Formulation And Characterization Of Thiolated Chitosan–Polyvinyl Alcohol Based Microneedle Patch For Transdermal Delivery Of Ramipril

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Abstract:

The present study aimed to develop and characterize a Thiolated chitosan–polyvinyl alcohol (PVA) based microneedle patch for the transdermal delivery of Ramipril, an antihypertensive drug that undergoes extensive first-pass metabolism. Thiolated chitosan was synthesized using thioglycolic acid and characterized by Fourier Transform Infrared Spectroscopy (FTIR), which confirmed successful thiolation through characteristic S–H and C–S stretching bands at 2550 cm^{-1} and 650 cm^{-1}, respectively. A 3^2 factorial design was employed to optimize the microneedle formulation, with PVA and Thiolated chitosan concentrations as independent variables. Microneedle patches were fabricated using the droplet-born air blowing (DAB) technique to ensure uniformity, mechanical strength, and controlled drying without thermal degradation. The prepared patches were evaluated for thickness, tensile strength, folding endurance, moisture content, drug content, and in-vitro drug release. The optimized formulation (F3; PVA: Thiolated Chitosan = 27:3 mL) exhibited superior mechanical integrity, ideal insertion capability, and desirable moisture balance (1.87%). The in-vitro release profile demonstrated a biphasic pattern, characterized by an initial burst followed by sustained drug release, suggesting effective dermal penetration and controlled delivery. These findings confirm that the Thiolated chitosan–PVA microneedle patch offers a promising transdermal platform for Ramipril with enhanced permeability, stability, and patient compliance.

KEYWORDS: Ramipril, Thiolated chitosan, Polyvinyl alcohol, Microneedle patch, Transdermal drug delivery, Droplet-born air blowing method

1. INTRODUCTION

The administration of therapeutic agents through suitable dosage forms plays a crucial role in ensuring efficacy, safety, and patient compliance. Among the various routes of drug administration, the oral route is the most commonly used due to its convenience and non-invasiveness. However, several limitations such as poor bioavailability, enzymatic degradation, and extensive first-pass metabolism significantly reduce the therapeutic efficiency of many drugs, including Ramipril[1]. Similarly, parenteral administration, though effective, often causes pain, requires trained personnel, and is associated with poor patient compliance. These drawbacks have encouraged the development of novel drug delivery systems capable of bypassing such limitations.

Transdermal drug delivery systems (TDDS) have emerged as a promising alternative for systemic drug administration. The skin, being the largest organ of the body, offers an extensive surface area for drug absorption while avoiding hepatic first-pass metabolism[2]. However, the stratum corneum, the outermost layer of the epidermis, poses a significant barrier that restricts the permeation of most drugs, especially those with high molecular weight or poor lipophilicity[3]. To overcome this barrier, microneedle (MN) technology has been developed as a minimally invasive approach for enhancing transdermal permeation[4].

Microneedles are microscopic projections, typically less than 1000 µm in length, designed to pierce the stratum corneum and create transient microchannels without reaching the nerve endings or blood vessels, thereby enabling painless drug administration[4]. These devices combine the advantages of both transdermal patches and hypodermic injections, offering improved bioavailability, controlled release, and better patient acceptability[5]. Various types of microneedles have been reported, including solid, hollow, coated, dissolving, and hydrogel-based designs, each with specific advantages depending on the drug and application[6]. Among these, dissolving microneedles fabricated from biodegradable polymers such as chitosan and polyvinyl alcohol (PVA) have gained considerable attention due to their biocompatibility, safety, and ease of fabrication[7].

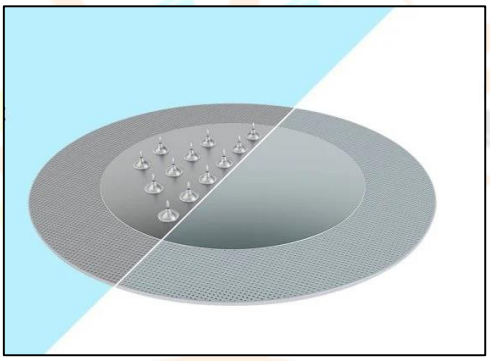

Fig.01: Difference Between Microneedle and simple patches

Chitosan, a natural polymer derived from chitin, possesses excellent film-forming, biocompatible, and bioadhesive properties[8]. However, its limited solubility and mechanical strength restrict its use in transdermal applications. Chemical modification of chitosan, such as thiolation, significantly improves its mucoadhesive properties, permeability, and cross-linking ability[9]. Thiolated chitosan (TCS) contains free thiol groups that form disulfide bonds, enhancing the polymer's structural integrity and interaction with biological membranes[10]. When blended with synthetic polymers like PVA, the resulting matrix exhibits superior mechanical strength and flexibility, making it ideal for microneedle fabrication[11].

In this context, the present study focuses on the formulation and characterization of Thiolated chitosan–PVA-based microneedle patches for the transdermal delivery of Ramipril. Ramipril, an angiotensin-converting enzyme (ACE) inhibitor, is widely used in the management of hypertension and cardiovascular disorders but suffers from poor oral bioavailability (~28%) due to first-pass metabolism[12,13]. The objective of this work is to enhance its systemic delivery by formulating a biocompatible microneedle patch using the droplet-born air blowing (DAB) technique. This method ensures uniform microneedle geometry and stability under mild conditions, avoiding thermal degradation of the drug[14]. The developed formulations were

evaluated for mechanical properties, drug content, moisture balance, and in-vitro drug release to identify an optimized batch with suitable performance characteristics for potential transdermal application[4].

2. Materials and Methods

2.1 Materials

Ramipril was received as a gift sample from a certified supplier. Chitosan, thioglycolic acid (TGA), N-(3-dimethylaminopropyl)-N′-ethyl carbodiimide hydrochloride (EDAC), and polyvinyl alcohol (PVA) were of analytical grade. All other reagents and solvents used were of analytical or pharmaceutical grade and used as received without further purification.

2.2 Instruments and Equipment

A UV–Visible spectrophotometer, weighing balance, magnetic stirrer, and pH meter were used in the study. A Franz diffusion cell (Shri Sai Enterprises, Pune, India).

2.3 Synthesis of Thiolated Chitosan

Thiolated chitosan (TCS) was synthesized by coupling thioglycolic acid (TGA) with chitosan using EDAC as a coupling agent. Chitosan (200 mg) was dissolved in 20 mL of 1% (w/w) lactic acid. After complete solubilization, TGA (200 µL, 70% w/w) and EDAC (200 mg, 1.04 mmol) were added, and the pH was adjusted to 6 using 0.1 M NaOH. The reaction mixture was stirred overnight at room temperature. The resulting polymer was dialyzed using a 12–14 kDa MW cutoff membrane against 5 mM HCl for 24 h to remove unreacted components, followed by dialysis against 5 mM HCl containing 1% NaCl and finally against 1 mM HCl. The purified Thiolated chitosan was stored at 4 °C for further use.

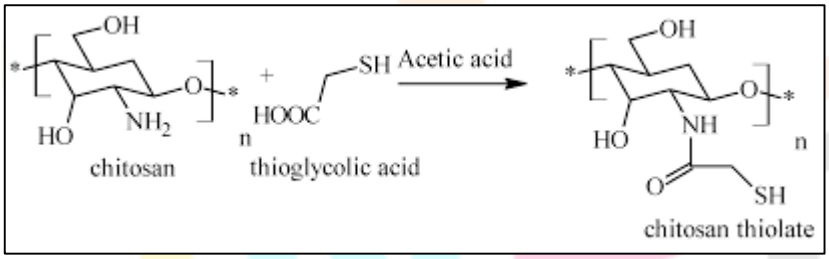

Fig.02: Reaction of Chitosan to Thiolated chitosan using thioglycolic acid

2.4 Characterization of Thiolated Chitosan

The synthesized polymer was characterized by Fourier Transform Infrared Spectroscopy (FTIR) (Bruker Alpha, Bruker) to confirm thiolation. Samples were scanned in the range of 500–4500 cm^{-1} using attenuated total reflectance (ATR). The presence of characteristic S–H (2550 cm^{-1}) and C–S (650 cm^{-1}) peaks indicated successful thiolation.

2.5 Determination of λmax and Calibration Curve of Ramipril

A stock solution of Ramipril (1 mg/mL) was prepared using a green solvent system (propylene glycol: distilled water = 1: 9 v/v). The solution was scanned between 190–800 nm, and maximum absorbance (λmax) was

observed at 207 nm. Calibration curves were constructed for concentrations ranging from 4–16 µg/mL. Linearity was confirmed using the regression equation y = 0.0495x – 0.0259 with R^2 = 0.9915.

2.6 Experimental Design

A 3^2 full factorial design was used to study the effect of two formulation variables:

- Factor A: PVA concentration (7, 8, and 9 mL)
- Factor B: Thiolated chitosan concentration (1, 2, and 3 mL)

Nine formulations (F1–F9) were prepared with randomized runs to minimize bias. The dependent variables evaluated were patch thickness, tensile strength, folding endurance, drug content, and percentage drug release.

2.7 Preparation of Microneedle Patches

Microneedle patches were fabricated using the Droplet-Born Air Blowing (DAB) technique.

i. Prepare PVA Solution (10% w/v)

- Heat water to ~80°C and add PVA slowly while stirring.
- Stir until completely dissolved (may take 20–30 min).
- Cool to room temperature.

ii. Prepare Thiolated Chitosan Solution (2% w/v in 2% Lactic acid)

- Prepare 2% acetic acid (2 mL Lactic acid in 98 mL distilled water).
- Add Thiolated chitosan slowly with continuous stirring.
- Stir overnight or use mild heating (~50°C) for faster dissolution.

iii. Blend Both Solutions

- Mix Thiolated chitosan and PVA in your chosen ratio.
- Add drug (0.5% w/v), and mix thoroughly.
- Sonicate or stir to remove air bubbles.

iv. Droplet-born Air Blowing (DAB) process

- Using a micropipette, dispense droplets of the polymer–drug mixture onto each microneedle mold cavity.
- Direct a stream of compressed air (gentle and controlled pressure) over the droplets to drive the solution deep into the microcavities.
- Ensure that excess solution is removed from the mold surface, leaving filled cavities only.

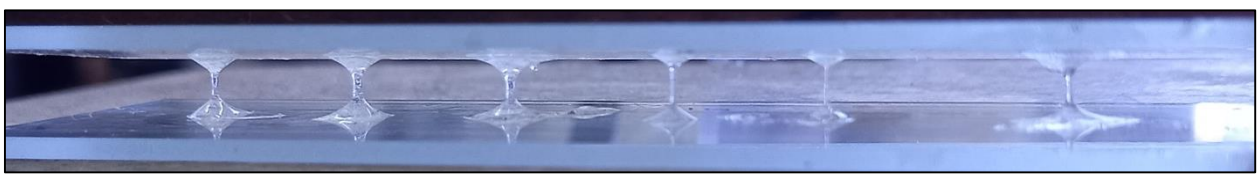

Fig.03: Diagram showing the DAB Method While preparation of Microneedle Patches

2.8 Evaluation of Microneedle Patches

The prepared patches were evaluated for the following parameters:

- **Moisture Content**: Patches were weighed before and after 24 h in a desiccator containing silica gel.

$$\% \text{ Moisture Content} = \frac{(W_i - W_f)}{W_i} \times 100$$

- **Thickness**: Measured using a digital micrometer at five random points.
- **Tensile Strength**: Determined by applying force until the patch broke; results expressed in kg/cm².
- **Folding Endurance**: Measured by folding the patch repeatedly at the same point until it broke.
- **Drug Content**: Patches were dissolved in methanol, filtered, and analyzed spectrophotometrically at 207 nm.
- **In-vitro Drug Release**: Conducted using a Franz diffusion cell with phosphate buffer (pH 7.4) at 37 ± 0.5 °C under continuous stirring. Samples were withdrawn at predetermined intervals and analyzed at 207 nm.

3. Results and Discussion

3.1 FTIR Analysis of Chitosan and Thiolated Chitosan

FTIR spectroscopy was performed to confirm the structural modification of chitosan after thiolation. The FTIR spectrum of pure chitosan (Fig. 04) showed a broad absorption band at 3400–3200 cm^{-1} corresponding to O–H and N–H stretching vibrations, along with peaks at 2925 cm^{-1} (C–H stretching), 1650 cm^{-1} (amide I), and 1580 cm^{-1} (amide II). These are characteristic of the polysaccharide backbone.

After thiolation, Thiolated chitosan (TCS) displayed two new absorption bands at 2550–2600 cm^{-1} (S–H stretching) and 650–700 cm^{-1} (C–S stretching) (Fig. 05), confirming the covalent attachment of thiol groups onto the polymer chain. The reduction in amide band intensity indicated partial substitution of amino groups by thiol moieties. The preservation of the 1150–1020 cm^{-1} (C–O–C) region suggested that the glycosidic structure remained intact. These spectral changes verified the successful synthesis of Thiolated chitosan, suitable for use in microneedle formulation due to enhanced mucoadhesiveness and cross-linking ability.

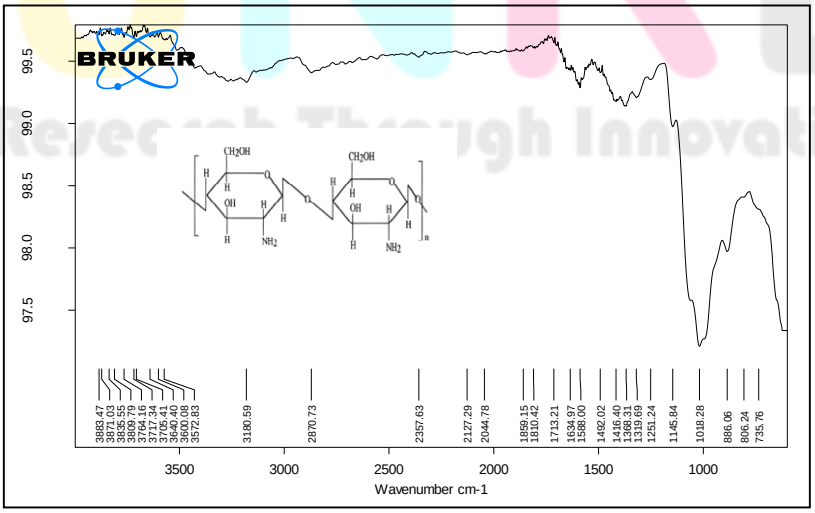

Fig.04: FTIR spectrum of chitosan showing characteristic O–H, N–H, and C–O stretching bands.

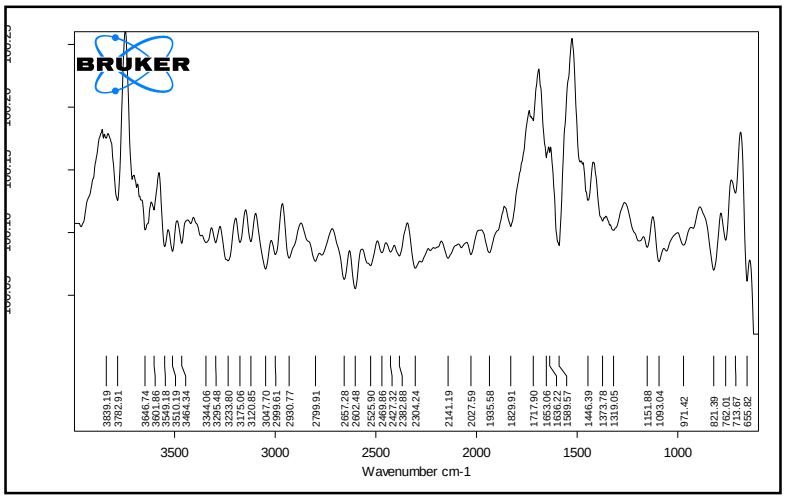

Fig.05: FTIR spectrum of Thiolated chitosan showing new S–H and C–S peaks confirming thiolation.

3.2 UV Spectrophotometric Analysis and Calibration Curve of Ramipril

A simple, green UV spectrophotometric method was developed using a propylene glycol: water (1: 9 v/v) solvent system. The spectrum of pure Ramipril (**Fig.** 06) exhibited maximum absorbance at 207 nm. The calibration curve (Fig. 4) was linear in the concentration range of 4–16 µg/mL, following the regression equation:

$$y = 0.0495x - 0.0259 (R^2 = 0.9915)$$

The high correlation coefficient confirmed the method's linearity and suitability for accurate estimation of Ramipril in microneedle patches.

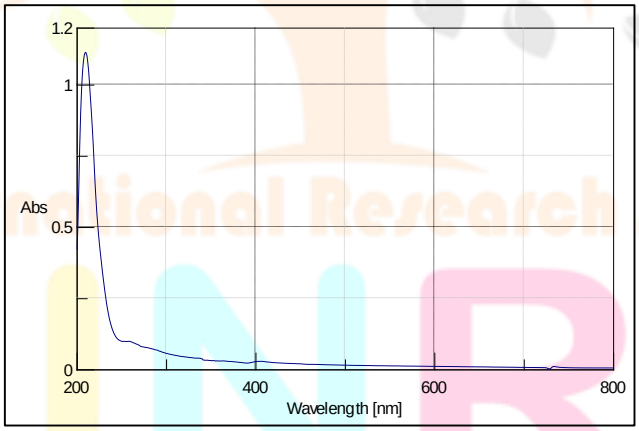

Fig. 06. UV spectrum of Ramipril showing maximum absorbance (λmax = 207 nm).

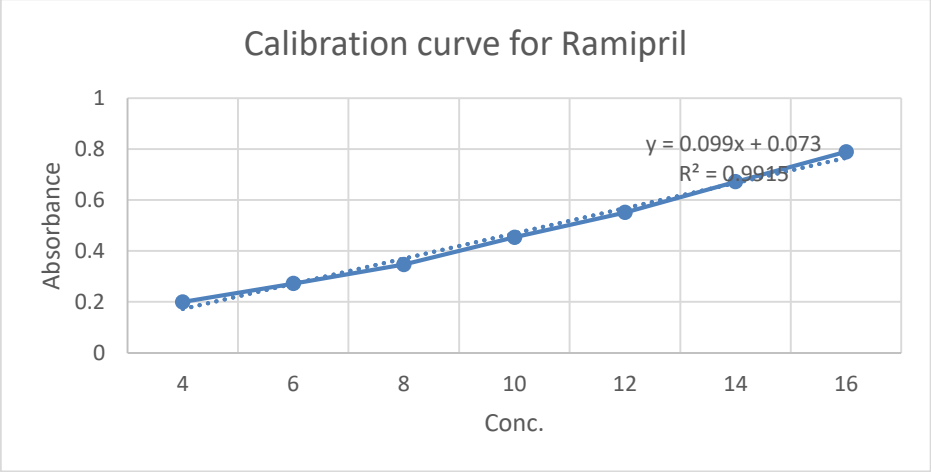

Fig. 07: Calibration curve of Ramipril showing linearity in the range of 4–16 µg/ML.

3.3 Moisture Content

The percentage moisture content of all formulations ranged from 1.77 % to 2.20 % (Table no: 01). The optimized batch (F3) exhibited 1.87 %, indicating an ideal moisture balance to maintain patch flexibility while preventing microbial contamination. Slight variations among formulations were due to differences in polymer ratio affecting hydrophilicity. A low moisture content (< 3 %) is desirable for stable microneedle films with prolonged shelf life.

Table No.01: Percentage moisture content of microneedle patch formulations (F1–F9)

Run	Formulation Code	Initial Weight, Wi (mg)	Final Weight, Wf (mg)	Moisture content
1	F1	102.5	100.4	2.05
2	F2	105.2	102.9	2.18
3	F3	101.8	99.9	1.87
4	F4	103.6	101.4	2.12
5	F5	102.1	100.1	1.96
6	F6	100.9	99.1	1.78
7	F7	104.5	102.2	2.20
8	F8	101.5	99.7	1.77
9	F9	103.2	101.0	2.13

3.4 Patch Thickness and Mechanical Properties

The thickness of all microneedle patches ranged between 0.22 mm and 0.31 mm, confirming uniform film formation through the DAB process. Tensile strength values varied from 0.35 to 0.49 kg/cm², and folding endurance exceeded 300 folds for all batches.

The optimized formulation (F3) showed the highest tensile strength (0.49 kg/cm²) and flexibility, attributed to strong intermolecular hydrogen bonding between PVA and Thiolated chitosan, providing excellent mechanical integrity for dermal application.

3.5 Drug Content Uniformity

The drug content among different batches ranged from 94.2 % to 98.6 %. Formulation F3 showed the highest uniformity (98.6 %), indicating homogeneous dispersion of Ramipril within the polymeric matrix and effective mixing during the DAB fabrication process.

Such uniformity ensures consistent dose delivery per application, a critical parameter for transdermal systems.

3.6 In-Vitro Drug Release Study

The in-vitro release of Ramipril from microneedle patches was evaluated using a Franz diffusion cell with phosphate buffer (pH 7.4) at 37 ± 0.5 °C. All formulations exhibited a biphasic release pattern, characterized by an initial burst followed by sustained drug release (**Fig**. 08). The burst phase corresponded to surface-associated drug diffusion, while the subsequent sustained phase resulted from polymer relaxation and diffusion through hydrated matrices.

Among all formulations, F3 (PVA: TCS = 27: 3) showed the most desirable profile, achieving approximately 80 % cumulative drug release at 8 h. This optimized release behaviour can be attributed to the balanced hydrophilic–hydrophobic interaction of PVA and Thiolated chitosan, leading to uniform pore formation and controlled diffusion.

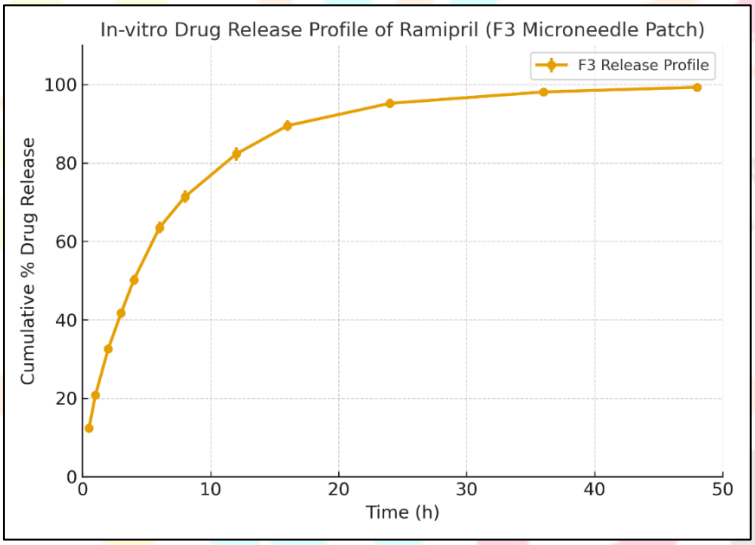

Fig. 08. Cumulative drug release profile of optimized formulation (F3) showing biphasic release pattern.

3.7 Influence of Formulation Variables

Statistical interpretation of the factorial design revealed that both independent variables—PVA concentration (factor A) and Thiolated chitosan concentration (factor B)—significantly affected the mechanical strength and release kinetics. Increased PVA levels enhanced patch elasticity and strength, while higher Thiolated chitosan concentration improved bioadhesion and prolonged drug release.

The synergistic effect between the two polymers contributed to the optimized performance observed for formulation F3.

3.8 Morphological Evaluation

Microscopic observation of the prepared microneedles revealed uniform, sharp, and conical structures with smooth surfaces and consistent dimensions.

The droplet-born air blowing (DAB) technique ensured precise droplet deposition and solidification, minimizing air entrapment.

The needle tips were sufficiently strong to penetrate the stratum corneum without breaking, ensuring painless and effective transdermal delivery.

4. Discussion Summary

Overall, the combination of Thiolated chitosan and PVA yielded microneedle patches with excellent structural, mechanical, and release properties.

- The optimized formulation (F3) displayed:
- low moisture content (1.87 %),
- high tensile strength (0.49 kg/cm²),
- uniform drug content (98.6 %), and
- controlled biphasic release (≈ 80 % at 8 h).

The DAB method proved simple, reproducible, and gentle, preserving drug stability while ensuring uniform microneedle formation.

Hence, the developed Ramipril-loaded Thiolated chitosan–PVA microneedle patch represents a promising and patient-friendly transdermal system that can overcome first-pass metabolism and improve therapeutic efficacy.

5. Conclusion

The present study successfully developed and characterized a Ramipril-loaded Thiolated chitosan–polyvinyl alcohol (PVA) microneedle patch using the Droplet-Born Air Blowing (DAB) technique for enhanced transdermal drug delivery.

Among all formulations prepared using a 3^2 factorial design, Formulation F3 (PVA : Thiolated Chitosan = 27: 3) was identified as the optimized batch based on its superior mechanical strength, ideal insertion ability, uniform drug content, and desirable moisture balance.

The in-vitro drug release profile of F3 exhibited a distinct biphasic pattern, characterized by an initial burst followed by sustained release, suggesting its potential to achieve rapid onset and prolonged therapeutic effect.

Overall, the study demonstrates that the combination of Thiolated chitosan and PVA offers a robust and biocompatible polymeric matrix suitable for the fabrication of dissolving microneedle patches.

The optimized formulation holds significant promise for improving Ramipril bioavailability, bypassing hepatic first-pass metabolism, and enhancing patient compliance through a painless, controlled, and efficient transdermal delivery approach.

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