, OPTIMIZATION AND SCALE UP OF AN INNOVATIVE TOPICAL CREAM FORMULATION OF TERIFLUNOMIDE FOR THE TREATMENT OF ATOPIC DERMATITIS

VenkataNookaraju Sreedharala,
Dept of Pharmaceutics, GITAM School of Pharmacy, GITAM deemed to be University, Hyderabad, Telangana, India

***Dr Pavan Kumar Chintamaneni,**
Assistant Professor, Dept of Pharmaceutics, GITAM School of Pharmacy, GITAM deemed to be University, Hyderabad, Telangana, India

Cite this paper as: VenkataNookaraju Sreedharala, Dr Pavan Kumar Chintamaneni (2024) DEVELOPMENT, OPTIMIZATION AND SCALE UP OF AN INNOVATIVE TOPICAL CREAM FORMULATION OF TERIFLUNOMIDE FOR THE TREATMENT OF ATOPIC DERMATITIS. *Frontiers in Health Informatics, 13* (8), 834-855

Abstract:
The skin, as the largest organ of the body, plays a crucial role in protection and appearance. Its health has been a primary focus of scientific exploration, with the goal of offering better treatment and aesthetic products. Skin diseases such as atopic dermatitis (AD), psoriasis, acne, vitiligo, and skin cancers significantly impact individuals' quality of life, influencing their socioeconomic and psychological well-being. Atopic dermatitis, a common inflammatory skin condition, affects 15-20% of children and 1-3% of adults. It often appears before the age of 5, with environmental and lifestyle changes being major contributing factors, alongside genetic mutations like those affecting the filaggrin gene. AD typically manifests as pruritus, erythema, and dermatitic plaques, with different presentations depending on age. Treatments include emollients, moisturizers, topical corticosteroids, calcineurin inhibitors, and newer options like Janus Kinase inhibitors (JAKi) and phosphodiesterase-4 inhibitors (PDE4i). However, there remains a significant need for more effective treatment options. Considering the need of new treatment options, a topical teriflunomide cream was developed, by repurposing the teriflunomide drug substance which was approved for multiple sclerosis as 7mg and 14mg tablets, for AD treatment. The formulation was optimized, scaled up, and tested for stability according to ICH guidelines, with further safety and efficacy studies planned.

Key Words: Teriflunomide, Atopic dermatitis, Topical cream, Drug targeting, Drug repurposing, DMARDs, DHODHi.

1. INTRODUCTION:

Skin being largest organ of the body protecting and giving the perception of appearance of individuals1, its protection and management has always been on top of scientific community's focus to explore providing new offerings for better treatment options or aesthetic products. Atopic dermatitis (AD), psoriasis, acne, vitiligo, leprosy, skin cancers, ulcers, bullous disorders, urticarial, onchomycosis, hair disorders, vulval

diseases, various microbial and fungal infections are the various skin diseases leading to individual's discomfort, their socioeconomic and psychological aspects affecting the quality of life2. AD, psoriasis and acne are reported to result in psychological problems such as depression, anxiety, and isolation and social problems such as disability, unemployment and loss of productive life3.

Atopic dermatitis is a common inflammatory skin disease affects 10 – 30% of children and about 2-10% of skin related concerns4. Sleepless nights of AD affected children effects their performance in academics and their parents and elder patients lead to loss of their productive life5.

There is an urgent need for new topical medications to treat atopic dermatitis (AD), a chronic skin condition that significantly impacts the lives of millions worldwide6. Despite the availability of current treatments like topical corticosteroids, calcineurin inhibitors, and emollients7,8, many patients still struggle to achieve long-term symptom control. These treatments, while effective to a degree, often come with significant drawbacks, such as skin thinning, irritation, burning sensations, and a risk of systemic side effects with prolonged use9. Moreover, some patients develop resistance to these therapies over time, leading to a diminished therapeutic response and persistent symptoms. The burden of AD, including intense itching, inflammation, and the psychosocial impact of visible skin lesions, underscores the need for new, innovative therapies. New treatments should aim to provide more targeted and sustained relief with a better safety profile, especially for long-term use and in sensitive skin areas. Advances in understanding the molecular pathways involved in AD have opened the door for novel therapeutic approaches, such as topical JAK inhibitors and PDE4 inhibitors10, yet there is still room for further innovation. The development of new topical medications that can offer both efficacy and safety, while being easy to use, would significantly improve the quality of life for those affected by atopic dermatitis.

2. MATERIALS AND METHODS

2.1. Materials and reagents

Teriflunomide (MSN laboratories limited, Hyderabad, India), cetostearyl alcohol, stearic acid, dimethyl sulfoxide (Panreac), glycerin, Tween 80, Span 60, methyl paraben, propyl paraben (Kredense), coconut oil, carbopol (Lubrizol), pharmacopeial grades were procured and used for formulation development. Analytical grade reagents and chemicals procured from Merck, Sigma were used in generating analytical data.

2.2. Methods

2.2.1. Preformulation

2.2.1.1. Physical characterization and identification

Proposed repurposed Teriflunomide drug substance was tested for physicochemical parameters to identify and qualify using analytical methods provided by supplier and/ or tests described in pharmacopeia general

chapters. Well established HPLC method was used to estimate assay of teriflunomide.

2.2.1.2. Solubility studies

Semi-quantitatively solubility of the proposed teriflunomide drug molecule was studied in various polar, non-polar and aprotioc solvents. The study was to identify the nature of the drug molecule and thereby to select the best suitable solvents and excipients for development of the topical cream formulation.

Known quantity of teriflunomide was added to 10 ml of the solvent and sealed in a glass tube. The mixture was agitated using mechanical agitator and observed for drug solubility visually. On complete solubilization, same procedure was repeated and recorded the total quantity of the drug solubilised in 10ml of the solvent. The data was reported in our previous publication11.

2.2.1.3. Drug excipient compatibility studies

As explained and published in our previous publication drug excipients compatibility studied using Shimadzu corporation differential scanning calorimeter. DSC plots were generated to study the compatibility for physical mixtures of drug and solvent and/or excipients, 10°C/min ramp to a temperature up to 250°C was used in generating the thermograms. DSC thermograms captured in Figure1.

2.2.2. Formulation of teriflunomide cream

Purified water, glycerin was taken as aqueous phase, carbopol was added under stirring to get uniform clear dispersion, followed by parabens were added and dissolved under stirring. Coconut oil, cetostearyl alcohol and surfactant were taken as oily phase. Both phases were heated to 70°C separately and oil phase was added to aqueous phase under stirring. While stirring temperature was brought down to about 30°C. pH was adjusted and drug in DMSO was added under stirring to get microemulsion based cream.

2.2.3. Optimization of the teriflunomide topical cream

Critical formulation factors namely penetration enhancer, concentration of gelling agent and pH of the formulation was optimized. To optimize these formulation factors, teriflunomide topical cream formulations were manufactured as described in the section 2.2.2 using different concentrations of gelling agents, permeation enhancers and at different pH values. The prepared cream batches were characterized for optimization of the formulation factors.

2.2.4. Evaluation of the formulations

2.2.4.1. Physical examination

Description including colour, clarity, homogeneity, grittiness and dispersion uniformity were observed physically for all formulations. Polarized light microscopic images were captured and presented in Figure 5.

2.2.4.2. pH

10% dispersion of the developed teriflunomide cream was prepared in purified water and pH of the same was recorded using glass electrode and electro lab pH meter.

2.2.4.3. Viscosity measurement

Antonpaar rheometer (Model: MCR 102E) was used to study the viscosity of the developed teriflunomide cream with spindle CP-25 at temperature and shear rate of 25°C and Ramp Linear 1-100 1/sec respectively.

2.2.4.4. Freeze thaw study

The developed cream formulation was exposed to freeze thaw study to study its stability at different atmospheric fluctuations. The samples were exposed to accelerated temperature and humidity (40±2°C and 75±5% RH) followed by freezing temperature (-40±2°C) for 24hrs at each condition and was considered as one cycle (together accelerated and freezing conditions). Such cycle was repeated second time as 2nd cycle. 3rd cycle was also carried out and in 3rd cycle, the sample was exposed for 72 hours to accelerated temperature and humidity (40°±2°C and 75±5% RH) and subsequently the same samples was exposed to freezing temperature (-40°±2°C) for 24hrs. After 3 cycles, the sample's physical parameters, crystal growth and drug release were studied. Studied for crystal growth by performing the XRD of the samples and the characteristic peak intensities was compared with the initial data. The data presented in Table 1.

2.2.4.5. Assay (Percentage purity)

Percentage purity of the API in the formulations directly influences on the therapeutic efficacy of the formulation. Hence it was assessed in the developed teriflunomide topical cream. Using buffer and acetonitrile mixture (30:70) as diluent, prepared 70ppm solution of the developed teriflunomide cream and was sonicated for 30min. Intermittent shaking was performed for every 5 min during sonication and after 30min of sonication, solution was filtered using nylon filter having pore size of 0.45µm. Validated HPLC method was used to quantify the API content in the developed cream formulation using C18 Kromasil-100 column having dimensions of 250nm x 4.6nm, 5µm. Analysis was performed using 35°C, 1.0mL/min and 5µL as column temperature, flow rate and injection volume respectively and areas were recorded at 250nm for quantifying the teriflunomide content (Percentage purity /Assay).

2.2.4.6. *In vitro* drug release test (IVRT)

In vitro diffusion study was performed to find out the drug release across the synthetic semi permeable membrane. Nylon net with pore size 0.20µm was used as semipermeable membrane arranged between donor and receptor compartments. Test sample was placed on the surface of the membrane in donor compartment and receptor compartment was filled with receptor medium.

Experiment was caried out at 37±0.5°C for 6 hours and 0.3ml of samples were withdrawn at 1, 2, 3, 4, 5 and 6th hour from receptor medium. After every withdrawal, same volume of fresh receptor medium was added and sink conditions was maintained. All precautions were taken to avoid the formation air bubbles in the receptor compartment during sample withdrawing and replacing fresh sample to avoid the erroneous results.

The samples were analyzed to find out the quantity of the drug released at each time point. Mathematical

plot was constructed with average amount of drug release against time (t, hrs) and the same drug release data was used to find out the order & mechanism of the drug release from test formulation. The data is presented in Table 4.

2.2.4.7. Optimization of the formulation parameters

Optimization of the penetration enhancer

Penetration enhancers are critical ingredients in the topical dosage forms to modulate the diffusion parameters and release profiles. Amount of drug substance distributed across the layers of skin and/or the concentrations achieved in the systemic circulation up on topical administration can be designed by proper selection of type and/or concentration of penetration enhancer. Two different concentrations of penetration enhancer, dimethyl sulfoxide i.e., 7.5% and 15% w/w were selected, and cream formulation was manufactured using same procedure described in the section 2.2.2. These cream formulations were analyzed for physical properties and release profiles. The data was compared with each other to study the effect of the permeation enhancer and optimum concentration was selected based on the results. The data presented in Figure 2.and data is presented in Figure 2.

Optimization of the gelling agent

Gelling agent/ viscosity builders are one of the important ingredients in the semisolid dosage forms which directly influence on many of the characteristics such as consistency, viscosity, spreadability, drug release/ permeation, etc. Considering this, the gelling agent was optimized in the current formulation.

Three different concentrations of gelling agent, Carbopol, was selected within its acceptable concentration range. i.e. 0.50%, 0.60% and 0.75%w/w and manufactured the cream formulations using the same procedure as described in the section 2.2.2. The cream formulations were characterized for physical properties, drug release and exposed to freeze thaw studies. Freeze thaw studies are explained in section 2.2.4.4. IVRT data of three formulations prepared with three different concentrations Carbopol are presented in Figure 3.

Optimization of the pH of the formulation

During the solubility studies of teriflunomide, it was observed that the Teriflunomide API has pH dependent solubility. API solubility is utmost important factor while developing the formulations irrespective of dosage forms. i.e. tablets, capsules, Aqueous gels, etc. The nature of the API solubility directly impacts on the drug release and/ or permeation from the dosage forms and across the biological layers. Moreover, in semisolid preparations pH of the formulation is critical parameter to be studied and monitored as it influences on the solubility, viscosity, drug release/ permeation, etc. Proposed formulation was manufactured with different pH values i.e. 5.28, 5.33, 6.01 and 6.43 by adding different volumes of the alkali. The manufactured cream formulations physical appearance and in vitro drug release (as described in section 2.2.4.6) was studied. IVRT data of the four teriflunomide topical cream formulations having four different pH values are presented in Figure 4.

2.2.4.8. *Ex vivo* diffusion study and drug distribution across the skin layers

Pork ear skin was used as semi permeable membrane and performed the ex vivo diffusion study of the developed topical cream. Skin was mounted between the compartments (donor and receptor) facing epidermis towards the donor compartment and dermis exposed to receptor medium. Ensured there was no hair and no cuts on epidermal surface. Also ensured that there was no flesh debris on the dermis region. During processing, cutting of suitable sized membranes, etc the membranes was placed in saline phosphate buffer.

Receptor medium (20ml) was filled in the receptor compartment and ensured that there were no air bubbles in the receptor medium as they impact on the drug permeation by decreasing the permeation area. It was also ensured that the receptor medium was in contact with the skin at every time point without air bubbles. Test sample i.e. teriflunomide (Around 300mg) cream was placed on the surface on the skin in donor compartment.

Experiment was initiated and continued for 24 hours at receptor medium temperature of 31±0.5°C. At specific time points i.e. 0.5, 1, 2, 4, 6, 8, 12, 18 and 24hrs, 0.3ml of the receptor medium was withdrawn and equal volume of the fresh buffer was replaced to maintain the sink conditions.

After 24hours drug permeation study, the pork skin samples were collected with utmost care from each diffusion cell. The test sample retained on the surface of the skin samples was transferred carefully and completely into the specific volume of the diluent separately. The skin samples were rinsed using the fresh diluent and transferred into their respective solutions. Further, epidermis and dermis layers of the skin sample's, were separated individually by exposing the skin samples to the hot surface at 60°C. The epidermis and dermis layers were chapped carefully and transferred the chapped pieces into the specific volume of the diluent to find out amount of the drug permeated into the skin layers and did not permeate into the receptor medium.

All the samples were analyzed after suitable dilutions using validated analytical method to find out the amount of the drug permeated across the skin, retained on the skin and drug retained in the skin layers. Ex vivo diffusion parameters such as flux, % drug release, etc and drug distribution between layers was presented in table 3.

2.2.4.9. *Drug* permeation kinetics study[12]

(1) Zero–order equation

$$Q_t = k_0 t$$

Where Q_t stands for the percentage of drug released at time t and

k_0 is the release rate constant.

(2) First–order equation

$$\text{Log Q}=\text{Log } Q_0 - \frac{Kt}{2.303}$$

Where, Q_0= is the initial concentration of drug,

K= is the first order rate constant, t = release time;

(3) Higuchi equation

$$Q_t = kH^{t1/2}$$

Where, k_H represents the Higuchi release rate constant;

(4) Korsmeyer-Peppas equation

$$\frac{Q_t}{Q_e} = Kt^n$$

Where Qt/Qe is the fractional drug release from the cream (donor compartment) into the receptor solution, K is a constant corresponding to the structural and geometric characteristics of the device and n is the release exponent which is indicative of the mechanism of the drug release. The (n) value of 0.5 indicates the Quasi-Fickian diffusion mechanism, while if (n>0.5) then anomalous or non-Fickian diffusion mechanism exists, and if it is (=1) then the Zero order release one exists.

(5) Hixon Crowel equation

The Hixon-Crowell model equation is as follows:

$$m_0^{1/3} - m_{left}^{1/3} = k_{H-C}t$$

where m_{left} is the amount of drug left in the formulation over time t, and

k_{H-C} is the Hixon-Crowell rate constant.

2.2.5. Scale up of the formulation

Based on the optimization studies at R&D, established an optimum concentration range for critical parameters i.e. permeation enhancer as 15% w/w, gelling agent as 0.5-0.75%w/w and pH as 4.0-6.0 respectively. Prototype formulation was designed where mid points (approx.) of established optimized ranges of critical parameters were selected. Gelling agent concentration was selected as 0.6%w/w, and the pH was decided as 6.0.

Considering these parameters, the prototype formulation was scaled up to 5kgs using ointment manufacturing unit (Make: Bowman Archer). The process parameters were established during the scale up of the formulation. The scaled-up formulation was characterized at initial stage and the same was subjected for stability studies as described in section 2.2.5.1. The results are presented in Table 5.

2.2.5.1. Stability study

The developed cream formulation was filled in the selected final packs i.e. laminated tubes and are exposed to accelerated (40±2 °C and 75±5% RH) and real time (30±2 °C and 75±5% RH) as per ICH tropical region guidelines. The stability samples were analysed for physical parameters such as viscosity, assay, IVRT, etc.

3. RESULTS AND DISCUSSION

3.1. Physical characterization and identification

The teriflunomide API appeared as white to pale yellow powder. It was identified by the validated HPLC method where the retention time of the sample was compared with the retention time specified by the vendor. It was concluded that the test sample was teriflunomide as its retention time was matched with the vendor specified retention time.

3.2. Solubility studies

Based on the solubility study of the teriflunomide as reported in our previously published article and based on the cream requirements, the excipients were selected which were GRAS approved as safe and were within the acceptable range as specified by the FDA (IIG limits).

3.3. Drug excipient compatibility studies

Based on the formulation needs, API physico chemical properties such as solubility, melting point, etc few excipients were selected for development of the formulation. DSC thermograms of such excipients and API mixture was recorded to study their compatibility. The mixtures of API and individual excipients did not show neither new peaks nor absence of existing peaks in their DSC thermograms. Nevertheless, very few mixtures of API and excipients exhibit change in the API/excipient melting point. This suggests that there are no interactions between the teriflunomide and the selected excipients. Thus, topical cream was developed using the selected excipients.

Moreover, for three months accelerated and real stability period, the cream formulation did not show variations in its characteristics and not shown any interactions. Figure 1 displays the DSC thermograms of the excipients and API.

3.4. Physical examination

The topical cream appeared as white to off white viscous in nature and had adequate consistency. It was easy to spread and found free from gritty particles. No separation of oil and aqueous phases was observed and appeared as uniform semisolid cream to the naked eye. Physical examination details are captured in Table 1.

3.5. pH

The cream was diluted with purified water and prepared 10% w/w solution. pH of the same was recorded and found within the prefixed range. i.e. 6.0±1.0 which was ideal to the skin pH. pH of the Initial and freeze thaw samples presented in Table 1.

3.6. Viscosity

The cream viscosity was studied with rheometer and found within the prefixed specification range i.e. Not less than 5000cps. Initial and freeze thaw samples viscosities are recorded and presented in Table 1.

3.7. Freeze thaw study

After exposing the cream formulation to three cycles of accelerated and freezing conditions, the cream stability was studied physically. There was no specific change was observed with naked eye and under polarized light microscope. However, there was slight increase in the percentage drug release after freeze thaw study but was not significant statistically. It was studied using student t-test and the p value found more than 0.05.

3.8. Assay (Percentage purity)

Validated HPLC method was used, and the samples were analysed for quantifying the percentage purity of the API in the developed formulation. The percentage purity found within the acceptance range i.e. 90.0-110.0 as per the regulatory norms. Assy values are reported in the Table 1.

3.9. *In vitro* drug release test (IVRT)

The study demonstrated that the teriflunomide release across the synthetic semi-permeable membrane i.e. nylon net was satisfactory. During 6 hours of the *in vitro* release study the cream formulation exhibited around 60% drug release. The release data was constructed as a plot between the cumulative amount of drug released (µg/cm^2) and the square root of time (Table 2 and Figure 3).

3.10. Optimization of the formulation parameters

The concentration of the permeation enhancer did not show any variation in the physical appearance. However slight change in the texture and spreadability was observed. Smoothness and spreading intensity of the cream was decreased with decreased concentration of the DMSO. The drug release from cream formulations prepared using 7.5% w/w and 15.0% w/w of DMSO was studied and found significant variation between the two formulations. *In vitro* drug release from the formulations was directly proportional to the concentration of the DMSO among two formulations. The details presented in Figure 2.

During physical observation, variation in the physical appearance, texture and viscosity of the formulations prepared with the different concentration of the carbopol. Formulation prepared with 0.6% w/w carbopol exhibited adequate consistency, spreadability, viscosity rather than the other two preparations. The *in vitro* drug release did not show significant variation among three formulations statistically. The drug release from the prepared formulations was compared statistically using student t-test considering two tailed hypothesis and significance level p-value as 0.05. Among three formulations the drug release was observed as non-significant as the p value found P>0.05 (0.75% vs 0.6%, p<0.7315, 0.75% vs 0.5%, p<0.6997 and 0.6% vs 0.5%, p<0.9639)[13].

Drug release from the formulations prepared at different pH values are studied physically and drug release was compared. The drug release was increased with increase in the pH of the formulation. This is because of the increased solubility of the teriflunomide with increased pH value as the drug available in solution form at higher pH. The same was depicted in the Figure 4. However, with the increased pH, the colour of the formulation was changed. As pH increased, the formulation turned from white/ off white to light brown colour. Considering these factors the optimum pH for the formulation was fixed. i.e. 5.00±1.00.

3.11. *Ex vivo* permeation test

The effectiveness of transdermal drug delivery systems differs significantly in their capacity to penetrate a drug through the skin. For *ex vivo* penetration studies in this work, the cream formulation consisting of the three different concentrations of the Carbopol, gelling agent i.e. 0.5%, 0.6% and 0.75% were compared regarding the skin penetration profiles as studied in IVRT. As the drug release not shown significant variation in the three formulation, permeation study was carried out with three formulations to confirm the pattern of drug permeation. Table 3 depicts the penetration profiles of drugs from the three developed cream formulations. The permeation profiles of three formulations were compared statistically using student t-test with 0.05 as significance level.

Drug permeation from three cream formulations was found as non-significant and the p-value found P>0.05 (0.75% vs 0.6%, p<0.3864, 0.75% vs 0.5%, p<0.7176 and 0.6% vs 0.5%, p<0.5998)[13].

The drug permeation was poor comparatively drug release from the three formulations. Around 60±15%w/w drug released from three formulations in 6hours across the synthetic semipermeable membrane where the same amount of the drug permeated in 24hours across the pork skin. Though there is variation in intensity of drug release/ permeation from the three formulations, the pattern was same. This substantiated that the studied range of gelling agent i.e. 0.5-0.75%w/w not influencing on the drug

release and permeation from cream formulation.

Cumulative amount of the teriflunomide permeated from unit area (µg/cm^2) of the skin into the receptor fluid and time (hr) was arranged on y and x axis respectively and constructed a graph. Amount of the drug permeated from unit area (µg/cm^2) in unit time (hr) at steady state was obtained from the slope of the above graph and was considered as the flux (J_{ss}, µg/cm^2/h) of the optimized cream formulation and was found as 50.2 J_{ss}, µg/cm^2/h. The different permeation parameters recorded and are reported in Table 3. The obtained results substantiated that the cream formulation was effectively permeating across the skin.

3.12. Drug distribution across different layers of skin[14]

The optimized formulation's *ex vivo* permeation study confirmed that around 60% of the drug permeated across the pork ear skin and the same will be the responsible for systemic therapeutic effect. As the AD is the topical disease and to maximize therapeutic efficacy, physicians prescribe both systemic and topical medications based on the severity of the disease. Topical formulation will have better efficacy in treating the topical diseases if it produces both systemic and localized effect simultaneously. In the current formulation around 60% of the drug permeated across the skin, around 15-20% of drug retained in the skin layers i.e. epidermis and dermis and around 25-30% of the retained on the skin. Drug permeated across the skin elicit the systemic effect whereas the drug retained in skin layers will elicit the localized effect. Drug retained on the skin acts as reservoir and deliver the drug slowly and prolonged period to produce both systemic and localized therapeutic effect. Considering this drug distribution pattern, it is assuming that the developed topical cream more effective and drug of choice for the treatment of the Atopic dermatitis to the patients and dermatologists. However, this concept must be studied preclinically and need to confirm the therapeutic efficacy.

3.13. Drug permeation kinetics study

Drug permeation mechanism and kinetics of the developed formulation was identified by fitting the *ex vivo* permeation study data in various mathematical models. The data was fitted in different mathematical models like zero, first, Higuchi, Hixon Crowel, and Korsemeyer –Peppas models.

Many physical and chemical laws control drug transport from pharmaceutical systems, making it difficult to fit the events that occur into a suitable mathematical model. Zero-order kinetics may be applied if the medication is released gradually from a pharmaceutical dosage form that does not disintegrate. When a drug is weakly soluble and embedded in a water-soluble matrix, the first-order model will be used, and drug release will occur as a result of dissolution rather than diffusion.

Several assumptions underpin the Higuchi equation: the drug's solubility is greater than its initial concentration in the formulation; it transfers only in one dimension; system swelling, and drug dissolution are minimal; drug diffusivity is constant; and sink conditions are satisfied. The Hixon-Crowell model is employed when the drug is administered on parallel planes of the dosage form surface, like in the case of a tablet, where the size decreases proportionately and the geometric shape stays constant[15, 16].

By accounting for non-Fickian mechanisms, Korsemeyer-Peppas mathematics is useful in explaining drug release from a polymeric system when multiple types of drug release mechanisms are involved or the drug release phenomenon is unknown[17, 18, 19].

By contrasting the R^2 values of these mathematical models, the best-fitting model was found. The kinetic model, which is suitable for the drug penetration, is shown by the R^2 values. Table 4 displays the values of the R^2.

The R^2 results demonstrated that the zero order and Higuchi equations, which matched the kinetics of the numerous topical creams that have been reported, best fit the drug release from the topical cream. The polymeric system's release mechanism will be determined by looking at models with R^2 values close to 1. The best kinetic model for the current cream formulation was found to be Korsmeyer-Peppas model, followed by the zero-order, because the plots showed strong linearity, mostly verifying the diffusion process.

This model states that when the n value is less than 0.5, the drug release is Fickian diffusion; if the n value is between 0.5 and 1.0, anomalous passing is indicated. The teriflunomide permeability from the optimized cream formulation was proven by many procedures rather than a single step in the current formulation since the n value was above 0.5, i.e. n=1.428. The details of R^2 and n values are presented in table 4.

3.14. Stability study

Shelf life of the any newly developed formulations were fixed based on the stability data of the respective formulations. Three (3) months stability of the proposed formulation was established in both accelerated and real time conditions. The stability study still under progress.

Three months stability samples were characterized for physical parameters and assay using validated analytical methods. The results were compared with the initial data and not found any major variation in the data compared to the initial data statistically. The characterization data of the three months stability samples found satisfactory met the prescribed guidelines and specifications. The data presented in the Table 5.

4. CONCLUSION

Teriflunomide topical cream was developed successfully and established satisfactory stability, *in vitro* drug release and *ex vivo* drug permeation. It was also established the acceptable concentration/range of the critical parameters i.e. permeation enhancer of 15% w/w, gelling agent of 0.5 - 0.75%w/w and pH range of 4.00 – 6.00. The formulation was scaled up effectively and well established the manufacturing process. The developed formulation must be explored further for its therapeutic efficacy against atopic dermatitis preclinically and clinically.

5. CONFLICT OF INTEREST

Authors declare that we have no Conflict of Interest.

6. ACKNOWLEDGMENT

The author thanks the Apramitha Innovations Private Limited, Hyderabad, Telangana, and Aizant Drug Research Solutions Private Limited, Hyderabad, Telangana for their support in conducting the *s*tudies at their labs.

LIST OF FIGURES

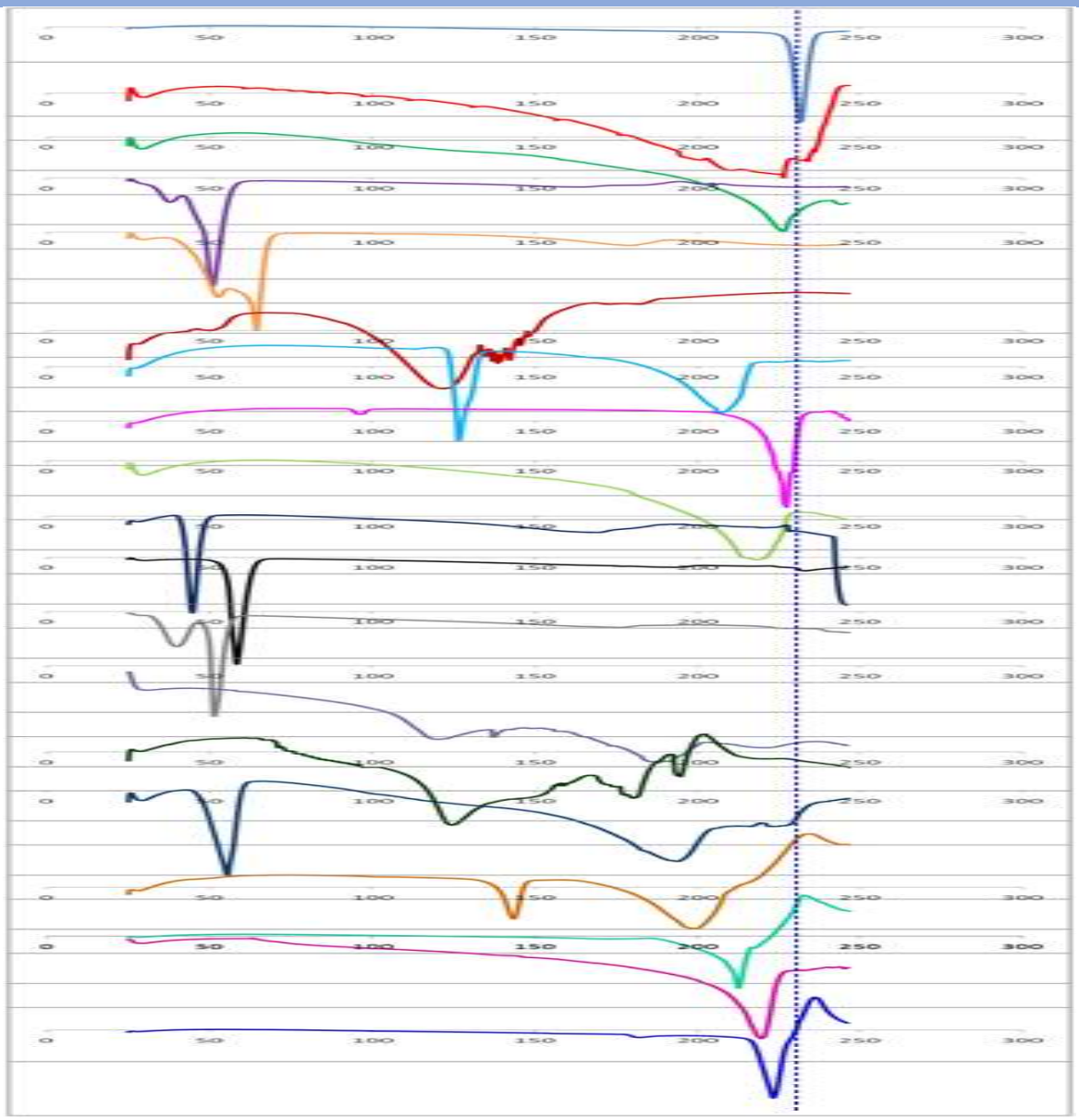

Figure 1: DSC thermo grams of API and physical mixture of API and excipients (From top to bottom): 1. Teriflunomide API, 2.Teriflunomide:Labrafac-pg, 3. Teriflunomide: Labrafac lipophile, 4. Teriflunomide+ Emulcire (2:9), 5. Teriflunomide+ Gelot 64 (2:3.6), 6. Teriflunomide+ DMSO (2:10), 7. Teriflunomide+ Methyl Paraben (2:0.5), 8. Teriflunomide+Propyl paraben(2:0.06), 9. Teriflunomide+ Coconut Oil(2:3), 10. Teriflunomide+ L-Menthol (2:5), 11. Teriflunomide+Stearic acid (2:9) 12. Teriflunomide+Cetosteryl alcohol (2:6), 13. Teriflunomide+Tween80 (2:4), 14. Teriflunomide+Glycerin (2:10), 15. Teriflunomide+Span 60 (1:1), 16. Teriflunomide+Cholesterol (1:0.5), 17. Teriflunomide+ Soyalecithin (1:0.199), 18. Teriflunomide+ Eucalyptus oil (2:3), 19. Teriflunomide+Carbapol 10NF (2:0.5)

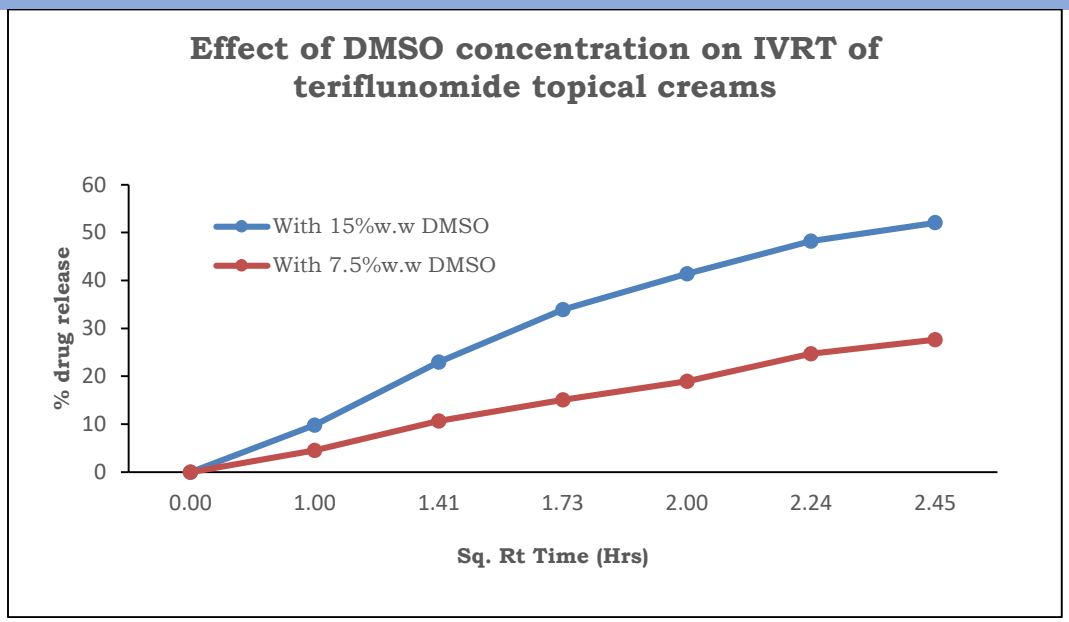

Figure 2: Effect of DMSO concentration on IVRT of teriflunomide topical creams

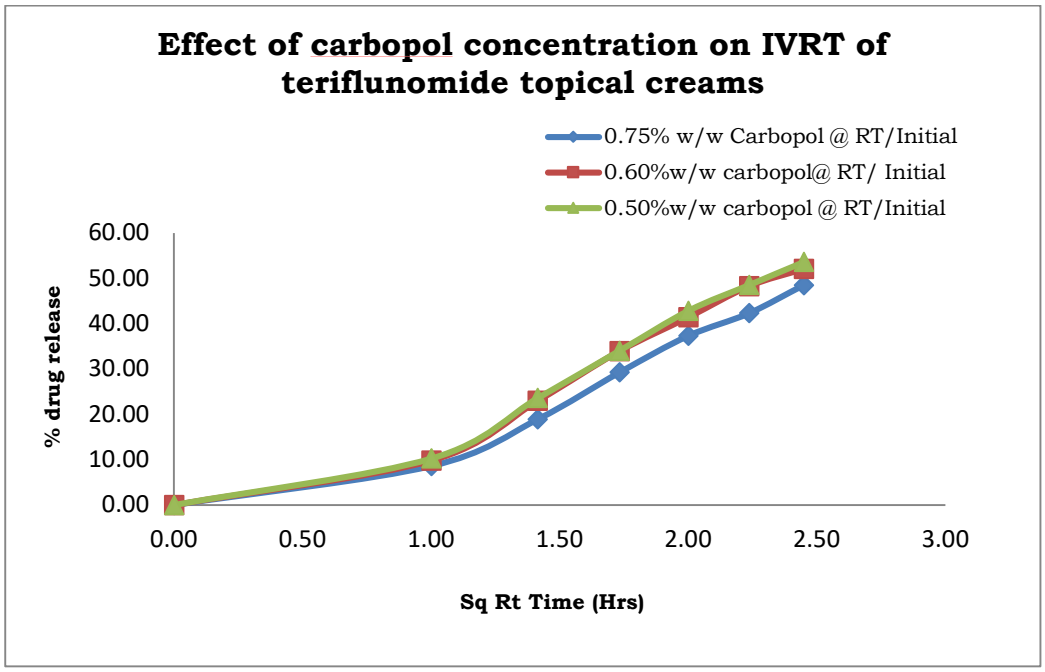

Figure 3: Effect of carbopol concentration on IVRT of teriflunomide topical creams

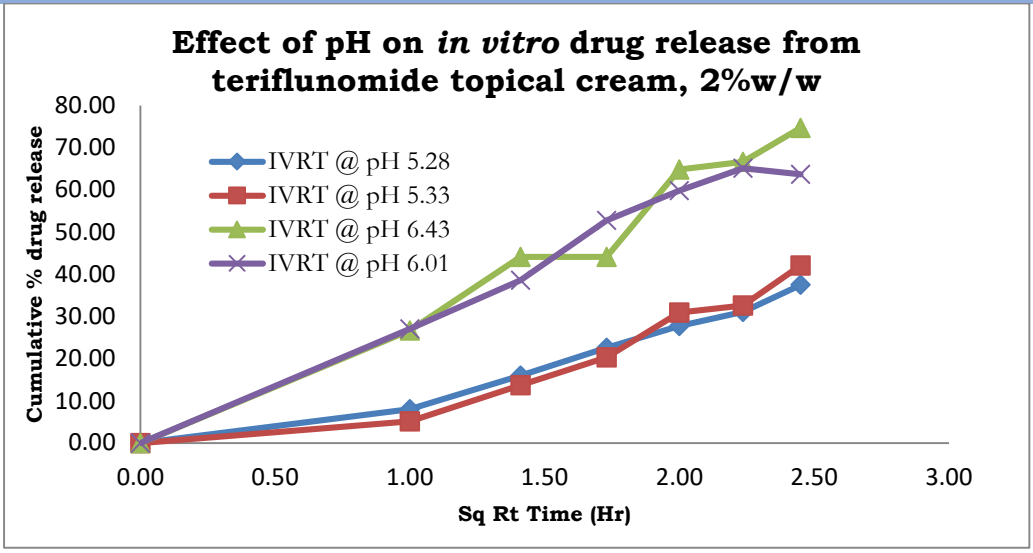

Figure 4: Effect of pH on the IVRT of teriflunomide topical creams, 2%w/w

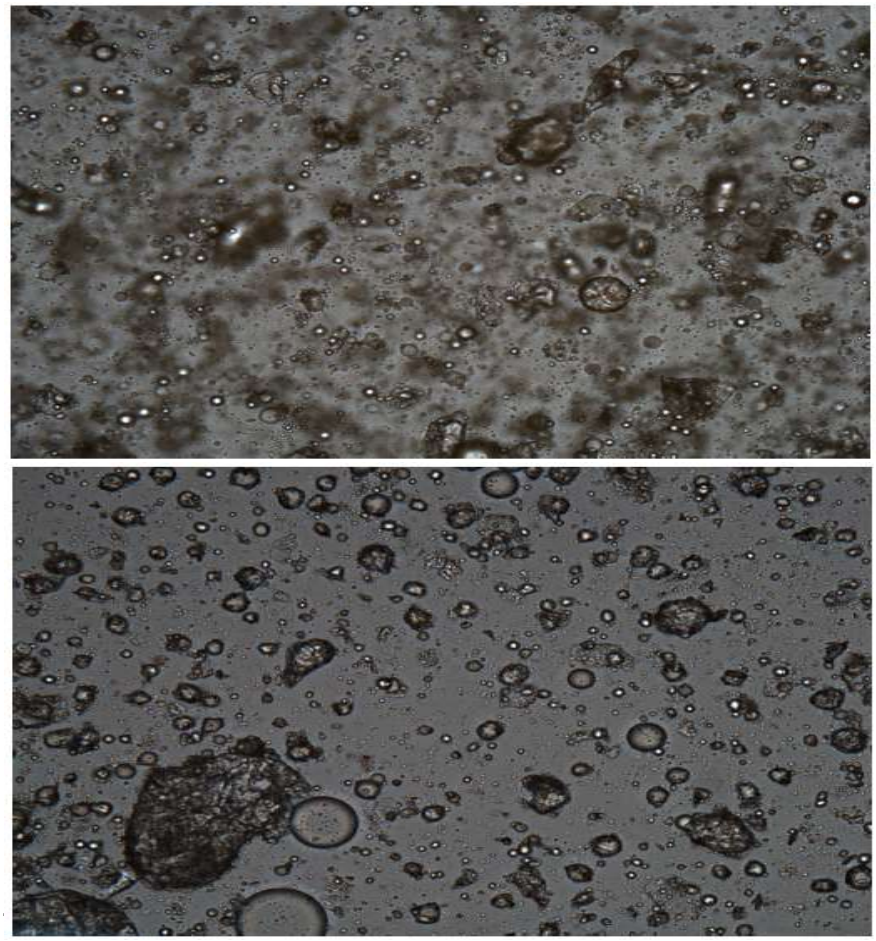

Figure 5. Polarized light microscope images (Magnification: 50X)

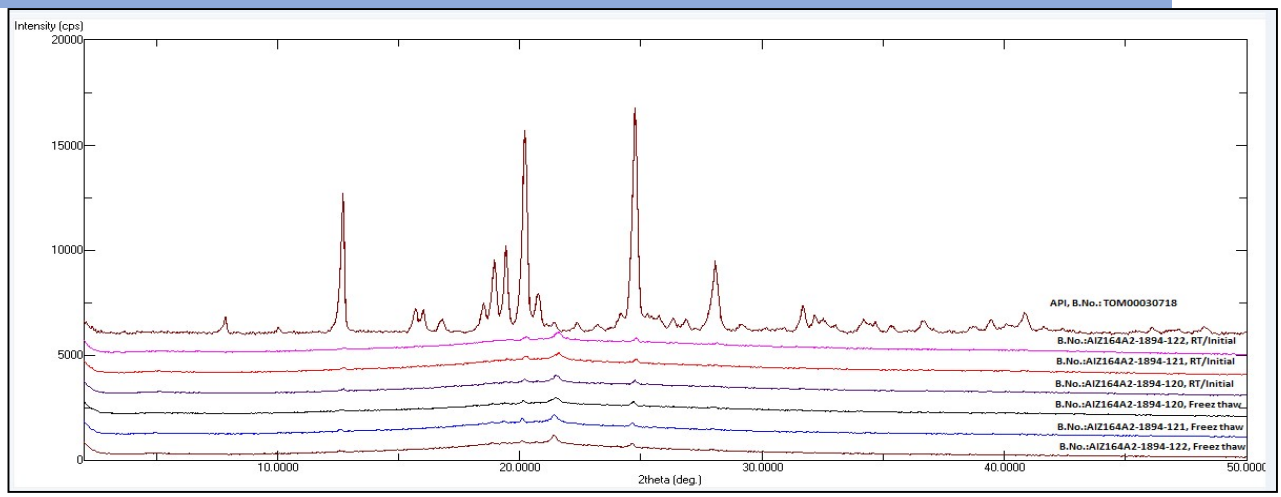

Figure 6. XRD plots of teriflunomide API and RT/ Initial samples and Freeze thaw samples of the teriflunomide topical creams, 2%w/w

LIST OF TABLES

Table 1: Comparison of physical parameters of the initial and freeze thaw samples

Batch No		AIZ164A2-1894-120		AIZ164A2-1894-121		AIZ164A2-1894-122	
Description		Batch with 0.75%w/w carbopol		Batch with 0.60%w/w Carbopol		Batch with 0.50%w/w carbopol	
Condition		Initial/RT	Freeze thaw	Initial/RT	Freeze thaw	Initial/RT	Freeze thaw
Physical parameters							
SNo	Parameter	Remark	Remark	Remark	Remark	Remark	Remark
1	Description	White to off white viscous cream	White to off white viscous cream	White to off white viscous cream	White to off white viscous cream	White to off white viscous cream	White to off white viscous cream
2	pH	6.69	5.04	6.45	5.12	6.70	5.20
3	Consistency	Adequate cream consistency and not pourable	Adequate cream consistency and not pourable	Adequate cream consistency and not pourable	Adequate cream consistency and not pourable	Adequate cream consistency and not pourable	Adequate cream consistency and not pourable
4	Gritty particles	Absent	Absent	Absent	Absent	Absent	Absent

5	Phase separation	Not observed		Not observed		Not observed		Not observed		Not observed		Not observed	
6	XRD	2θ value	Intensity	2θ value	Intensity	2θ value	Intensity	2θ value	Intensity	2θ value	Intensity	2θ value	Intensity
		12.68	541	12.68	508	12.72	594	12.60	540	12.72	573	12.58	488
		20.20	980	20.16	960	20.26	1123	20.12	1074	20.26	1080	20.10	967
		24.76	937	24.68	887	24.80	995	24.64	840	24.78	1014	24.60	817

Table 2. IVRT data and drug release parameters of the cream formulations stored at RT/ Initial and subjected Freeze thaw study

Batch No		**AIZ164A2-1894-120**		**AIZ164A2-1894-121**		**AIZ164A2-1894-122**	
Description		**Batch with 0.75%w/w Carbopol**		**Batch with 0.60%w/w carbopol**		**Batch with 0.50%w/w carbopol**	
Condition		**Initial/RT**	**Freeze thaw**	**Initial/RT**	**Freeze thaw**	**Initial/RT**	**Freeze thaw**
S No	**Sq Rt Time (Hrs)**	**Cumulative % Drug release (%)**					
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1.00	8.10	14.09	9.29	15.20	9.76	15.58
3	1.41	18.35	26.25	23.40	28.87	23.00	24.90
4	1.73	28.62	38.48	33.28	40.88	33.37	36.16
5	2.00	36.64	45.59	40.72	47.92	42.12	44.63
6	2.24	41.59	53.33	47.50	55.39	47.78	52.72
7	2.45	47.75	59.04	51.29	60.16	52.83	59.23
***In vitro* diffusion parameters**							
1	Average flux (J_{ss}, mg/cm²/h)	471.20	535.10	505.50	525.60	523.00	522.00
2	Average % drug release (%)	47.75	59.04	51.29	60.16	52.83	59.23
3	Average cumulative amount of drug released (µg/cm²)	1099.80	1257.45	1150.91	1224.20	1202.61	1251.23

Table 3. Details of the *ex vivo* permeation, diffusion parameters and drug distribution across the skin layers

Product	**Teriflunomide topical cream, 2%w/w**		
Batch No	**AIZ164A2-1894-120**	**AIZ164A2-1894-121**	**AIZ164A2-1894-122**

Batch size		**100g**	**100g**	**100g**
Description		**Batch with 0.75%w/w carbopol**	**Batch with 0.60%w/w carbopol**	**Batch with 0.50%w/w carbopol**
S No	**Time (hr)**	**% *Ex vivo* drug permeation (IVPT, N=2)**		
1	0.00	0.000	0.000	0.000
2	0.50	0.439	0.285	0.000
3	1.00	0.470	0.842	0.122
4	2.00	0.647	2.070	0.763
5	4.00	2.467	6.077	3.276
6	6.00	4.917	10.964	6.673
7	8.00	7.850	16.744	10.975
8	12.00	14.617	28.017	20.439
9	18.00	27.318	45.526	34.757
10	24.00	41.221	60.725	48.418
S No	***Ex vivo* drug parameters study: Diffusion parameters**			
1	Average flux (J_{ss}, µg/cm²/h)	35.25	50.16	43.76
2	Average % drug release (%)	41.22	60.73	48.42
3	Average cumulative amount of drug permeated (µg/cm²)	838.25	1152.71	1003.19
***Ex vivo* drug parameters study: Mass balance**				
S No	**No of cells (N)**	**2**	**2**	**2**
1	% drug permeated	41.25	60.72	48.28
2	% drug present on the skin	38.45	26.70	28.20
3	% drug present in epidermis	10.70	9.00	12.10
4	% drug present in dermis	5.50	4.40	5.30
5	% Mass balance	95.90	100.82	93.88

Table 4: Mathematical models and their regression coefficient values of the teriflunomide topical cream, 2%w/w

S No	Mathematical model	R^2	N
1	Zero order	0.9921	N/A
2	First order	0.9732	N/A
3	Higuchi	0.9114	N/A
4	Hixon-Crowell	0.9308	N/A
5	Korsemeyer- Peppas	0.9989	1.428

Table 5: Accelerated and real time stability data of the scale up batch of teriflunomide topical cream, 2% w/w

Station			Initial/	1Month/	2Month/	3Month/	3Month/
Condition			RT	40°C/75% RH	40°C/75% RH	40°C/75% RH	30°C/75% RH
Physical parameters							
S No	**Parameter**	**Stability Specification**	**Remark**	**Remark**	**Remark**	**Remark**	**Remark**
1	Description	White to off white viscous cream	White to off white viscous cream	White to off white viscous cream	White to off white viscous cream	White to off white viscous cream	White to off white viscous cream
2	pH	4.5 – 5.5	5.33	5.05	4.96	5.05	5.06
3	% Assay	90.0 - 110.0	111.9	115	109.1	108.0	113.9
4	Viscosity (m.Pas)	Report the results	15937	14524	13882	UP	UP
IVRT data							
S No	**Sq RT Time (Hr)**		**% Drug release**				
1	0.00	Not applicable	0.000	Not performed	Not performed	0.000	0.000
2	1.00		5.144			8.972	9.748
3	1.41		13.685			16.542	18.931
4	1.73		20.294			22.984	27.103
5	2.00		30.931			27.941	33.786
6	2.24		32.577			33.953	41.338
7	2.45		42.092			38.859	47.611
IVRT data							
S No	**Drug release parameters**						
1	Average flux (J_{ss}, µg/cm²/h	Not applicable	360.7	Not applicable	Not applicable	328.2	387.2

	)						
2	Average % drug release		42.092			38.859	47.611
3	Average cumulative amount of drug released (µg/cm²)		876.159			792.742	932.175

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