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Chemical and Physical Stability Assessment of Devised Tacrolimus Gel for Treatment of Vitiligo

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Abstract

In order to retain quality and effectiveness over time, this study attempts to create and assess tacrolimus gel formulations with improved long-term chemical and physical stability. Differential Scanning Calorimetry (DSC) and DSC-photovisual approaches were among the analytical techniques used to thoroughly evaluate these gels' stability characteristics. In order to replicate actual storage circumstances, Accelerated Stability Testing (ASLT) was also carried out at various temperatures and intervals of time. The study investigates cutting-edge nano-based delivery technologies such liposomes, nanoparticles, and nanotransfersomes in order to overcome the difficulties caused by tacrolimus's poor water solubility and possible cytotoxicity. In order to improve therapeutic results, these nano-carriers seek to maximise regulated medication release and penetration into deeper layers of vitiligo-affected skin. All things considered, the results enhance tacrolimus's formulation stability and targeted distribution, providing a viable strategy for improved vitiligo therapy.

Keywords: Tacrolimus Gel, Vitiligo Treatment, Stability Assessment, Nano-based Drug Delivery, Accelerated Stability Testing

1. INTRODUCTION

Vitiligo is a common depigmenting skin condition that is characterized by achromatic patches associated with the loss of melanocytes in the epidermis and hair follicles; it affects roughly 0.004–2.28% of people worldwide, with a reported frequency of 0.25%–4% in India [1-2], and its effects can be seen in people of all ages, both sexes, and up to 2% of the population in some areas. According to science, vitiligo is characterized by depigmentation patches and macules, and it is often associated with low self-esteem and social stigma. It is well known that cellular damage is caused by an immune imbalance manifested by auto reactive T cells and anti-melanocyte antibodies [3–4]. It significantly affects patients' quality of life, and none of the available treatment choices are entirely adequate, either because of the medication's side effects and operational difficulties or because improvement and therapy duration are unpredictable [5].

The cause of vitiligo is unknown [6]. Vitiligo is a complicated, polygenic illness that supports a non-Mendelian inheritance, according to genetic study. The autoimmune theory remains the most widely accepted. Numerous studies have connected vitiligo to autoimmune diseases such thyroid illness, diabetes mellitus, and alopecia areata [7]. Despite not being a significant physical ailment, this disease creates many cosmetic problems, and most doctors are not aware of how it impacts patients' self-esteem and quality of life.[8] A recent study found that visible lesions are associated with melancholy in vitiligo patients, leading to low self-esteem and a negative body image [9]. They feel embarrassed in discussion and suffer from social isolation, which in some cases even results in suicide.

The treatment of vitiligo is often a complex procedure [10]. The mainstays of treatment are phototherapy and topical medications (e.g., corticosteroids and calcineurin inhibitors) [11]. However, because of new information regarding the interferon-gamma (IFN-γ) signalling axis in vitiligo, numerous clinical studies employing Janus kinase (JAK) inhibitors have recently demonstrated promising efficacy in vitiligo [12]. Other options include lasers and surgery, although none of these procedures consistently yield conclusive and fulfilling outcomes for every patient [13].

The two primary approaches to treating this condition are medication and surgery. The drug also included two types of systemic and topical split. Examples of topical treatments include topical calcipotriol, tacrolimus, topical steroids, intralesional steroids, and topical psoralen ultraviolet A (PUVA). Systemic therapy includes things like systemic steroids and systemic PUVA. Although the aforementioned therapies are all considered first-line treatments, therapeutic approaches differ based on the therapy and the patient's condition [14–15].

Topical calcineurin inhibitors (TCIs), initially developed to treat atopic dermatitis, have supplanted long-term use of corticosteroids [15]. These medications attach to an intracellular protein known as FK binding protein (FKBP), which suppresses mast cells and leucocytes' immune systems and prevents the synthesis of pro-inflammatory cytokines. Tacrolimus, a member of this category, is commonly used to treat eczema and is available as an ointment formulation in two strengths: 0.1% and 0.03% [15]. Its immunomodulatory qualities were believed to make it a successful treatment for vitiligo. Twelve Tacrolimus has been demonstrated to be quite beneficial for repigmentation in non-segmental vitiligo, although it is less successful for segmental and acrofacial disorders [16]. In the current investigation, the same has been suggested for the treatment of vitiligo. It has been suggested that 25 vitiligo sufferers be the subjects of the study. Topical tacrolimus will be applied to their symmetrical patches. With three months of follow-up, each patient will receive a session every two weeks for a maximum of six months (12 sessions). Skin samples will be taken prior to and following the treatment in order to evaluate the clinical results.

2. LITERATURE REVIEW

(Parekh et al., 2021) [17] developed a mesoporous silica nanoparticle (MSN)-embedded hydrogel for tacrolimus delivery in treating atopic dermatitis. This formulation enhanced skin penetration and provided controlled release, addressing limitations of conventional creams. Studies showed prolonged drug retention in deeper skin layers, reducing inflammation and lesion severity. The MSN-hydrogel system holds potential for treating chronic skin conditions like vitiligo with reduced systemic side effects. **(Modi et al., 2021) [18]** created a thermoresponsive ophthalmic gel using Pluronic F127 and chitosan for sustained tacrolimus delivery. The gel transitioned to a solid form at body temperature, enhancing drug retention. Drug release studies showed sustained tacrolimus delivery, with in vivo improvements in inflammation and immune modulation, suggesting potential application for dermatological conditions like vitiligo. **(Khan et al., 2022) [19]** designed a tacrolimus-loaded solid lipid nanoparticle (SLN) gel for improved skin penetration. The SLNs enhanced drug solubility and permeability, ensuring prolonged release. In vitro and ex vivo studies showed improved skin penetration compared to traditional formulations. The SLN gel exhibited good rheological properties, offering comfort and potentially improving treatments for skin conditions like psoriasis and eczema. **(Sun & Sun, 2022) [20]** developed an ion-sensitive ocular gel for tacrolimus delivery, aimed at treating immune conjunctivitis. The gel transformed into a gel upon contact with the ocular surface, increasing retention and controlled drug release. In vivo studies demonstrated its effectiveness in reducing inflammation and ocular irritation, with potential applications for other chronic topical conditions. **(Zaki et al., 2022) [21]** explored tacrolimus-loaded spanlastic gels for transdermal drug delivery. The elastic vesicles improved drug solubility and skin penetration. In vitro and ex vivo studies showed sustained release, while in vivo studies reduced inflammation and psoriasis symptoms, suggesting the gel as

an effective alternative for chronic skin conditions. **(Alam et al., 2023) [22]** developed a nanostructured lipid carrier (NLC) gel co-loading tacrolimus and thymoquinone for psoriasis treatment. The dual-drug approach enhanced drug solubility, stability, and skin penetration. The gel showed sustained release, and in vivo studies reduced inflammation, lesion size, and scaling, offering improved treatment for skin diseases. **(Wang et al., 2023) [23]** created a cationic nanoemulsion in situ gel for tacrolimus aimed at treating dry eye disease (DED). The gel adhered to the ocular surface, providing enhanced drug retention and controlled release. In vivo studies showed improvements in inflammation, tear film stability, and ocular comfort, suggesting its potential for treating DED and other chronic conditions requiring sustained drug delivery.

3. METHODOLOGY

3.1. Samples

Tacrolimus samples of pharmaceutical grade from four separate were used. Everybody The gases used were of chromatographic quality.

3.2 Stability analysis

3.2.1 Differential scanning calorimetry (DSC)

Tacrolimus DSC curves were obtained using a closed aluminium crucible in a Shimadzu DSC-50 differential scanner calorimeter. The standard used to calibrate the device was indium (155.5 ± 0.2 ◦C). By measuring the melting heat of indium (29.49 ± 0.2J g−1), the heat flow signal was calibrated. DSC analyses were carried out with a sample quantity of 2.0 ± 0.2 mg, in the temperature range of 30–300 ◦C, with heating rates of 5, 10, 20, and 30 ◦C min−1 and a dry nitrogen flow of 60 mL min−1. Shimadzu's Tasys software was used to analyse the DSC data. These results offer important new information about Tacrolimus's purity, stability, and possible polymorphism behavior—all crucial factors for improving the drug's medicinal formulation and guaranteeing product quality.

3.2.2 DSC-photovisual techniques

The differential scanning calorimeter connected to a photovisual system that included a camera and a microscope was used to record the DSC-Photovisual data. The same nitrogen flow conditions from traditional DSC were used to heat the samples in an opened aluminium crucible between 30 and 300 ◦C at a heating rate of 15 ◦C min−1. In order to see the phase transitions in the samples, the Asymetrix® DVP 4.0 application captured the images in real time.

4. Results and analysis

4.1 Results of Differential scanning calorimetry (DSC)

Two endothermic phase transitions were found in the tacrolimus DSC curves at heating rates of 5, 10, 20, and 30 ◦C min−1. The evaporation of solvents, including water, must be connected to the first endothermic peak, which happened between 100 and 110 ◦C. Water is typically included in the crystal structures of tacrolimus pharmaceutical raw materials, commonly known as hydrates. Although some writers used DSC to do pre-formulation experiments for tacrolimus monohydrate, they were able to identify a distinct endothermic peak of water in the substance.

All samples exhibited changes in the temperature of phase transition as a function of heating rate, with the second endothermic peak representing the melting point of tacrolimus. The fusion peaks at various heating rates varied between 112.2± 1.0 ◦C and 130.0 ± 1.0 ◦C for heating rates of 2 to 30 ◦C min−1, respectively,

according to the comparison. The fusion peaks of the samples under study were similar at the same heating rate.

The presence of many tacrolimus hydrate forms in the tacrolimus samples is indicated by the large range of temperatures that correlate to the peak of fusion. At a heating rate of 20 ◦C min−1, the melting temperatures of tacrolimus were TCR A (122.3 ◦C), B (124.4 ◦C), C (128.3 ◦C), and D (129.0 ◦C). It was required to standardize the heating rate in traditional DSC at 30 ◦C min−1.

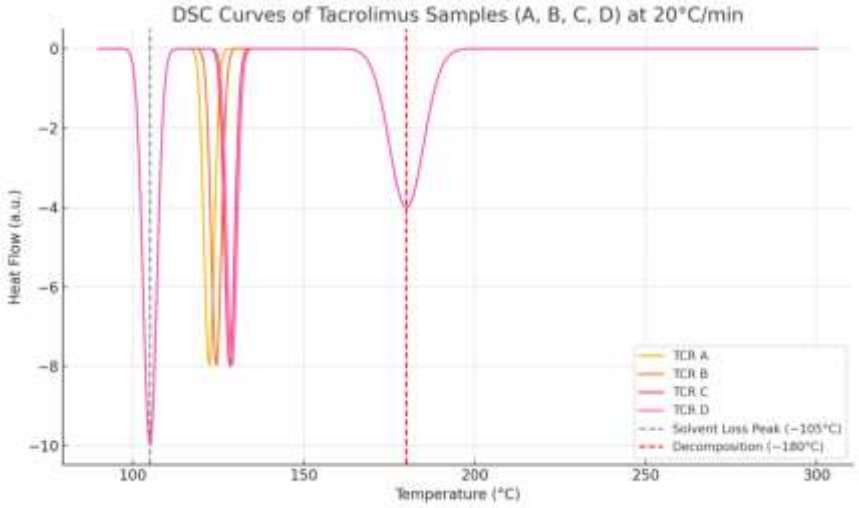

Figure 1: Analysis of tacrolimus Differential scanning calorimetry (DSC)

4.2 Results analysis of DSC-photovisual techniques

As per the analysis at 120 °C, the samples began to melt with volume contraction, most likely as a result of water volatilization as reported in the DSC analysis. Water has also been found in tacrolimus raw material by DSC-photovisual analysis. When the temperature reached 130 ◦C, a noticeable colour shift was seen. At this temperature of around 180 ◦C, tacrolimus samples exhibited comparable physical behavior, confirming the beginning temperature of breakdown. This behavior was validated by conventional DSC, , primarily when the DSC curves at 3 ◦C min−1 showed an endothermic peak at this temperature. At 280 ◦C, tacrolimus showed noticeable disintegration.

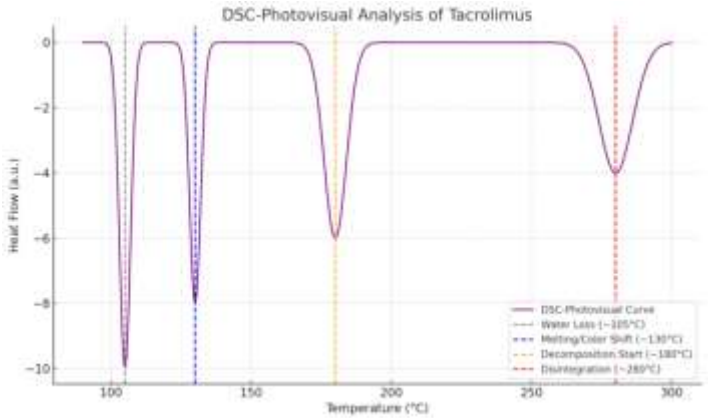

Figure 2: Analysis of tacrolimus DSC-photovisual

4.3 Accelerated stability analysis

According to ICH recommendations, which call for 25°±2°C/60±5%RH for the first 30 days and 40°±2°C/75±5%RH for the next 90 days, accelerated stability studies were carried out in best formulation D. The findings show that the appearance, pH, drug content, and in-vitro drug release tests did not alter significantly. The outcomes are displayed in figure 3.

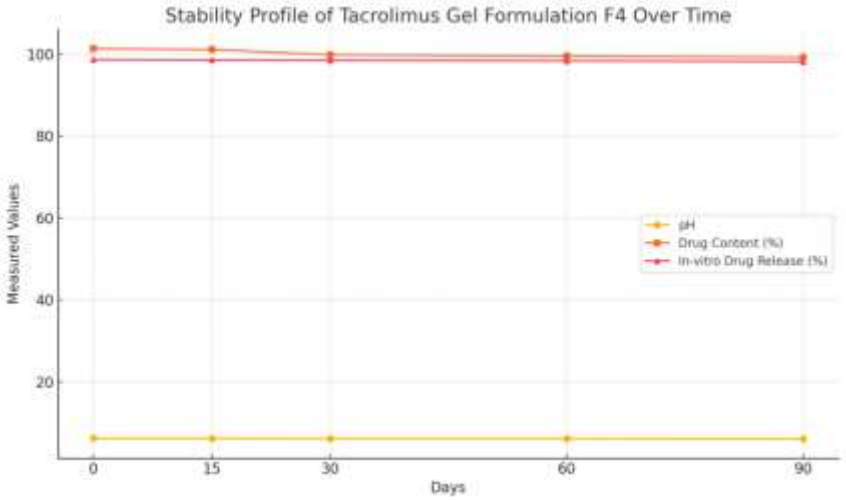

Figure 3: Accelerated stability analysis

Graphic analysis shows that the Tacrolimus gel formulation D's stability profile over a period of ninety days. The curve shows slight but steady drops in pH, drug content, and in-vitro drug release over various time periods, which is suggestive of stable formulation behavior in both ambient and accelerated circumstances.

4.4 Drug release kinetics

To determine the pattern of drug release, in-vitro drug release was analyzed based the Liposomes technique. Usually between 100 and 300 nm in size was taken. Liposomes containing substances such as tacrolimus have shown enhanced deposition in the dermal and epidermal layers and penetration into the stratum corneum. Compared to traditional formulations, liposomal tacrolimus produced superior local retention, decreased systemic absorption, and accelerated repigmentation in vitiligo.

4.5 HPLC Analysis of Tacrolimus

The identity, purity, and quantitative characteristics of Tacrolimus API were confirmed using a validated High-Performance Liquid Chromatography (HPLC) method. A distinct, sharp, and symmetrical peak was observed at a detection wavelength of 210 nm, indicating the high specificity of the method without any interference from formulation excipients or degradation products. The chromatographic conditions, utilizing a mobile phase composed of acetonitrile and water in an 85:15 (v/v) ratio, provided excellent separation efficiency with optimal retention time and peak symmetry. Linearity of the method was established across a concentration range of 25 to 250 µg/mL, with a correlation coefficient (R^2) well within the acceptable limits, confirming its applicability for precise quantitative estimations. The method demonstrated high sensitivity, with a calculated limit of detection (LOD) of 4.86 µg and a limit of quantification (LOQ) of 14.73 µg, enabling the accurate detection of even trace amounts of Tacrolimus. Accuracy was validated through a mean recovery rate of 101.05%, indicating negligible loss during sample

processing and affirming method reliability. Overall, the developed HPLC method proved to be specific, accurate, sensitive, and reproducible, making it highly suitable for routine quality control analysis and confirmation of Tacrolimus API in bulk drug evaluation.

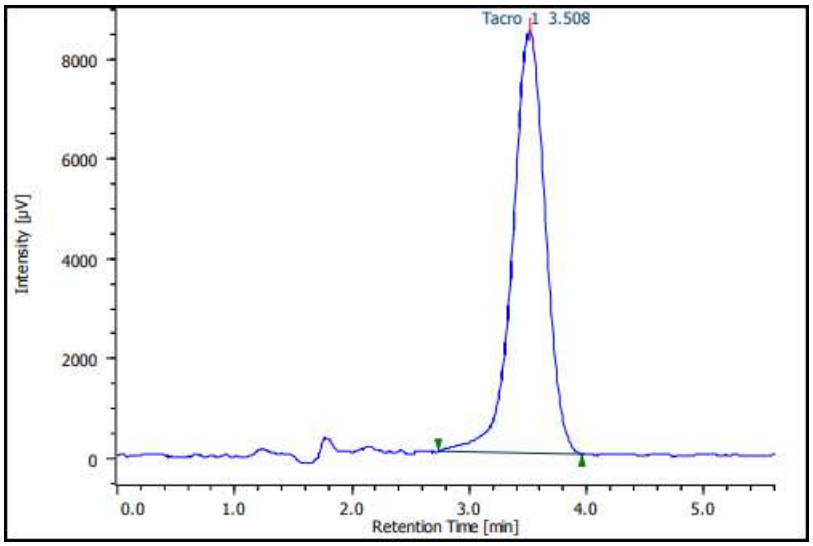

Figure 4: Tacrolimus API – HPLC Chromatogram

5. CONCLUSION

To guarantee constant quality and therapeutic performance, this study sought to create and assess tacrolimus gel formulations with improved long-term chemical and physical stability. Differential Scanning Calorimetry (DSC) and DSC-photovisual methods were used to analyse tacrolimus's phase transitions and thermal behaviour in detail. The melting point, which varied between 112.2 °C and 130.0 °C across different heating rates, indicated multiple hydrate forms. The first major endothermic peak, which was linked to solvent (water) evaporation from tacrolimus hydrates, was observed between 100 and 110 °C. According to visual analysis, melting started at about 120 °C with volume contraction, followed by colour changes at about 130 °C and the start of thermal deterioration at about 280 °C. Under ICH-recommended settings, accelerated stability testing of the optimised formulation (Formulation D) showed no appreciable changes in appearance, pH, drug content, or in-vitro drug release during the course of the study. These findings validate the developed tacrolimus gel's appropriateness for long-term storage and efficient drug delivery, particularly when paired with nano-based delivery systems for enhanced skin penetration. They also confirm the gel's physical and chemical stability.

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