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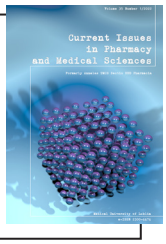

Formulation and development of Tacrolimus nanosponges-loaded hydrogel for the treatment of atopic dermatitis

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ABSTRACT

Targeted drug delivery to specific locations is a significant challenge encountered by researchers. Nanosponges have emerged as a promising solution for delivering drugs to their target sites. Nanosponges are nanoparticles with a large surface area, allowing for enhanced penetration compared to conventional drug delivery systems. This new technology offers controlled drug release and has several advantages over other methods. This project aimed to prepare and evaluate a Tacrolimus-loaded Nanosponges hydrogel. The goal was to create a dermatological gel suitable for local drug delivery after topical application, aiming for improved therapeutic effects, better dispersibility and good storage stability. Tacrolimus-loaded Nanosponge was prepared using beta-cyclodextrin and ethylcellulose as polymers, along with polyvinyl alcohol (PVA) as a surfactant. The solvent emulsion diffusion technique is a method for preparing nanosponges, and the variations in the formulation using different polymers and drug-polymer ratios offer the opportunity to fine-tune the nanosponge's properties to meet specific requirements for drug delivery applications. A Tacrolimus-loaded nanosponge hydrogel was formulated using carbopol 934. The results of the study indicated that the optimized batch of nanosponges with high entrapment efficiency was chosen for formulating the gel incorporating carbopol 934. The gel that was prepared was subjected to assessments for pH, viscosity, spreadability, *in-vitro* diffusion, permeation and stability. The findings suggest that Tacrolimus can be effectively formulated in low doses of nanosponges-loaded hydrogel for atopic dermatitis.

INTRODUCTION

Transdermal drug delivery systems are designed to be administered as individual units or doses that regulate the drug release from the skin into systemic circulation when used for topical purposes. The oral route of administration is widely used due to its ease of administration; however, it has drawbacks such as low bioavailability and rapid spikes in blood levels, which necessitate high or frequent doses. The task of precise drug distribution has posed a persistent hurdle for scientific investigators in the medical field. Their goal is to transport medications to precise locations within the body, synchronizing the release of drugs effectively, managing their impact and curative outcomes, guaranteeing safety, and averting the risk of excessive effects. Developing a new drug delivery system is crucial to overcome these challenges and to enhance drug efficacy and safety [1].

Recently, nanosponges have emerged as a promising drug delivery tool. Nanosponges can solubilize drugs that exhibit poor water solubility, resulting in extended drug release and enhanced bioavailability. This is because nanosponges possess an internal hydrophobic cavity and an external hydrophilic branch, allowing them to encapsulate drug molecules that are both hydrophilic and hydrophobic [2].

The FDA has approved (Tacrolimus) in dermatological medications at different concentrations. Topical calcium neuro inhibitors do not affect skin development and have a selective mechanism that does not interfere with collagen synthesis, making them suitable as corticosteroid-sparing agents [3,4]. Although patients with autoimmune skin diseases usually tolerate topical Tacrolimus preparations well, they might encounter notable adverse reactions, among others, sensations of itchiness, heat, and burning at the site of application during the initial treatment [5].

Modern pharmaceutical research has advanced significantly with the creation of a Tacrolimus-loaded nanosponge hydrogel, notably in targeted topical drug delivery. A flexible carrier for drug encapsulation and controlled release, the hydrogel is a three-dimensional network structure that is

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swelled with water [6]. This innovative hydrogel system accommodates the stable suspension of Tacrolimus-loaded nanosponges, thus enabling efficient transdermal drug administration. Through rigorous experimentation and analysis, this study endeavors to provide valuable insights, contribute to the advancement of medical science and patient care, evaluate the *in vitro* release of Tacrolimus-loaded nanosponges, and offer a novel alternative to conventional therapy.

MATERIAL AND METHODS

Material

The Tacrolimus sample was gifted by Glenmark Pharmaceuticals, Nashik. Ethyl Cellulose, Beta-Cyclodextrin, Carbopol 934, Polyvinyl Alcohol, Propyl Paraben, Triethanolamine, and Methyl Paraben were purchased from a local vendor in Nashik (Modern Science).

Experimental work

Pre- formulation study

In the formulation development process, having a comprehensive understanding of ensuring that the properties of the drugs are well understood and characterized is of utmost importance to preventing formulation issues in later stages of drug development.

Characterization of Drug

Organoleptic properties of tacrolimus

The color of the tacrolimus sample was evaluated visually. We observed the appearance and odor of a small sample of tacrolimus using visual methods.

Melting point

The Tacrolimus melting point was settled using the capillary tube method. A small quantity of the Tacrolimus sample was introduced into a sealed capillary tube, and its open end was subsequently sealed shut. The capillary tube was then inserted into a Thiele's tube, and a thermometer was securely attached to the Thiele's tube using a rubber band. The heating process was initiated to gradually increase the temperature, and the thermometer recorded the temperature changes.

Determination of λmax by UV spectrophotometer method

A Shimadzu UV-visible spectrophotometer (Shimadzu UV1800) with a scanning range of 200-400 nm was employed to achieve Tacrolimus's maximum absorbance. The sample solution of Tacrolimus was prepared using methanol, and readings were recorded using a digital double-beam recording spectrophotometer. A 10-mm pair of quartz cells was utilized for the measurement of the spectral absorbance, and the UV spectrum was visually determined in a graph [9].

Fourier Transform Infrared Spectroscopy (FT-IR)

The IR spectrum of untainted Tacrolimus was determined using a Shimadzu 8400 FT-IR spectrometer. The sample was prepared using the KBr disc method, where a small amount of Tacrolimus was mixed with potassium bromide (KBr) to create a thin disc. The IR spectrum was obtained in transmission mode, meaning that the IR radiation passed through the sample. The assessment was carried out in the infrared region, encompassing frequencies ranging from 4000 to 400 cm^1 [10].

Formula design

Tacrolimus-loaded nanosponges batches were formulated using the solvent emulsion diffusion technique. Two different polymers, beta-cyclodextrin and ethylcellulose, were utilized in the formulation. A total of 8 batches were formulated for optimization, with 4 batches formulated using beta-cyclodextrin and ethylcellulose and the other 4 batches using ethylcellulose as the polymer. Within these batches, the ratio of drug to polymer was altered, ranging from 1:1 to 1:4 [11]. The preparation procedure consisted of two stages: the organic phase and the aqueous phase. During the organic phase, 100 mg of Tacrolimus along with a designated quantity of ethylcellulose were added to dichloromethane. In the aqueous phase, a specific amount of beta-cyclodextrin (for F5-F6 batches) and polyvinyl alcohol (PVA) were dissolved in distilled water. The subsequent stage included slowly introducing the dispersed phase into the aqueous phase using dropwise addition, all the while maintaining stirring with a magnetic stirrer. The mixture was then kept to homogenize for 2 hours at 1000 rpm. The resultant nanosponges were gathered using filtration and subsequently subjected to drying in a hot air oven at a temperature of 40°C for 24 hours. To eliminate any remaining solvent, the nanosponges were placed in a vacuum desiccator. The same technique was used for all the batches, with only the drug-to-polymer ratios varying among them.

Table 1. Composition of tacrolimus nanosponges

Sr. No.	Formulation Batch	Drug (mg)	Ethyl Cellulose (mg)	Beta-Cyclodextrin (mg)	Polyvinyl Alcohol (%w/v)	DCM (ml)	Distilled water	Drug: Polymer Ratio
1	F1	30	100	-	0.5	20	100	1:1
2	F2	30	200	-	0.5	20	100	1:2
3	F3	30	300	-	0.5	20	100	1:3
4	F4	30	400	-	0.5	20	100	1:4
5	F5	30	100	100	0.5	20	100	1:1
6	F6	30	100	200	0.5	20	100	1:2
7	F7	30	100	300	0.5	20	100	1:3
8	F8	30	100	400	0.5	20	100	1:4

Evaluation of Nanoparticle

Percentage yield of Nanoparticle

The nanoparticles' yield was determined by subtracting the weight of the nanosponges from the overall weight of the polymer and drug utilized in the preparation process. The nanosponges percentage yield was computed using the subsequent formula:

$$\text{Percentage Yield} = (\text{Weight of Nanosponges} / \text{Total Weight of Polymer and Drug}) \times 100$$

Drug entrapment efficiency

To determine the drug entrapment efficiency, an adequate amount of Tacrolimus nanosponges was crushed using a mortar and pestle. Then, 10 mg of the nanosponges were suspended in 10 ml of phosphate buffer at pH 7.4. After 24 hours, the solution was filtered, and the filter was appropriately diluted with phosphate buffer. Subsequently, the solution that ensued was examined by employing a UV-Vis Spectrophotometer. Accordingly:

$$\text{Drug Entrapment Efficiency} = \frac{\text{Experimental drug loading}}{\text{Theoretical drug loading}} \times 100$$

Actual drug content

For one hour, an accurately measured amount of nanosponges containing drugs was held within a 100-ml volume of phosphate buffer having a pH of 7.4. The solution was constantly stirred during this time to ensure uniform mixing. Subsequently, the solution that ensured was examined by employing a UV-Vis Spectrophotometer. Here:

$$\text{Actual Drug Content (\%)} = \frac{\text{Nact}}{\text{Nms}} \times 100$$

where:
Nact – Actual drug content of nanosponges,
Nms – Weight of nanosponges.

FTIR Spectroscopy of formulation (F6)

The FT-IR spectrum of the formulation was obtained using an FT-IR spectrophotometer. To record the spectrum, an adequate amount of sample and potassium bromide were combined in a specified ratio of 1:99 to record the spectra. After that, a mixture was employed to calibrate the spectra between 400 and 400 cm (-1).

Particle size analysis

At 25°C, the light scattering technique is used to ascertain the average mean diameter and size distribution of the loaded nanosponges. To generate the light distribution intensity needed for the optimized Tacrolimus nanosponges, dried nanosponges are first dispersed in water.

Determination of zeta potential

The zeta potential is determined based on electrophoretic mobility. Zeta potential plays a pivotal role in assessing the surface charge and the nanosponge's stability. Zeta potential, also known as 'electrophoretic mobility', provides valuable information about the surface charge of nanoparticles. Its significance lies in understanding the nanosponge's stability.

Scanning Electron Microscopy (SEM)

The micro characteristics, such as the form and shape, of the optimized Tacrolimus nanosponges were analyzed using scanning electron microscopy (SEM) testing. SEM photos were obtained at various magnifications using nanosponges, which were produced and carefully dried to minimize moisture content.

Preparation of Tacrolimus nanosponge topical gel

The composition of the hydrogel formulation, including the specific amounts of each ingredient, is detailed in Table 2. This table provides a comprehensive overview of the different components and their respective quantities in the final nanosponge-loaded gel product. The formulation process of the nanosponge gel containing Tacrolimus involved several steps:

1. Preparation of gelling agent: Different amounts of carbopol 934, a gelling agent, were immersed and dissolved in an inadequate amount of water to achieve good dispersion. The soaking was done overnight to ensure proper hydration and swelling of the gelling agent. After 24 hours, the remaining ingredients were added to the gelling agent solution [19].
2. Dispersion of Tacrolimus nanosponges: In a separate beaker, the Tacrolimus-loaded nanosponge was dispersed in water to create a stable suspension.
3. Combining ingredients: The Tacrolimus-loaded nanosponge dispersion was then added to the beaker containing the gelling agent and other excipients, forming the Tacrolimus-loaded nanosponge hydrogel formulation. Triethanolamine was added drop by drop to achieve pH balance.

Table 2. Tacrolimus-loaded nanosponge Hydrogel's composition

Sr. No.	Ingredient	Formulation batch			
		C1	C2	C3	C4
1	Nanosponges (mg)	30	30	30	30
2	Carbapol 934 (gm)	0.5	1	1.5	2
3	Propylene Glycol (ml)	5	5	5	5
4	Methyl Paraben (mg)	0.1	0.1	0.1	0.1
5	Propyl Paraben (mg)	0.01	0.01	0.01	0.01
7	Distilled water (ml)	10	10	10	10
6	Triethanolamine (ml)	q.s	q.s	q.s	q.s

Evaluation of hydrogel

Physical evaluation

The hydrogel of Tacrolimus-loaded nanosponges was evaluated to ascertain their uniformity, color, pH level and the existence of any lumps through a visual inspection.

Actual Drug content

Using a UV-visible spectrophotometer (Shimadzu, UV2600), we measured the absorbance at 293 nm of a 5 ml sample extracted from a solution containing 10mg of gel dissolved in 100 ml phosphate buffer with a pH of 7.4 and further diluted to 10 ml. The purpose of this process was to ascertain the drug content.

Spreadability

The spreadability of the formulated hydrogel was assessed. The procedure involved placing the gel between two glass slides in the spreadability apparatus and attaching a weight to the upper glass slide. Subsequently, time was recorded for the gel to slide over both slides. To compute

the spreadability of the gel, the subsequent formula was employed:

$$S = M \cdot L/T$$

where: S = Spreadability
M = weight tied to upper slide
L = lengyh of the glass slide
T = Time taken toseparate the 2 slides (sec.)

In vitro drug release study

In the *in vitro* diffusion study, a hydrogel loaded with nanosponge was tested using the Franz diffusion cell apparatus. A cellophane membrane separated the donor compartment (gel side) from the receptor compartment. The receiving compartment was filled with a pH 7.4 phosphate buffer, and a consistent temperature of 37±0.5°C was maintained using a magnetic stirrer. To begin the experiment, 0.1 g of the hydrogel was put on the cellophane membrane. Over a time interval of 8 hours, a 1 ml sample was taken out of the receptor compartment and adequately diluted. To maintain the experimental conditions, at regular intervals, a sample was removed and replaced with an equal amount of pH 7.4 phosphate buffer[17].

The diluted sample was analyzed for the concentration of TAC (Tacrolimus) using a UV-visible spectrophotometer at a wavelength of 293 nm.

Permeation studies

In this study, a comprehensive comparison was conducted between a commercially available marketed product (TACROZ®) ointment and prepared hydrogel, utilizing a Franz diffusion cell apparatus as the experimental platform. The primary objective was to investigate the permeation characteristics of both formulations through pig ear skin, which serves as a relevant model for human skin due to its similarities in structure and permeability [20]. The experiments were conducted using a pH 7.4 phosphate buffer as the solvent to replicate physiological conditions. The diffusion cell apparatus was maintained at a constant temperature of 37.5°C to simulate human body temperature. Over 8 hours, samples were collected at regular intervals ranging from 5 minutes to 8 hours while maintaining sink conditions, enabling a comprehensive understanding of the permeation profiles over time. This rigorous testing methodology aimed to provide valuable insights into the comparative performance of the two formulations in terms of their skin permeation behavior and potential implications for topical application.

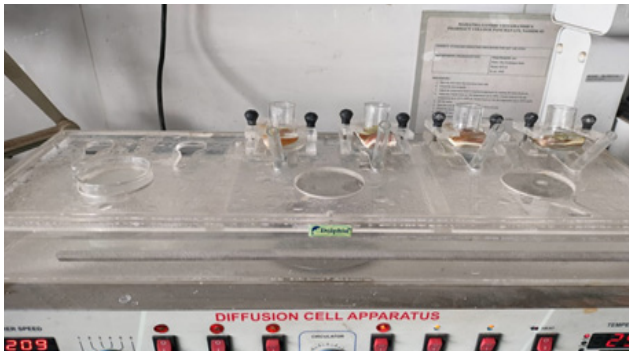

Figure 1. Permeation study by using Franz diffusion cell apparats

Stability study

By the guidelines set forth by the International Council for Harmonization (ICH), a comprehensive stability evaluation was conducted for the transdermal hydrogel formulation. Adhering to the ICH recommendations, accelerated stability studies were meticulously executed over six months, with assessment points at 1, 3 and 6 months. These studies were conducted under controlled conditions, specifically at a relative humidity of 60% and a constant temperature of 25°C. The primary focus of these accelerated studies was to investigate any potential changes in the pH of the hydrogel and alterations in its physical appearance.

RESULTS

Pre-formulation study of drug

Organoleptic Properties

Organoleptic characteristics of the drug samples were observed, and the outcomes are given in Table 3.

Table 3. Organoleptic properties of Tacrolimus

Identification Test	Observation	Reported Standard
Appearance	Amorphous powder	Amorphous powder
Color	White	White
Odor	Odorless	Odorless

Melting point

The provided information includes the melting point of Tacrolimus.

Table 4. Melting point of Tacrolimus against a reference value

Sr. No.	Observation	Reported Standard
1	126°C	126-128°C

UV – Visible spectroscopy of Tacrolimus

The UV spectrum of the solution of Tacrolimus dissolved in methanol exhibits a peak absorbance at 293nm. The spectrum of Tacrolimus is depicted in Figure 2.

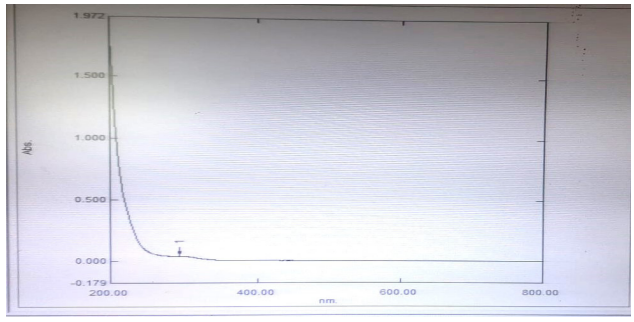

Figure 2. UV absorption spectrum of Tacrolimus in methanol

The Tacrolimus sample exhibited its maximum absorption at 293 nm, making it the chosen wavelength for further studies.

Construction of Beers-Lambert's plot in methanol

The concentration range of Tacrolimus in methanol exhibited a linear calibration curve with a linear calibration curve of 10 to 50 µg/ml, with a coefficient of regression value (R^2) of 0.9972

Table 5. Absorbance of different concentrations of Tacrolimus in methanol

Sr. No.	Concentration (µg/ml)	Absorbance (AUC) at 293 nm
1	Blank	0.000
2	10	0.0715
3	20	0.122
4	30	0.178
5	40	0.228
6	50	0.284

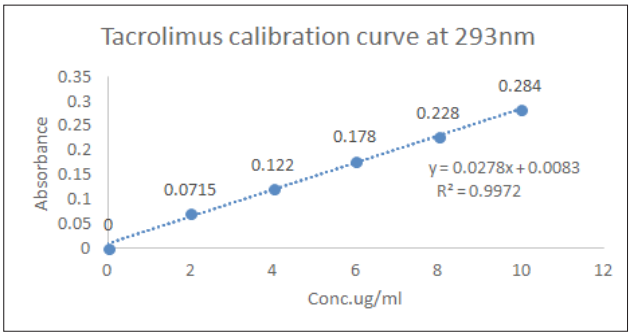

Figure 3. Calibration curve of Tacrolimus in methanol

Fourier- transform infrared spectroscopic studies (FT-IR)

The FT-IR spectrum of Tacrolimus was obtained using an FT-IR spectrophotometer. The recorded results are indicated in Figure 4 and Table 6

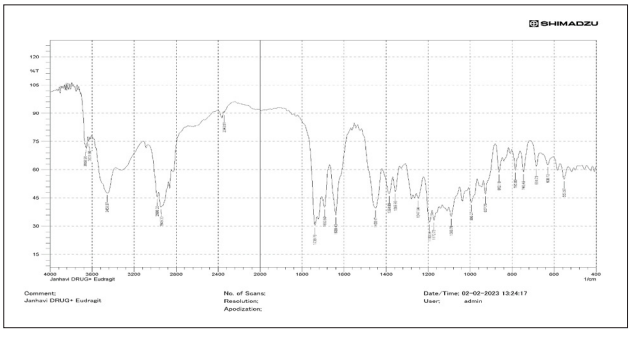

Figure 4. FTIR of Tacrolimus pure Drug

Table 6. Major peaks observed in the FTIR spectrum of Tacrolimus

Functional group	Observed ranges cm-1	Standard ranges cm-1
O-H Stretching	3458	2500-3300
C=O Stretching (Ester)	1739	1735-1750
C=C Stretching	1639	1626-1662
C-O-C Stretching (Ether)	1089	100-1300

Evaluation of formulate tacrolimus loaded nanosponges

Table 7. Production yield, entrapment efficiency and actual drug content (%) Tacrolimus loaded nanosponges

Sr. No.	Formulation Batch	% Production Yield	% Entrapment efficiency	Actual Drug Content (%)
1	F1	78.41	93.43±0.02	75.04
2	F2	88.24	94.90±0.04	59.45
3	F3	86.10	88.97±0.02	79.36
4	F4	75.9	94.05±0.02	83.21
5	F5	89.44	91.77±0.01	83.21
6	F6	90.5	95.33±0.07	88.43
7	F7	75.24	93.35±0.06	50.64
8	F8	68.38	90.12±0.06	44.25

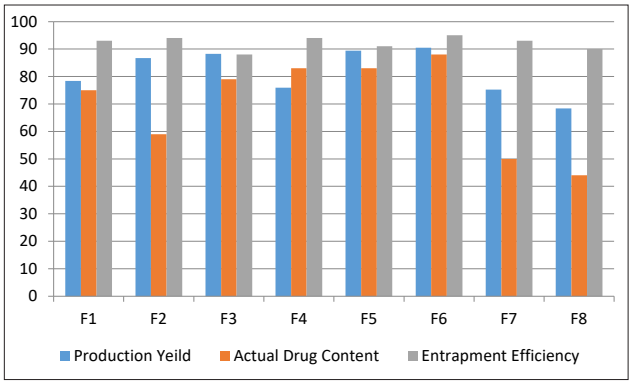

Figure 5. Production yield, entrapment efficiency and actual drug content (%)Tacrolimus loaded nanosponges

Figure 6. Formulation batches of Tacrolimus loaded nanosponges

FTIR spectroscopy of Tacrolimus nanosponges

The FTIR spectrum of Tacrolimus nanosponges from the F6 batch with ethyl cellulose and beta-cyclodextrin is depicted in Figure 7.

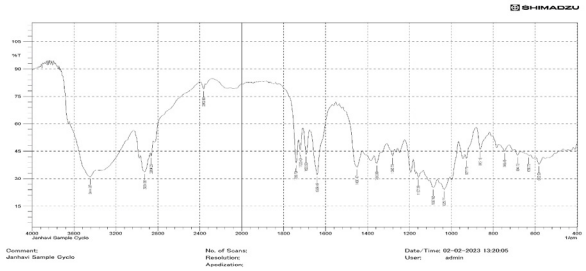

Figure 7. FT-IR spectra of Optimized batch (F6)

Particle size measurement

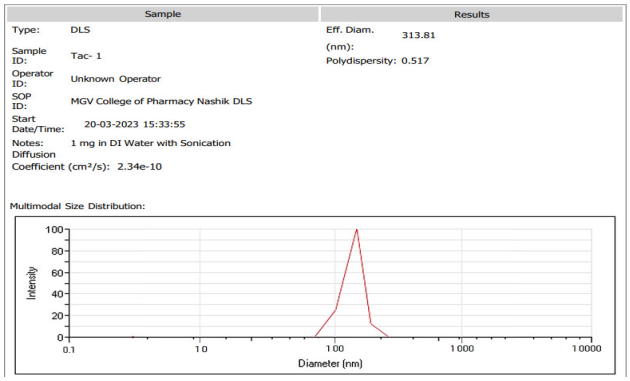

Figure 8. Particle size of an optimized batch of nanosponge (F6)

Zeta Potential

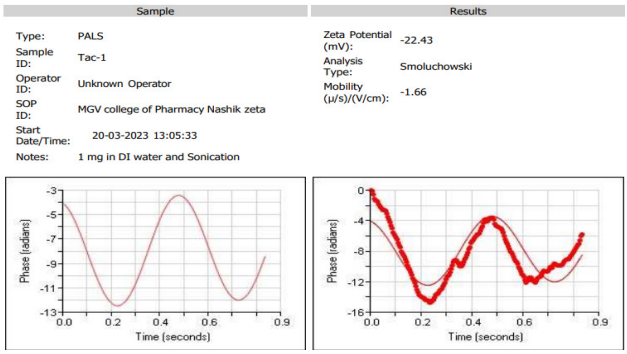

Figure 9. Zeta potential of an optimized batch of nanosponge(F6)

Scanning electron microscopy

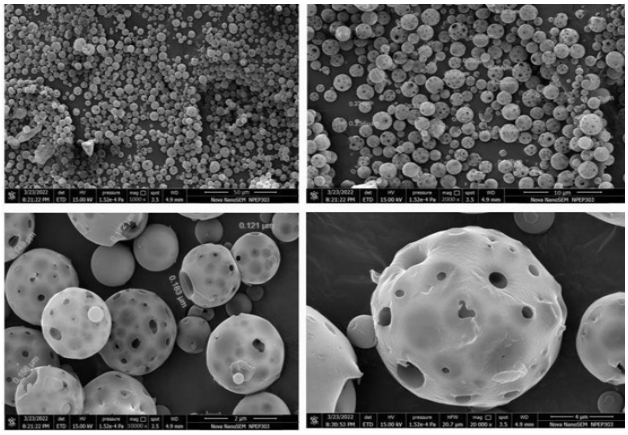

Figure 10. SEM images of an optimized batch of nanosponges (F6)

Evaluation of Topical Gel

Table 8. pH, actual drug content and spreadability of hydrogel

Formulation Batch	pH	Spreadability (gm.cm/sec)	Drug Content (%)	Homogeneity
C1	6.24	22.88	88.68	Good
C2	6.80	21.53	86.62	Good
C3	6.71	19.82	85.45	Good
C4	6.62	19.35	84.32	Good

Figure 11. Formulation batches of Tacrolimus nanosponge loaded hydrogel

In-vitro drug release study

Table 9. *In-vitro* drug release of hydrogel batch C1 to C4

Sr. No.	Time (min.)	Formulation Batch			
		C1	C2	C3	C4
1	5	12.26	10.32	7.52	6.766
2	15	21.68	17.67	10.73	10.186
3	30	26.78	21.75	17.86	15.69
4	60	30.20	25.87	22.70	20.83
5	120	35.92	33.06	28.02	25.80
6	180	41.38	37.30	34.91	33.85
7	240	46.68	44.29	40.144	37.35
8	300	53.01	48.86	46.40	44.76
9	360	58.43	53.27	54.344	51.29
10	420	67.14	57.179	57.189	54.75
11	480	75.73	70.94	67.18	64.76

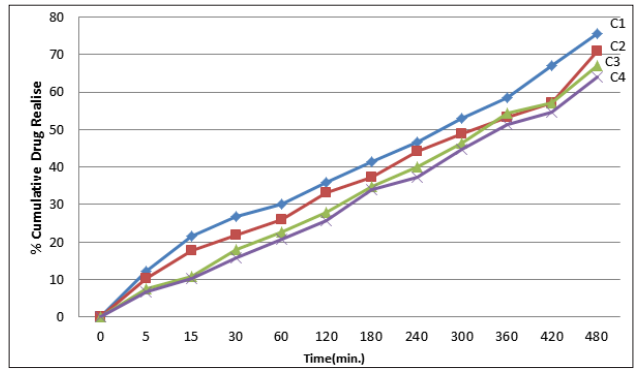

Figure 12. *In-vitro* drug release of hydrogel batch C1 to C4

Kinetic assessment of formulated Tacrolimus nanosponge-loaded hydrogel

Table 10. Regression coefficient value of *in vitro* studies

Batches	Coefficient of Regression R2					
	Zero-order	First order	Higuchi model	Hixon model	Korsmeyer Peppas	
					R^2	n
F1	0.9233	0.9138	0.9687	0.9493	0.8978	23.3

Permeation study

Table 11. Comparison of drug release between formulated hydrogel and marketed product

Batches	Time in Min.											
	0	5	15	30	60	120	780	240	300	360	420	480
Prepared Hydrogel	0	0.39	1.15	3.86	8.01	12.4	17.7	21.1	25.8	30.5	35.5	41.5
Marketed Batch	0	0.07	0.40	1.59	5.82	9.01	12.6	15.8	17.2	19.8	22.4	24.1

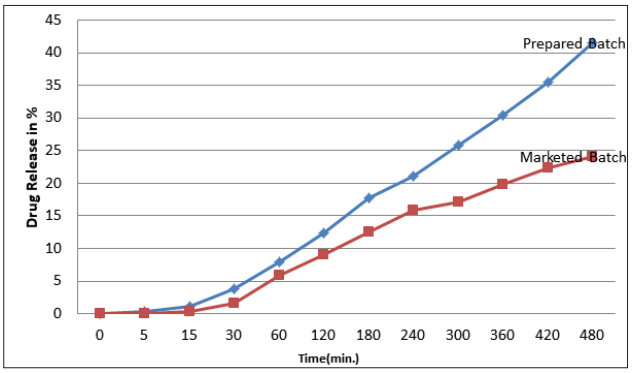

Figure 13. *In vitro* drug release of hydrogel and marketed product

DISCUSSION

Pre-formulation Study of Drug

Organoleptic properties

In the pre-formulation study, the identification of the sample was conducted, and the organoleptic properties of the drug were evaluated. The results of this assessment indicated that the organoleptic characteristics of the drug were consistent with those mentioned in relevant literature and books.

Melting point

The melting point of the sample is close to that mentioned in official standards. This indicates that the samples purchased are obtained as pure.

Fourier – transform infrared spectroscopic studies (FT-IR)

The figure displaying the FT-IR spectrum exhibited several characteristic bands for pure Tacrolimus. These include: A band related to the free stretching and vibrating at 3458 cm^-1. A band related to the C=O stretching and vibrating at 1739 cm^-1. Another band has C=O stretch vibrations of ketone at 1693 cm^-1. The band represents the C–O–C stretch of ether at 1089 cm^-1. Further validating the identity and purity of the provided Tacrolimus sample. The similarity of the obtained FT-IR spectrum with the reported literature data provides further confirmation of the correct identification of Tacrolimus.

Evaluation of formulate tacrolimus loaded nanosponges

Production yield (%)

Results suggest that the percentage yield may vary with the concentration of the polymer used in the formulations. The percentage yield values for all formulations are summarised in Table 7, and the graphical representation can be seen in Figure 5.

Entrapment efficiency (%)

The entrapment efficiency of the different formulations was evaluated, and the highest value was observed for the F6 formulation batch, which exhibited an entrapment efficiency of 95.33%. The entrapment efficiency values for all the formulations are presented in Table 7, and a graphical representation can be seen in Figure 5.

Actual drug content

Drug content analysis can be used to assess the uniform dispersion of Tacrolimus nanosponges. The optimized F6 batch of nanosponges demonstrated a uniform distribution of the drug, with 88.43% of the drug effectively incorporated into the nanosponges. Table 7 contains the drug content analysis results for all batches, and a graphical representation is in Figure 5.

FTIR Spectroscopy of Tacrolimus nanosponges

The FTIR spectrum of Tacrolimus nanosponges formulated with ethyl cellulose and beta-cyclodextrin is given in Figure 7. This spectrum is compared with the FTIR spectra of Tacrolimus. In the pure Tacrolimus spectrum, specific bands were observed. However, in the FTIR spectrum of the F6 formulation (Tacrolimus nanosponges), it was discovered that the peak corresponding to the C-O-C stretching at 1089 cm1 was either absent or had a lower intensity. The indication is that the drug was effectively encapsulated within the nanosponges of the F6 formulation.

Particle size measurement

The desired particle size for the nanosponges is less than 1. The particle size reports indicated that the size of the Tacrolimus nanosponges was within the specified standard diameter.

The results of the mean particle size analysis and the comparison to the desired size are presented in Figure 8.

Zeta potential

The Zetaplus zeta-sizer was employed to measure the zeta potential. For Tacrolimus with Beta-Cyclodextrin (Batch F6), the calculated zeta potential was found to be -21.4 mV, with a peak region indicating 100% intensity along with it. These outcomes demonstrate the F6 batch's stability. Figure 9 shows the zeta potential analysis for batch F6.

Scanning electron microscopy

The characteristic spongy and porous structure of the nanosponges is evident in the figures provided. Specifically, Figure 10 displays the SEM image of the Tacrolimus-loaded nanosponges.

Evaluation of topical gel

Physical evaluation

All batches of the formulated product exhibited a pleasing appearance, appearing transparent and having a smooth consistency. Additionally, the viscosity of all batches was uniform and homogeneous.

PH of topical gel

The pH values of all the formulated gel batches were found to be within a pH range that is suitable for the skin's typical pH range. Specifically, all hydrogel compositions pH values ranged from 6.80 to 6.24. These findings suggest that the hydrogel preparations were non-irritating to the skin.

Drug content in hydrogel

The nanosponges gel formulation's drug content examination produced satisfactory findings, suggesting that the drug was evenly distributed throughout all the formulations. The range of the drug content percentage was found to be between 84.32 and 88.68. Table 8 provides a list of the topical gel compositions' actual medication contents.

Spreadability of hydrogel

Spreadability is a crucial attribute of topical formulations as it guarantees accurate dosage application to the intended area and holds significant importance in hydrogel formulation. The spreadability of the formulated nanosponges hydrogel was determined to be within the range of 19.35 to 22.88 gm/sec. The spreadability values for the various topical gel formulations can be found in Table 8.

In-vitro drug release study

During the *in vitro* drug release assessment of formulations C1 to C4, it was observed that the rate of drug release reduced as the carbopol 934 concentration in the gel formulation increased. The *in vitro* release of gel formulations with varying concentrations of carbopol 934 was analyzed and is displayed in Table 9. The outcomes highlighted that the drug's release rate from these nanosponges-loaded topical gel formulations was influenced by the concentration of carbopol 934. The formulated gel demonstrated sustained drug release for up to 8 hours. The cumulative *in-vitro* drug release is shown graphically in Figure 12.

Kinetic assessment of formulated Tacrolimus nanosponge-loaded hydrogel

To understand how drug release from formulations works, the *in-vitro* diffusion study data was examined through different mathematical models. For the formulation C1 batch, the R2 values for zero order and first order were determined to be 0.92 and 0.91, respectively. Based on these results, the optimized formulation C1 followed the zero-order release, which was confirmed. In the Hixon Crowell plot, the drug release data for the optimized formulation C1 resulted in a regression coefficient of 0.9493. In the Higuchi plot, the R2 value for the optimized formulation C1 was found to be 0.9687. These results indicate that a time-dependent square root mechanism based on Fickian diffusion plays a crucial role in the drug release and is followed by the drug's release from the porous, insoluble matrix.

Permeation study

Analyses and comparisons were made between the drug release characteristics of the produced hydrogel and the commercial batch. In the comparative analysis of drug release profiles between the hydrogel and the marketed batch, distinctive patterns emerged, highlighting their varying release kinetics. Figure 13 shows the hydrogel's drug release at a steady and progressive pattern over time. In contrast, the marketed batch demonstrated a slower initial release, followed by a gradual yet comparatively slower release over time.

Stability study

The evaluation of the hydrogel's physical characteristics under accelerated conditions indicates that the hydrogel displayed a remarkable capacity to uphold its physical attributes. The absence of significant alterations further reinforces the formulation's potential to retain its efficacy. This stability-focused assessment reinforces the notion that the hydrogel maintains its inherent properties, thereby bolstering its suitability for sustained application and therapeutic benefit.

CONCLUSION

Using the solvent emulsion diffusion approach, Tacrolimus was successfully integrated into nanosponges in the current study. The developed nanosponges underwent comprehensive examination across multiple parameters, encompassing physical characteristics, yield of production, Zeta potential, entrapment efficiency and particle size. The findings demonstrated that all formulations displayed favorable attributes. Among the formulations, the F6 batch showed the highest entrapment efficiency. Particle size results showed the lowest particle size, establishing it as the optimized batch owing to the synergistic utilization of ethyl cellulose polymer and beta-cyclodextrin; hence, the study proved that the incorporation of cyclodextrin enhances the solubilization and encapsulation of poorly water-soluble drugs within the nanosponges and also ethyl cellulose for stability, contributing to controlled drug release and as a potential approach for improved bioavailability in the formulation process.

The average particle size and entrapment effectiveness of the optimized F6 batch were determined to be 95.33% and 313.81nm, respectively, after further analysis of the surface morphology. Tacrolimus nanosponges were incorporated into a hydrogel, which was then tested for pH, spreadability, and *in-vitro* drug diffusion. The prepared C1 hydrogel exhibited a gradual and sustained release pattern over time, while the marketed batch displayed a slower initial release followed by a comparably slower release rate. A stability study shows that the hydrogel possesses an inherent capacity to uphold its effectiveness over time.

In summary, the study focused on the development and characterization of nanosponges loaded with poorly water-soluble drugs, exploring the roles of different components in the formulation, and evaluating their potential for enhanced drug solubility and controlled release through topical gel formulations. The optimized F6 batch, utilizing a combination of ethyl cellulose and beta-cyclodextrin, appeared to be a promising candidate for further development and application. The findings suggest that Tacrolimus can be effectively formulated in low doses of nanosponges-loaded hydrogel for atopic dermatitis. However, the study recommends further extensive research to delve deeper into this formulation and its potential therapeutic applications.

Current and future aspects

Our findings suggest that Tacrolimus can be effectively formulated in low doses of nanosponges-loaded hydrogel

for the management of atopic dermatitis. However, we acknowledge the need for more extensive research to delve deeper into this formulation, assess its long-term efficacy, and explore its potential therapeutic applications. Future studies may focus on clinical trials and in vivo assessments to validate the practical utility of this innovative drug delivery system. This research contributes to the field of drug delivery, particularly for dermatological conditions, by offering a novel approach that holds promise for bettering patient outcomes in atopic dermatitis treatment and psoriasis.

LIST OF ABBREVIATIONS

FDA – Food and Drug Administration
DCM – Dichloromethane
TAC – Tacrolimus
SEM – Scanning Electron Microscopy
FT- IR – Fourier Transform Infrared Spectroscopy

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