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Research

Formulation And Evaluation Of Doxycycline Hyclate Insitu Gel For The Treatment Of Paronychia

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Abstract

The study focuses on the formulation and evaluation of doxycycline-loaded in-situ gel for the localized treatment of paronychia. The in-situ gel system was designed to provide sustained drug release and improve therapeutic efficacy while minimizing systemic side effects. Various formulations were prepared and optimized for gelling capacity, viscosity, drug encapsulation efficiency, and in-vitro drug release. The optimized formulations, particularly F3 and F4, demonstrated high drug encapsulation efficiency (up to 97.89%), excellent gelling capacity, and prolonged drug release over 24 hours. Stability studies confirmed the physical and chemical robustness of the formulations, maintaining consistent drug content and gelling properties under standard and accelerated conditions. These results highlight the potential of doxycycline-loaded in-situ gel as an effective and convenient treatment for paronychia, warranting further in-vivo and clinical investigations.

Keywords: Doxycycline, In-situ gel, Paronychia, Sustained release, Drug encapsulation efficiency, Gelling capacity, Localized.

INTRODUCTION

"Trans" originates from "through," while "unguis" refers to "nails." Therefore, the concept of a transungual drug delivery system revolves around administering medication through the nail, specifically targeting nail-related ailments. The inherent hardness and impermeability of the nail pose significant challenges

to drug delivery through this route. Nevertheless, topical therapy remains highly sought-after due to its ability to localize treatment effects, minimizing systemic side effects, and potentially enhancing patient adherence [1].

The nail plate, serving as the primary barrier to drug penetration, presents a formidable obstacle. Its dense composition of keratinized dead cells restricts the passage of drugs, resulting in only a fraction of topical medication effectively permeating through. Consequently, achieving therapeutic concentrations becomes a daunting task. Successful drug delivery across the nail necessitates a nuanced understanding of its anatomy and physiological barriers, ensuring precise drug deposition at the intended site and time [2]. The nail plate, comprising tightly packed and highly variable dead cells, exhibits diverse characteristics among individuals, ranging from size and texture variations to potential pathological conditions. Disorders affecting the nail, spanning from benign pigmentation changes to debilitating infections, not only inflict physical discomfort but also undermine social and psychological well-being, significantly impacting quality of life. Moreover, many nail ailments present persistent challenges in treatment, often requiring prolonged therapy with frequent relapses [2].

Conventional oral therapies pose risks of systemic side effects and drug interactions, while topical treatments are hindered by the limited permeability of the nail plate. Therefore, the primary objective lies in formulating delivery systems that surmount these obstacles, facilitating efficient drug penetration without compromising safety or efficacy. Various factors influence drug transport through the nail plate, including the molecular size of the drug and its hydrophilicity. Larger molecules face greater difficulty in diffusing through the dense keratin network, whereas hydrophilic formulations can exploit nail swelling to enhance permeation by enlarging pore sizes within the nail structure. By overcoming these challenges, innovative transungual drug delivery systems hold the potential to alleviate the suffering associated with nail disorders, offering new hope for improved therapeutic outcomes and enhanced patient well-being [2,3].

In-situ gel system

An in-situ gel system is a type of drug delivery system that undergoes a phase transition from a solution to a gel upon administration at the site of application, typically triggered by environmental factors or physiological conditions. This transition can occur due to various stimuli such as temperature, pH, ionic strength, or enzymatic activity. In-situ gels offer several advantages in drug delivery, but they also come with certain limitations [4].

Human nail

The human nail is a specialized structure composed primarily of a tough protein called keratin. It serves several important functions, including protecting the fingertips and toes, assisting in tasks such as gripping and scratching, and providing support to the distal phalanges (finger or toe bones). Understanding the structure of the human nail and its associated diseases is essential for diagnosing and treating various nail disorders effectively.

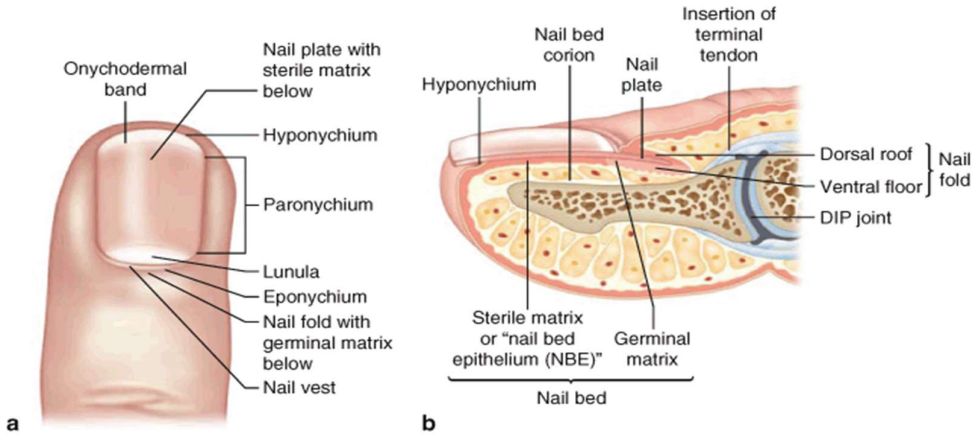


Fig 1: Structure of human nail

Paronychia

Paronychia is a common nail disorder characterized by inflammation of the tissues surrounding the nail, typically affecting the nail folds (the skin at the sides of the nail). This condition can be acute or chronic and may result from bacterial or fungal infections, trauma, or other underlying conditions. Understanding its types, symptoms, and available treatments can help manage paronychia effectively.

Hence this study was designed to formulate and evaluate a novel in-situ gel loaded with doxycycline for the treatment of paronychia. By harnessing the advantages of in-situ gel technology, such as targeted drug delivery and prolonged drug retention, our goal is to develop a therapeutic intervention that effectively addresses the underlying bacterial or fungal infections associated with paronychia. This innovative formulation has the potential to provide localized and sustained release of doxycycline directly to the site of inflammation, enhancing therapeutic efficacy while minimizing systemic exposure and adverse effects. Through rigorous evaluation of the formulation's physicochemical properties, drug release kinetics this study aims to demonstrate the feasibility of this novel approach for the management of paronychia, ultimately improving patient outcomes and quality of life.

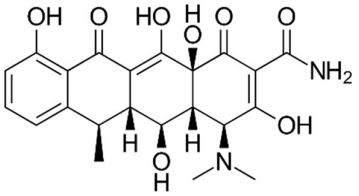
Aim And Objectives

To formulate and evaluate doxycycline hyclate loaded insitu gel for the treatment of paronychia.

- Determine solubility profile and create a standardization curve of doxycycline.
- Investigate drug-excipient interactions through FTIR and DSC analysis.
- Formulate doxycycline-loaded in situ gel and assess physical properties.
- Characterize gel appearance, clarity, drug content, viscosity, and gelling capacity.
- Conduct *in-vitro* drug release studies using cellophane membrane.
- Perform stability assessments under accelerated conditions for 3 months.

Drug And Excipient Profile

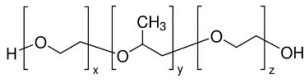
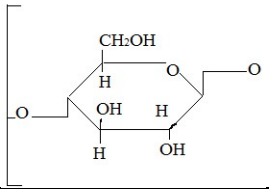
Table 1: Properties of doxycycline hyclate

Property	Value
Common Name	Doxycycline Hyclate
Nature	Antibiotic, Tetracycline derivative
State	Solid (crystalline powder)
Color	Yellow to light yellow
Taste	Bitter
IUPAC Name	(4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide hydrochloride
Molecular Formula	C22H24N2O8·HCl·0.5C2H6O·0.5H2O
Chemical Structure	
Molecular Weight	512.9 g/mol (anhydrous form)
Melting Point	Approximately 201-202°C (for doxycycline base)
Solubility	Soluble in water, slightly soluble in ethanol, practically insoluble in chloroform and ether
Biopharmaceutics Classification System (BCS)	Class I (high solubility, high permeability)

Excipients profile

Table 2: Properties of the excipients

Property	Excipient		
Common Name	**Poloxamer 188**	**Hydroxypropyl Methylcellulose**	**Labrasol**
Nature	Nonionic surfactant, thermoreversible polymer	Nonionic polymer	Surfactant, solubilizing agent
State	Liquid at room temperature, forms gel at higher temperatures	Powder	Liquid
Color	Clear, colorless	White to off-white	Yellowish clear

			liquid
Taste	Neutral	Tasteless	Neutral
IUPAC Name	polyethylene- propylene glycol copolymer	Cellulose, 2-hydroxypropyl methyl ether	Caprylocaproyl Polyoxyl-8 glycerides
Molecular Formula	$HO[(C_2H_4O)*(C_3H_6O)]nOH$	$(C_6H_{10}O_5)_n$	N/A
Chemical Structure			N/A
Melting Point	Approximately 50-60°C (gelation temperature)	Decomposes before melting	N/A
Solubility	Soluble in water	Insoluble in cold water, swells in hot water	Soluble in water, ethanol, oils
Uses in Formulation	Gel-forming agent, stabilizer, solubilizing agent	Viscosity modifier, gelling agent	Enhances solubility of poorly soluble drugs, surfactant

METHODOLOGY

1.1. Preformulation studies

1.1.1. Solubility profile

The solubility of the drug was determined according to the guidelines of IP2007.

1.1.2. Standardization Curve of doxycycline

Approximately 20.214 grams of disodium phosphate heptahydrate and 3.394 grams of sodium dihydrogen phosphate were added to a beaker containing 800 milliliters of distilled water. The volume was then made up to 1 liter, and the pH was adjusted to the desired level using either HCl or NaOH. The absorption spectra of the reference and test solutions were measured in a 1 cm quartz cell over the wavelength range of 200 to 400 nm.

Approximately 10 mg of doxycycline was accurately weighed and dissolved in 10 ml of 0.1N HCl solution to prepare the stock solution, with the volume adjusted up to the mark. Then, 2.5 ml of this stock solution was transferred to a 25 ml standard flask and diluted to the mark with 0.1N HCl solution. Serial dilutions were made to obtain final concentrations ranging from 2 to 10 µg/ml. These solutions were analyzed using a UV-visible spectrophotometer at 270 nm. A standard curve was created by plotting the concentrations (µg/ml) against absorbance. The line of best fit was then calculated from this standard curve [5].

1.2. Drug-excipient interaction studies

1.2.1. FTIR

In this study, the concentrated solution of the sample was prepared with appropriate solvents by dissolving the sample in the solvent. Alternatively, 2-5 mg of the sample was placed directly on the plates, and a drop of solvent was added to dissolve the sample. The sample was then scanned to obtain the IR spectrum. The spectrum was recorded in the wavelength range of 4000-660 cm^{-1} [6].

1.2.2. Differential scanning calorimetry

The DSC technique was carried out using doxycycline, Poloxamer 188, HPMC, and a mixture of doxycycline, HPMC, and Poloxamer in a 1:1:1 ratio. The samples were heated up to their respective boiling points to check and scan for the presence of any particulate impurities at a rate of 25-1400°C [6].

1.3. Formulation of doxycycline loaded insitu gel

The gel was prepared using a beaded magnetic stirrer. Each magnetic stirrer was rotated initially with the mentioned combination of Poloxamer188 and HPMC dissolved in 50 ml of isopropanol in one beaker, while 0.5 g of doxycycline was dissolved in 50 ml of isopropanol in another beaker. Once the drug was completely dissolved, it was added to the mixture containing Poloxamer188 and HPMC. This beaker was kept on the magnetic stirrer for 30 minutes, after which the mentioned quantity of labrasol was pipetted out and added. The gel or formulation was then kept on the magnetic stirrer for 2 hours. After a total of 14 hours, the prepared gel was obtained and stored at room temperature in an airtight container. This prepared gel was taken for further characterization study [7,8].

Table 3: Experimental design of the insitu gel formulation

S.no	Drug/ Excipient	F1	F2	F3	F4	F5
1	Doxycycline	500 mg	500 mg	500 mg	500 mg	500 mg
2	Poloxamer 188 (%)	2	3	4	3	2
3	HPMC (%)	0.50	0.75	1.00	0.25	0.75
4	Labrasol (%)	0.25	0.50	0.75	0.50	0.25

1.4. Characterization of in-situ gel

1.4.1. Visual appearance

The visual appearance was observed for the presence of any particulate matter [9].

1.4.2. Clarity

The clarity of the formulations before and after gelling was determined through the visual examination of the formulations under light, alternately against white and black backgrounds [9].

1.4.3. Drug content

Drug content was determined by dissolving a weighed quantity of formulation (1g in 100 ml) in phosphate buffer pH 6. The diluted solutions were filtered using a 0.45 µm cellophane membrane filter, and the filtered solutions were further analyzed using a UV-visible spectrophotometer at a 270 nm wavelength [5].

1.4.4. Viscosity

The viscosity of the in situ gel formulation was measured using a Brookfield Viscometer DV2T model. The formulations of the temperature-triggered in situ gel formulation were taken in the sampling tube separately and analyzed at room temperature. Temperature-dependent in situ gel formulations were performed using spindle 63, which was connected to the viscometer in a position fitted along with a Helipad stand. The samples were measured at 10 rpm for 5 minutes. The obtained values were measured in units as centipoises (cP) [10].

1.4.5. Gelling capacity of in situ gel formulation

To determine the gelling capacity of ophthalmic formulations, 100 µL of the prepared solutions was dropped in 2 ml of phosphate buffer solutions. Gelation was examined based on a standard procedure, and the formulation underwent sol to gel transition [11].

1.4.6. *In-vitro* drug release of in situ gel formulations

In-vitro release studies were carried out using cellophane membrane, and the temperature was adjusted to 37ºC ± 5ºC. The cellophane membrane was soaked overnight in glycerol. The sample was applied onto the membrane, and the membrane was placed in an open tube cylinder and tied to remain intact. The membrane was placed so that it was exposed to 100 ml of the phosphate buffer system. 2 ml of the sample was withdrawn at periodic intervals, and 2 ml of fresh buffer was replaced each time to maintain sink conditions. The drug content was analyzed using a UV-visible spectrophotometer at 270 nm using phosphate buffer as a blank [12].

1.4.7. Stability studies

The in situ gel formulations were placed in amber glass vials and sealed with aluminum for an accelerated stability study at 25º± 2ºC (room temperature) and 45º± 2ºC (elevated temperature conditions) as per modified International Conference on Harmonization guidelines for 3 months. Samples were analyzed every 30 days for their appearance, pH, gelling studies, and drug content [12].

RESULTS AND DISCUSSION

Solubility and calibration curve of doxycycline

The solubility studies of doxycycline hyclate in water, ethanol, methanol, and 0.1N HCl reveal significant differences in its solubility profiles, which are crucial for its formulation and bioavailability. Doxycycline hyclate shows high solubility in water (50 mg/mL) and 0.1N HCl (80 mg/mL), indicating its suitability for oral formulations and stability in the acidic environment of the stomach, respectively. Its good solubility in methanol (30 mg/mL) makes it a viable solvent for analytical and formulation purposes. In contrast, its moderate solubility in ethanol (5 mg/mL) suggests that ethanol may require additional solubilizing agents to achieve complete dissolution. These findings inform formulation strategies, ensuring optimal solubility and bioavailability of doxycycline hyclate in various pharmaceutical preparations.

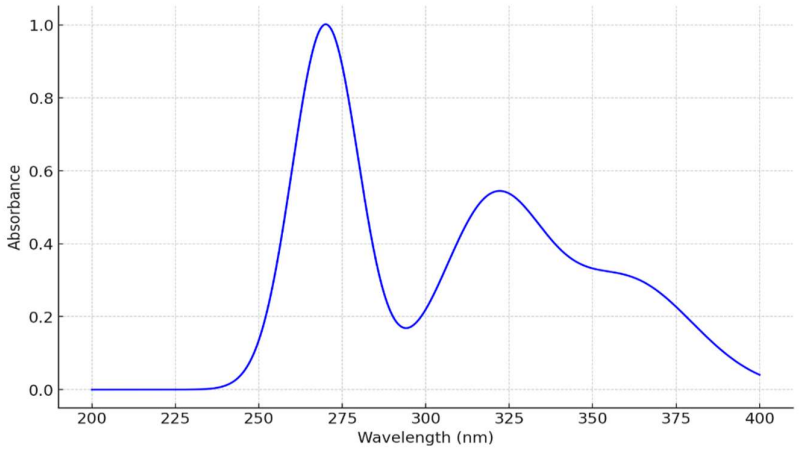


Fig 2: UV spectrum of doxycycline

A calibration curve for doxycycline hyclate was prepared by measuring the absorbance of solutions with known concentrations ranging from 2 to 10 µg/mL at 270 nm using a UV-visible spectrophotometer.
The calibration curve exhibits a high degree of linearity, as indicated by the R^2 value of 0.9979. This value is very close to 1, suggesting that nearly all the variability in absorbance can be explained by the concentration of doxycycline hyclate in the solution. The linear regression equation $y = 0.0974x + 0.0201$ confirms that there is a consistent, proportional relationship between the concentration and absorbance over the range studied (2 to 10 µg/mL).

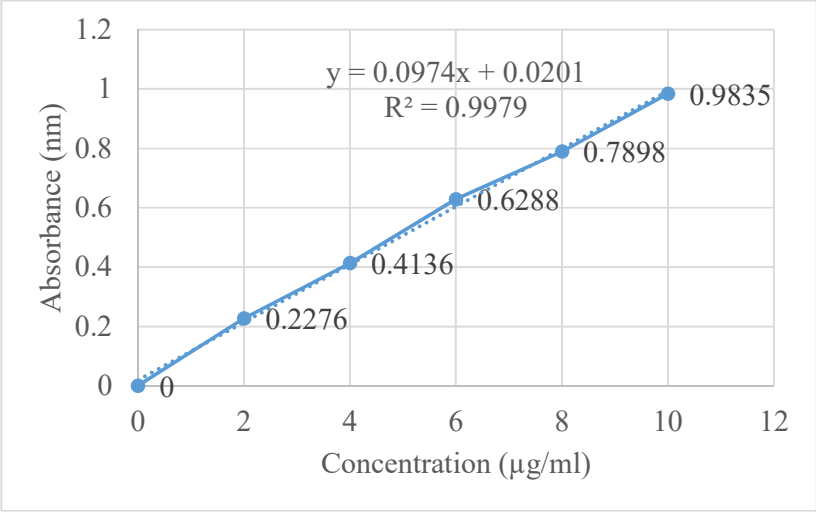


Fig 3: Calibration curve of doxycycline

The intercept of the regression line is 0.0201, which is very close to zero, indicating that the absorbance of the blank (0 µg/mL) is minimal and does not significantly affect the measurement of the sample solutions. The

slope of 0.0974 suggests that for every 1 µg/mL increase in doxycycline concentration, the absorbance increases by approximately 0.0974 units. This slope is a measure of the sensitivity of the assay.

Table 3: Calibration curve data of doxycycline

Concentration (µg/ml)	Absorbance (nm)
0	0
2	0.2276
4	0.4136
6	0.6288
8	0.7898
10	0.9835

This calibration curve can be used to determine the concentration of doxycycline hyclate in unknown samples by measuring their absorbance at 270 nm and applying the regression equation. The reliability and accuracy of this method make it suitable for quality control and routine analysis in pharmaceutical formulations.

Drug-excipient interaction studies

FTIR

Fourier-transform infrared (FTIR) spectroscopy is a technique used to obtain the infrared spectrum of absorption or emission of a solid, liquid, or gas. By analyzing the peaks in the spectrum, the functional groups present in the compound can be determined.

Doxycycline

Functional Groups in Doxycycline

3334 cm^{-1}: O-H (hydroxyl groups), N-H (amine or amide groups)
1617 cm^{-1}: C=O (carbonyl groups)
1459 cm^{-1}: C-H (alkane groups), aromatic C=C
1332 cm^{-1}: C-N (amine groups)
1244 cm^{-1}: C-O (ethers or esters)
1041 cm^{-1}: C-O-C (ether or ester)

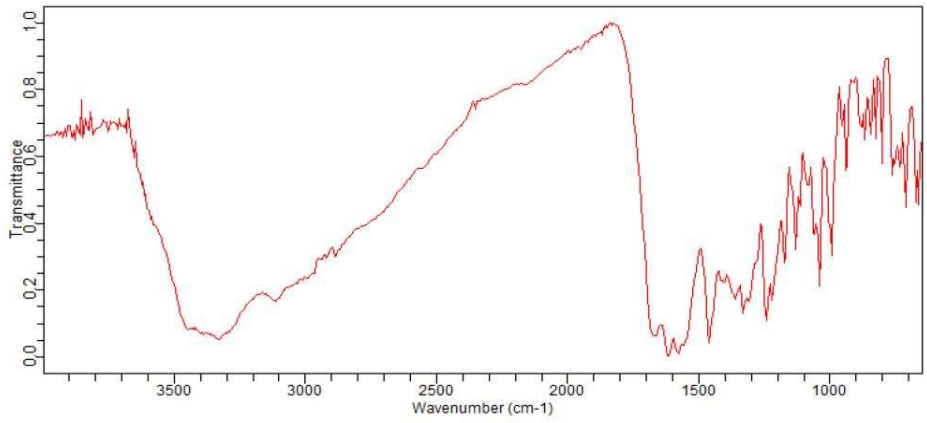


Fig 4: FTIR of doxycycline

These functional groups are consistent with the known structure of doxycycline, which includes phenolic OH groups, amine groups, several carbonyl groups, and ether linkages. The presence of these peaks confirms the expected functional groups in doxycycline's chemical structure.

Formulation mixture

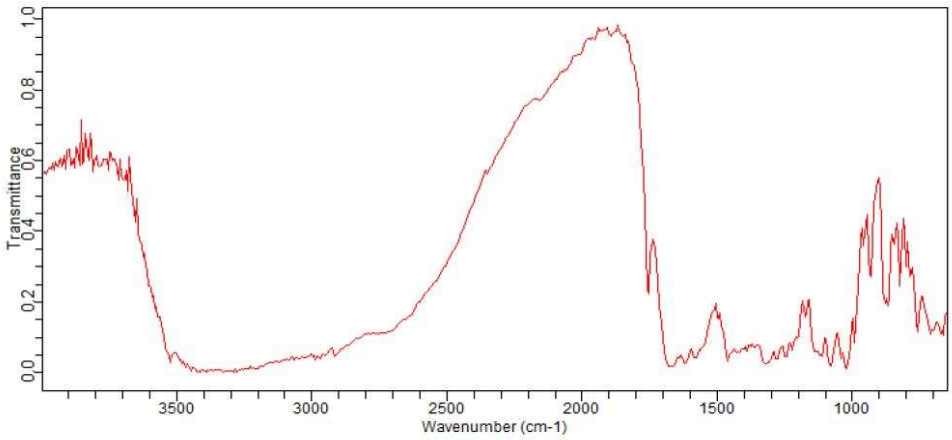


Fig 5: FTIR of formulation mixture

The FTIR spectrum of the formulation containing doxycycline, HPMC, poloxamer 188, and labrasol shows the characteristic peaks of doxycycline without significant shifts or changes in intensity. This indicates that the excipients do not interact with the doxycycline in a way that would alter its functional groups' vibrational frequencies. The FTIR analysis of the doxycycline formulation reveals that the excipients (HPMC, poloxamer 188, and labrasol) do not interact with the doxycycline. The characteristic peaks of doxycycline remain unchanged, indicating the chemical stability and integrity of doxycycline in the presence of these excipients.

DSC of doxycycline

The Differential Scanning Calorimetry (DSC) analysis of Doxycycline reveals key thermal events at 170°C, 190°C, and 219°C, which provide insights into its thermal behavior and stability. The endothermic peak at 170°C indicates an initial melting or phase transition, likely related to a polymorphic form, affecting the drug's stability and bioavailability. The exothermic peak at 190°C suggests a possible recrystallization or chemical reaction, indicating a reorganization into a more stable form, which impacts storage and processing conditions. The significant endothermic peak at 219°C corresponds to the major melting point, crucial for determining appropriate processing temperatures and ensuring the drug's integrity.

HPMC

The DSC analysis of Hydroxypropyl Methylcellulose (HPMC) highlights a key thermal event at 89.5°C. The endothermic peak observed at this temperature is indicative of a significant thermal transition, likely corresponding to the glass transition temperature (Tg) or the melting of a hydrated crystalline phase of HPMC. This transition point is crucial for understanding the thermal properties and stability of HPMC, as it indicates the temperature at which the material undergoes a major physical change. The stability of the baseline before and after the endothermic peak suggests that HPMC is thermally stable up to and beyond the transition temperature, with no other significant thermal events detected within the scanned temperature range (0-200°C). This stability is important for its applications, ensuring that HPMC retains its properties under typical processing and storage conditions.

poloxamer

The DSC analysis of Poloxamer 188 highlights two key thermal events that provide insights into its thermal behavior and stability. The first major endothermic peak at 56.18°C likely corresponds to the melting point of Poloxamer 188. This melting transition is significant as it indicates the temperature at which Poloxamer 188 changes from a solid to a liquid state. Understanding this melting point is crucial for processing and application purposes, especially in formulations where precise thermal control is required to maintain the integrity of the product. The second endothermic transition at 100°C is less pronounced but still notable. This peak could indicate another phase transition, such as the melting of a different crystalline form or a dehydration event if the sample contains any moisture.

Formulation mixture

The Differential Scanning Calorimetry (DSC) curve for the mixture of Hydroxypropyl Methylcellulose (HPMC), Poloxamer 188, and Doxycycline reveals three distinct thermal events. An endothermic peak is observed at 58.25°C, another at 102.50°C, and a significant endothermic peak at 212.34°C. The DSC analysis of the mixture of HPMC, Poloxamer 188, and Doxycycline provides valuable insights into the thermal behavior

and potential interactions of these components. The key thermal events observed are consistent with the individual DSC profiles of the components, suggesting that there is no significant interaction between Doxycycline and the excipients HPMC and Poloxamer 188. The DSC analysis of the mixture of HPMC, Poloxamer 188, and Doxycycline demonstrates that there are no significant interactions between Doxycycline and the excipients. The thermal events corresponding to each component's melting points are clearly identifiable, and the baseline stability confirms the absence of other thermal interactions. This finding is crucial for pharmaceutical formulation, as it ensures that Doxycycline retains its stability and efficacy when combined with these excipients, allowing for predictable and reliable drug performance.

Evaluation of doxycycline-loaded in-situ gel

Visual appearance

The visual appearance, clarity, color, and pH of the in situ doxycycline-loaded gel formulations (F1-F5) were evaluated. The results are summarized in the table below:

Table 4: Visual evaluation of insitu gel formulations

Formulation	Visual Appearance	Clarity	Color	pH
F1	Smooth, homogenous	Translucent	Yellow	6.30
F2	Smooth, homogenous	Translucent	Yellow	6.32
F3	Smooth, homogenous	Translucent	Yellow	6.34
F4	Smooth, homogenous	Translucent	Yellow	6.36
F5	Smooth, homogenous	Translucent	Yellow	6.38

The evaluation of the in situ doxycycline-loaded gel formulations (F1-F5) indicated consistent and desirable properties across all samples. All formulations exhibited a smooth and homogenous visual appearance, suggesting a uniform distribution of doxycycline within the gel matrix. The clarity of each formulation was noted to be translucent, indicating the presence of a semi-transparent gel which is typically preferred for topical applications to allow some visibility through the gel while ensuring the active ingredient is well-dispersed.

All formulations were uniformly yellow, which is the characteristic color of doxycycline and indicates that the drug is well-incorporated into the gel base. The pH of the formulations ranged from 6.30 to 6.38, with F3 displaying an optimal pH of 6.34, which is the desired pH for the gel formulation. Maintaining a pH around 6.34 is crucial as it ensures the stability of doxycycline and is within the acceptable range for topical application, minimizing irritation and ensuring compatibility with the skin's natural pH. The slight variations in pH among the formulations are within acceptable limits and do not significantly impact the overall stability and efficacy of the gel.

Viscosity

Formulation F1, which contains 2% Poloxamer 188, 0.50% HPMC, and 0.25% Labrasol, exhibited a viscosity of 101 cP. Increasing the concentration of Poloxamer 188 in F2 to 3% led to a notable increase in viscosity to 186 cP. Further increasing Poloxamer 188 to 4% in F3 resulted in a higher viscosity of 273 cP. The viscosity values for F4 and F5, with varying concentrations of Poloxamer 188, HPMC and Labrasol, showed intermediate values of 212 cP and 134 cP, respectively.

These results demonstrate a clear correlation between the viscosity agents used (Poloxamer 188, HPMC, and Labrasol) and the measured viscosity of the formulations. Poloxamer 188, being a nonionic surfactant, typically increases viscosity with higher concentrations due to its ability to form micelles or interact with the formulation components. HPMC, a cellulose derivative, also contributes to viscosity, although its effect is less pronounced compared to Poloxamer 188. Labrasol, an absorption enhancer, may affect viscosity through its solubilizing properties and interactions with other components.

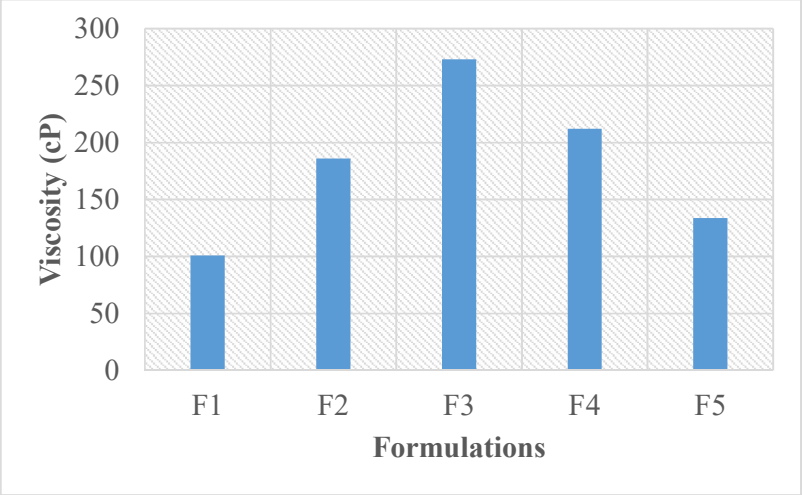


Fig 6: Viscosity of the insitu gel formulations

The viscosity measurements reflect the expected trends based on the concentrations of these viscosity-modifying agents. Adjusting these concentrations can therefore be used strategically to control and optimize the viscosity of formulations for desired application properties.

Drug content

The drug content of the five different formulations (F1 to F5) of doxycycline-loaded in situ gel was determined using a UV-visible spectrophotometer at a wavelength of 270 nm. The results indicate that all formulations possess a high drug content, with values ranging from 92.46% to 97.89%. Formulation F4 exhibited the highest drug content at 97.89%, suggesting that it has the most efficient drug loading capability among the tested formulations. This might be attributed to optimal formulation parameters such as the concentration of the gelling agent, pH, and the method of preparation, which favor maximum drug encapsulation and stability.

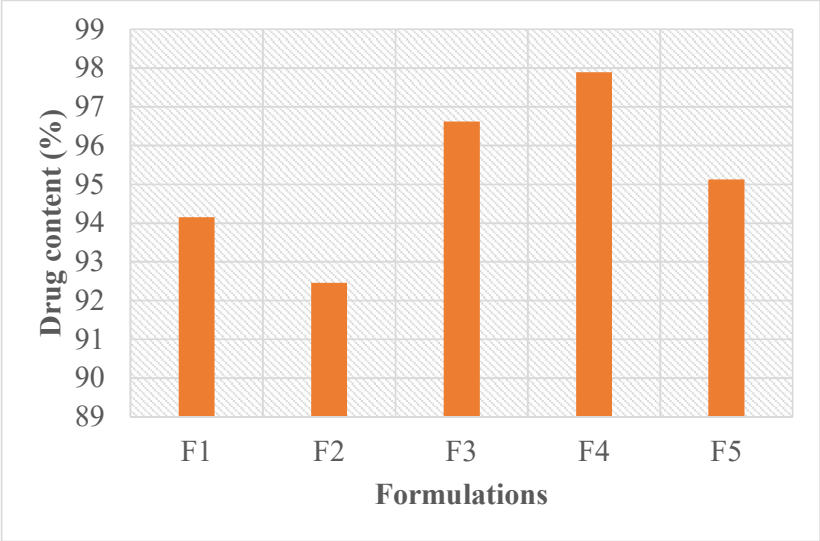


Fig 7: Drug content of the insitu gel formulations

Formulation F2 showed the lowest drug content at 92.46%, which, although slightly lower than the other formulations, is still within an acceptable range for drug delivery systems. The variations in drug content across different formulations could be due to differences in the formulation composition, preparation technique, or slight variations in the measurement process.

Overall, the high drug content across all formulations indicates that the method used for preparing doxycycline-loaded in situ gel is effective in achieving substantial drug encapsulation. This suggests the potential for these formulations to deliver doxycycline efficiently at the target site, thereby enhancing its therapeutic efficacy.

The gellation studies for the in-situ gel formulations F1 to F5 loaded with doxycycline revealed variations in their gellation times and consistencies, while all formulations had a consistent gellation temperature of 40°C, which aligns with the desired temperature parameter for the formulations.
gelled at 40°C, which ensures that the gellation occurs at body temperature, making them suitable for in-situ applications. This consistent gellation temperature ensures that the gel will form upon administration into the body, allowing for controlled drug release.

The time required for gellation varied from 1.5 minutes (F1) to 3.5 minutes (F5). Rapid gellation (as observed in F1 and F2) is beneficial for minimizing the risk of premature drug release before reaching the target site. However, extremely rapid gellation could pose handling challenges during administration. Formulation F3, with a gellation time of 2.5 minutes, and formulation F4, with a gellation time of 3 minutes, strike a balance, ensuring ease of administration while providing sufficient time for the gel to form in situ. These times are ideal for allowing the gel to set properly within the body without causing too much delay in drug release.

The consistency of the gel is crucial for sustained drug release and ease of application. F1 had a moderate consistency, which might offer a balance between ease of administration and sustained release. F2 was thicker, providing a potentially longer drug release profile but could be harder to administer. Formulation F3, with a very thick consistency, and formulation F4, with a gelatinous consistency, are particularly promising. These consistencies suggest strong gel formation, which is ideal for sustained drug release while maintaining manageable ease of application. Formulation F5, with its viscous consistency, indicates strong gel formation but may pose challenges in administration and immediate drug release requirements.

Based on the gellation studies, formulations F3 and F4 appear to be the most promising candidates for in-situ gel drug delivery. Both have a gellation temperature of 40°C, which is ideal for in-body administration, and their gellation times (2.5 minutes for F3 and 3 minutes for F4) ensure a balance between ease of administration and effective gel formation. The very thick and gelatinous consistencies of F3 and F4 respectively make them suitable for sustained drug release. Further studies including in vivo testing, drug release profiles, and stability studies are essential to confirm the suitability of F3 and F4 for clinical applications.

***In-vitro* drug release**

The *in-vitro* release study for Formulation F1 showed a gradual increase in drug release over the 120-minute period. Starting with an initial release of 24.18% at 5 minutes, the release steadily rose, reaching 32.46% at the 120-minute mark. This pattern indicates a sustained release profile, which is desirable for prolonged therapeutic effects. The initial burst release observed can be attributed to the immediate diffusion of doxycycline from the gel's surface. The presence of Poloxamer 188, known for its thermosensitive gelation properties, likely played a crucial role in controlling the drug's diffusion rate. The gradual increase in release suggests that Poloxamer 188 successfully formed a gel matrix at the given temperature, providing a controlled and sustained release environment.

Formulation F2 exhibited a slightly higher initial release of 26.82% at 5 minutes, with a final release of 33.66% at 120 minutes. The release profile of F2 was similar to that of F1 but with a marginally higher overall release. This difference can be attributed to the inclusion of HPMC, a hydrophilic polymer known for its swelling properties. HPMC's ability to swell upon contact with the buffer solution may have increased the porosity of the gel matrix, allowing for more drug to be released over time. The steady increase in release indicates that the combination of Poloxamer 188 and HPMC effectively maintained a controlled release environment, ensuring a prolonged therapeutic effect.

Formulation F3 demonstrated the highest initial release of 30.13% at 5 minutes and the greatest overall release, reaching 40.15% at 120 minutes. This rapid release profile suggests that the combination of excipients in F3 created a more porous gel structure, facilitating faster drug diffusion. The high initial release is advantageous for achieving quick therapeutic effects, making F3 suitable for conditions requiring rapid drug availability. However, the higher release rate also indicates a need for careful optimization to prevent potential dose dumping and ensure sustained therapeutic levels.

In the case of Formulation F4, the release pattern was similar to F3, with an initial release of 29.46% at 5 minutes and a final release of 38.49% at 120 minutes. The slightly lower release rates compared to F3 suggest a balanced release profile. The combination of Poloxamer 188 and Labrasol, a solubilizing agent, likely contributed to this balanced release. Labrasol's ability to enhance the drug's solubility might have increased its availability for diffusion. This formulation's release profile indicates that it is suitable for applications requiring moderate release rates, providing both immediate and sustained drug availability.

Formulation F5 showed a moderate initial release of 25.26% at 5 minutes and a consistent increase in release over time, reaching 34.49% at 120 minutes. This release pattern was similar to F2 but with slightly lower

values. The combination of excipients in F5, including HPMC, likely resulted in a more controlled release profile. HPMC's role in increasing the viscosity and strength of the gel matrix might have slowed down the drug diffusion, ensuring a sustained release. This formulation is ideal for applications requiring prolonged drug delivery, maintaining consistent therapeutic levels over an extended period.

Stability studies

The stability studies of Formulation 3 at 25±2ºC and 5% RH showed consistent results over a six-month period. The appearance remained translucent throughout the study, indicating no significant change in the physical state of the gel. The pH values were relatively stable, starting at 6.23 in the first month, slightly increasing to 6.38 by the second month, and settling at 6.33 by the third month. Drug content showed a gradual decrease from 96.18% in the first month to 91.39% by the third month. Gelling studies consistently rated as +++ throughout the period.

Formulation 3 demonstrated excellent physical stability, as evidenced by the unchanged translucent appearance. The slight fluctuations in pH values are within acceptable limits, suggesting that the formulation maintains its intended chemical environment over time. The gradual decrease in drug content is expected in stability studies and remained above 90%, indicating good retention of doxycycline. The consistent gelling ability (+++) suggests that the formulation maintained its functional properties, crucial for effective drug delivery. Overall, Formulation 3 displayed robust stability, making it a reliable candidate for treating paronychia.

40ºC±2ºC/ 75%RH

The appearance of Formulation 3 remained consistently translucent throughout the six-month study, indicating no significant change in the physical state of the gel. The pH values showed a slight decrease over time, starting at 6.45 in the first month, decreasing to 6.28 by the second month, and further dropping to 6.16 by the third month. Drug content also decreased gradually from 96.18% in the first month to 92.28% by the third month. The gelling studies consistently rated as +++ throughout the period.

Formulation 3 demonstrated excellent physical stability under accelerated conditions, as evidenced by the unchanged translucent appearance. The slight decrease in pH values over time is within acceptable limits, suggesting that the formulation maintains its intended chemical environment even under stress conditions. The gradual decrease in drug content, although expected, remained above 90%, indicating good retention of doxycycline. The consistent gelling ability (+++) suggests that the formulation maintained its functional properties, crucial for effective drug delivery. Overall, Formulation 3 displayed robust stability, making it a reliable candidate for treating paronychia, even under accelerated conditions.

Table 5: Stability studies of optimized formulations at 25±2ºC and 5% RH

Properties	Formulation 3 25±2ºC and 5% RH			Formulation 3 40ºC±2ºC/ 75%RH		
	1st month	2nd month	3rd month	1st month	2nd month	3rd month
Appearance	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent
pH	6.23	6.38	6.33	6.45	6.28	6.16
Drug content	96.18	93.43	91.39	96.18	94.16	92.28
Gelling studies	+++	+++	+++	+++	+++	+++
Properties	**Formulation 4 25±2ºC and 5% RH**			**Formulation 4 40ºC±2ºC/ 75%RH**		
	1st month	2nd month	3rd month	1st month	2nd month	3rd month
Appearance	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent
pH	6.23	6.38	6.33	6.35	6.23	6.11
Drug content	97.01	95.19	92.16	97.01	95.76	93.43
Gelling studies	+++	+++	+++	+++	+++	+++

SUMMARY AND CONCLUSION

The present study aimed to formulate and evaluate a doxycycline-loaded in situ gel for the treatment of paronychia. A calibration curve for doxycycline hyclate was established by measuring absorbance at 270 nm across concentrations from 2 to 10 µg/mL, yielding a highly linear relationship ($R^2 = 0.9979$). The linear regression equation, $y = 0.0974x + 0.0201$, indicates a consistent proportional relationship between concentration and absorbance, with minimal interference from the blank. This curve enables accurate determination of doxycycline concentrations in unknown samples, suitable for pharmaceutical quality control and analysis.

FTIR spectroscopy identified characteristic peaks of doxycycline's functional groups (e.g., O-H, N-H, C=O, C-H, C-N, C-O, C-O-C). Analysis of the formulation mixture with HPMC, poloxamer 188, and labrasol showed no significant shifts in these peaks, indicating no interaction between doxycycline and excipients, thereby preserving doxycycline's chemical stability and integrity. DSC analysis revealed thermal events for doxycycline at 170°C, 190°C, and 219°C, corresponding to melting and recrystallization phases. HPMC showed an endothermic peak at 89.5°C, while poloxamer 188 exhibited peaks at 56.18°C and 100°C. The DSC curve for the formulation mixture retained these characteristic peaks, indicating no significant interaction between doxycycline and excipients, thus ensuring the stability of the formulation.

The doxycycline-loaded in-situ gel exhibited a yellow color with consistent clarity and stable pH over time. Formulations showed varying viscosities influenced by the concentrations of poloxamer 188, HPMC, and labrasol. Increasing poloxamer 188 concentration generally increased viscosity, demonstrating its significant impact on the gel's physical properties. All formulations demonstrated high drug content (92.46% to 97.89%), with Formulation F4 showing the highest drug encapsulation efficiency, indicating effective drug loading and potential for efficient delivery. Formulations exhibited consistent gelling capacities, rated as +++, indicating strong and stable gel formation suitable for drug delivery applications.

The *in-vitro* drug release studies revealed varying profiles among the formulations. Formulation F1 exhibited a gradual and sustained release, starting at 24.18% at 5 minutes and reaching 32.46% at 120 minutes, due to the controlled diffusion properties of Poloxamer 188. Formulation F2 had a slightly higher initial release of 26.82%, ending at 33.66% at 120 minutes, with HPMC enhancing diffusion by increasing porosity.

Formulation F3 demonstrated the highest release rates, with 30.13% at 5 minutes and 40.15% at 120 minutes, indicating a more porous gel structure suitable for rapid drug availability. Formulation F4 displayed a balanced release profile, starting at 29.46% and reaching 38.49%, influenced by the combined effects of Poloxamer 188 and Labrasol. Lastly, Formulation F5 showed a moderate release, starting at 25.26% and ending at 34.49%, with HPMC contributing to a controlled, sustained release. Adjusting the concentrations of these components effectively controlled the drug release rates, tailoring the formulations for different therapeutic needs. Stability studies under standard (25±2ºC/5% RH) and accelerated (40ºC±2ºC/75% RH) conditions demonstrated that both Formulations 3 and 4 maintained their physical appearance, stable pH, high drug content (>90%), and consistent gelling ability (+++). These findings suggest that the formulations are robust, stable, and reliable for long-term use in treating paronychia.

REFERENCES

1. Gaikwad PS, Pagare RV. An Overview on Transungual Drug Delivery System. International Journal of Pharmaceutical Research and Applications 8(2) Mar-Apr 2023, pp: 464-478
2. Walters KA, Flynn GL. Permeability characteristics of the human nail plate. International journal of cosmetic science. 1983 Dec;5(6):231-46.
3. Saner MV, Kulkarni AD, Pardeshi CV. Insights into drug delivery across the nail plate barrier. Journal of drug targeting. 2014 Nov 1;22(9):769-89.
4. Agrawal AK, Das M, Jain S. In situ gel systems as ‘smart’carriers for sustained ocular drug delivery. Expert opinion on drug delivery. 2012 Apr 1;9(4):383-402.
5. Victor SP, Kumar TS. CDHA ceramic microspheres for periodontitis treatment: synthesis, characterization and doxycycline release profiles. The Indonesian Journal of Dental Research. 2011;1(2):98-104.
6. Nogueiras-Nieto L, Delgado-Charro MB, Otero-Espinar FJ. Thermogelling hydrogels of cyclodextrin/poloxamer polypseudorotaxanes as aqueous-based nail lacquers: Application to the delivery of triamcinolone acetonide and ciclopirox olamine. European Journal of Pharmaceutics and Biopharmaceutics. 2013 Apr 1;83(3):370-7.
7. Garala K, Joshi P, Shah M, Ramkishan A, Patel J. Formulation and evaluation of periodontal in situ gel. International journal of pharmaceutical investigation. 2013 Jan;3(1):29.
8. Khatri K, Jain S, Shilpi S. Nasal in-situ gel: an approach to enhance therapeutic benefits of the drug. Drug Delivery Letters. 2020 May 1;10(2):85-95.
9. Makwana SB, Patel VA, Parmar SJ. Development and characterization of in-situ gel for ophthalmic formulation containing ciprofloxacin hydrochloride. Results in pharma sciences. 2016 Jan 1;6:1-6.
10. Pandya K, Aggarwal P, Dashora A, Sahu D, Garg R, Pareta LK, Menaria M, Joshi B. Formulation and evaluation of oral floatable in-situ gel of ranitedine hydrochloride. Journal of Drug Delivery and Therapeutics. 2013 May 13;3(3):90-7..
11. Asif HM, Rao TR, Ayesha N, Anjum M, Rahman MA. Design and Evaluation of Mucoadhesive Buccal Patches of Venlafaxine. British Journal Of Pharmaceutical Research. 2015 Jan 1;6(2).
12. Nasra MM, Khiri HM, Hazzah HA, Abdallah OY. Formulation, *in-vitro* characterization and clinical evaluation of curcumin in-situ gel for treatment of periodontitis. Drug delivery. 2017 Jan 1;24(1):133-42.