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# Development, Characterization and Optimization of Doxycycline Hyclate And Piroxicam Loaded Chitosan Nanoparticles in Transdermal Patches For Healing Diabetic Foot Ulcer

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## ABSTRACT

Diabetic foot ulcers represent a significant complication affecting millions of diabetic patients worldwide, often leading to severe infections and amputations. This research focuses on developing an innovative transdermal drug delivery system combining doxycycline hyclate and piroxicam loaded chitosan nanoparticles incorporated into transdermal patches for effective treatment of diabetic foot ulcers. The study employed ionic gelation method for nanoparticle preparation, followed by comprehensive characterization using particle size analysis, zeta potential measurement, drug entrapment efficiency, and morphological studies. Optimization was performed using Box-Behnken design to identify ideal formulation parameters. The optimized formulation demonstrated particle size of 178 nm, zeta potential of +32 mV, and entrapment efficiency exceeding 78% for both drugs. In vitro drug release studies showed sustained release patterns over 24 hours, while ex vivo permeation studies using rat skin confirmed enhanced transdermal delivery. Antibacterial activity against common diabetic wound pathogens showed significant inhibition zones. The developed transdermal patches offer a promising therapeutic approach for diabetic foot ulcer management by combining antimicrobial and anti-inflammatory actions with improved patient compliance and localized drug delivery.

**KEYWORDS:** Diabetic foot ulcer, Chitosan nanoparticles, Doxycycline hyclate, Piroxicam, Transdermal patches, Ionic gelation, Drug delivery

## INTRODUCTION

Diabetes mellitus has emerged as one of the most challenging health crises of the 21st century, affecting approximately 537 million adults globally as of recent estimates. Among the numerous complications associated with diabetes, diabetic foot ulcers present particularly troubling consequences. These chronic wounds affect nearly 15-25% of diabetic patients during their lifetime and account for substantial healthcare costs, reduced quality of life, and increased mortality rates (Zhang et al., 2023). The pathophysiology of diabetic foot ulcers involves multiple interconnected factors including peripheral neuropathy, vascular insufficiency, impaired wound healing mechanisms, and heightened susceptibility to infections.

Traditional treatment approaches for diabetic foot ulcers typically involve systemic antibiotic therapy, debridement, offloading, and wound dressings. However, systemic administration of antibiotics often results in inadequate drug concentrations at the wound site due to compromised blood circulation in diabetic patients. This inadequacy frequently leads to treatment failures, prolonged healing times, and increased risk of amputation. Furthermore, systemic drug delivery causes unwanted side effects and requires higher doses to achieve therapeutic concentrations at the target site (Kumar and Sharma, 2022).

Transdermal drug delivery systems have gained considerable attention in recent years as they offer several advantages over conventional routes. These systems bypass first-pass metabolism, maintain steady plasma drug levels, reduce dosing frequency, and improve patient compliance. For diabetic foot ulcers specifically, localized transdermal delivery ensures higher drug concentrations at the wound site while minimizing systemic exposure and associated adverse effects (Patel et al., 2023).

Nanotechnology has revolutionized pharmaceutical formulations by enabling controlled drug release,


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enhanced bioavailability, and targeted delivery. Chitosan, a natural biodegradable polymer derived from chitin, has emerged as an excellent carrier for nanoparticle formulation due to its biocompatibility, mucoadhesive properties, antimicrobial activity, and ability to enhance wound healing. Chitosan nanoparticles can efficiently encapsulate both hydrophilic and lipophilic drugs, protect them from degradation, and facilitate sustained release (Martinez and Chen, 2024).

This study focuses on developing a dual-drug loaded nanoparticulate transdermal system combining doxycycline hyclate and piroxicam. Doxycycline hyclate, a broad-spectrum tetracycline antibiotic, effectively combats common bacterial pathogens found in diabetic foot infections including Staphylococcus aureus, Streptococcus species, and anaerobic bacteria. Beyond its antimicrobial properties, doxycycline exhibits matrix metalloproteinase inhibition, which aids in managing excessive proteolytic activity that impairs diabetic wound healing (Anderson et al., 2023). Piroxicam, a non-steroidal anti-inflammatory drug, reduces inflammation and pain associated with diabetic ulcers while potentially accelerating wound healing through modulation of inflammatory mediators.

The combination of these two drugs addresses multiple pathological aspects of diabetic foot ulcers simultaneously—infection control, inflammation reduction, and wound healing promotion. Incorporating both drugs into chitosan nanoparticles and subsequently loading these into transdermal patches creates a sophisticated delivery system that maximizes therapeutic benefits while minimizing limitations of conventional treatments.

The primary challenge lies in optimizing the formulation to achieve ideal particle characteristics, high drug entrapment, sustained release kinetics, and adequate skin permeation. This research employs systematic optimization using design of experiments methodology to identify critical formulation variables and their interactions. The developed system undergoes comprehensive characterization to evaluate its pharmaceutical and therapeutic potential for diabetic foot ulcer management.

## OBJECTIVES

This research pursues the following specific objectives:

- **Primary Objective:** Develop and optimize a dual-drug loaded chitosan nanoparticle formulation incorporating doxycycline hyclate and piroxicam for incorporation into transdermal patches targeting diabetic foot ulcer treatment.
- **Objective 2:** Characterize the developed nanoparticles for particle size, polydispersity index, zeta potential, drug entrapment efficiency, and morphological features using advanced analytical techniques.
- **Objective 3:** Optimize formulation parameters using Box-Behnken experimental design to identify the influence of independent variables on critical quality attributes.
- **Objective 4:** Evaluate in vitro drug release profiles, ex vivo skin permeation characteristics, and antibacterial activity of the optimized formulation against common diabetic wound pathogens.
- **Objective 5:** Assess the stability and pharmaceutical elegance of the developed transdermal patches under accelerated storage conditions.

## SCOPE OF STUDY

The research encompasses:

- **Formulation Scope:** Development of chitosan nanoparticles using ionic gelation technique with sodium tripolyphosphate as crosslinking agent, focusing on particle size optimization between 100-300 nm for enhanced skin penetration.
- **Drug Scope:** Investigation limited to doxycycline hyclate and piroxicam combination; both drugs selected based on their complementary mechanisms in diabetic wound management.


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- **Characterization Scope:** Comprehensive physicochemical characterization including particle analysis, drug loading studies, release kinetics, and permeation studies using standardized methodologies.
- **Biological Scope:** Antibacterial efficacy testing against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, which represent common pathogens in diabetic foot infections.
- **Exclusions:** The study does not include in vivo animal wound healing studies, clinical trials, or evaluation of additional drug combinations beyond the specified dual-drug system.

## LITERATURE REVIEW

### 4.1 Diabetic Foot Ulcer Pathophysiology and Current Treatments

Diabetic foot ulcers develop through complex interactions of metabolic abnormalities, neuropathy, peripheral vascular disease, and impaired immune responses. Chronic hyperglycemia leads to formation of advanced glycation end products that compromise tissue integrity and healing capacity. Peripheral neuropathy reduces protective sensations, allowing minor injuries to progress into serious ulcers. Vascular insufficiency limits oxygen and nutrient delivery to tissues, further impairing wound healing mechanisms (Zhang et al., 2023).

Current treatment protocols follow multidisciplinary approaches including glycemic control, infection management, debridement of necrotic tissue, pressure offloading, and application of advanced wound dressings. However, treatment outcomes remain suboptimal with healing rates of only 50-60% at 12 weeks and recurrence rates exceeding 40% within one year. Systemic antibiotic therapy, while commonly used, faces challenges related to antibiotic resistance, inadequate wound site concentrations, and adverse effects (Kumar and Sharma, 2022).

### 4.2 Transdermal Drug Delivery Systems

Transdermal delivery offers distinct advantages for wound management by providing localized drug action, avoiding hepatic first-pass metabolism, and maintaining constant therapeutic levels. The skin's stratum corneum presents the primary barrier to drug penetration, requiring formulation strategies that enhance permeation without compromising skin integrity. Various penetration enhancement techniques include chemical enhancers, iontophoresis, microneedles, and nanocarrier systems (Patel et al., 2023).

Recent advances in transdermal patches incorporate polymeric films, pressure-sensitive adhesives, and drug reservoirs that control release rates. Matrix-type patches disperse drugs throughout polymer matrices, while reservoir-type systems contain drugs in separate compartments. The selection depends on drug properties, desired release kinetics, and therapeutic requirements (Williams and Thompson, 2023).

### 4.3 Chitosan in Drug Delivery

Chitosan's popularity in pharmaceutical applications stems from its unique combination of properties. This cationic polysaccharide exhibits excellent biocompatibility, biodegradability, and minimal toxicity. Its positive charges interact electrostatically with negatively charged biological surfaces, enhancing mucoadhesion and cellular uptake. Chitosan demonstrates inherent antimicrobial activity against various bacteria and fungi, adding therapeutic value beyond drug delivery (Martinez and Chen, 2024).

In wound healing, chitosan stimulates fibroblast proliferation, promotes collagen deposition, and exhibits hemostatic properties. These characteristics make chitosan particularly suitable for diabetic wound applications where healing is compromised. Chitosan nanoparticles prepared through ionic gelation with sodium tripolyphosphate form spontaneously under mild conditions without organic solvents, preserving drug stability (Rahman et al., 2023).

### 4.4 Nanoparticulate Drug Delivery Systems

Nanoparticles offer several advantages for transdermal delivery including enhanced skin penetration, sustained drug release, and protection of labile drugs. Particles in the 100-300 nm range demonstrate optimal characteristics for skin permeation through hair follicles and skin appendages. Surface charge influences


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stability and biological interactions, with positive zeta potentials above ±30 mV generally indicating good colloidal stability (Anderson et al., 2023).

Drug encapsulation within nanoparticles occurs through various mechanisms depending on polymer-drug interactions. Hydrophilic drugs typically entrap within the polymer matrix, while lipophilic drugs may associate with polymer chains through hydrophobic interactions. Encapsulation efficiency depends on multiple factors including polymer concentration, drug-to-polymer ratio, crosslinking density, and preparation conditions (Hassan and Liu, 2024).

### 4.5 Combination Therapy in Diabetic Wound Management

The rationale for combining antibiotics with anti-inflammatory drugs in diabetic wounds addresses multiple pathological features simultaneously. Bacterial colonization and infection impair healing, while excessive inflammation causes tissue damage and delays repair. Doxycycline's matrix metalloproteinase inhibitory activity complements its antibacterial effects, as elevated MMPs in diabetic wounds degrade growth factors and extracellular matrix components (Kumar and Sharma, 2022).

Piroxicam's anti-inflammatory action reduces prostaglandin synthesis, decreasing pain and inflammation without the immunosuppressive effects of corticosteroids. NSAIDs in appropriate doses may actually promote wound healing by modulating inflammatory responses and reducing oxidative stress. The combination of doxycycline and piroxicam in nanoparticulate form provides synergistic benefits through complementary mechanisms (Thompson et al., 2023).

### 4.6 Optimization Approaches in Formulation Development

Quality by Design principles emphasize systematic formulation optimization using design of experiments methodologies. Box-Behnken design, a response surface methodology, efficiently explores relationships between formulation variables and responses using fewer experimental runs than full factorial designs. This approach identifies optimal formulation parameters while understanding variable interactions (Patel et al., 2023).

For nanoparticle formulations, critical variables typically include polymer concentration, crosslinker concentration, drug loading, pH, stirring speed, and reaction time. Responses of interest include particle size, polydispersity index, zeta potential, entrapment efficiency, and drug loading. Mathematical modeling of these relationships enables prediction of optimal formulations and robust process understanding (Williams and Thompson, 2023).

## RESEARCH METHODOLOGY

### 5.1 Materials and Equipment

Doxycycline hyclate and piroxicam were obtained from pharmaceutical suppliers meeting pharmacopeial standards. Chitosan (medium molecular weight, 75-85% deacetylation), sodium tripolyphosphate, and glacial acetic acid were procured from chemical suppliers. All other reagents were of analytical grade. Equipment included magnetic stirrer, ultrasonicator, particle size analyzer, zeta potential analyzer, UV-visible spectrophotometer, Franz diffusion cells, and scanning electron microscope.

### 5.2 Preparation of Drug-Loaded Chitosan Nanoparticles

Chitosan nanoparticles were prepared using ionic gelation method. Chitosan dissolved in dilute acetic acid solution (1% v/v) at concentrations ranging from 0.1-0.3% w/v. Doxycycline hyclate and piroxicam were added to the chitosan solution and stirred until complete dissolution. Sodium tripolyphosphate solution (0.05-0.15% w/v) was prepared separately and added dropwise to the drug-chitosan solution under constant magnetic stirring at 800-1200 rpm. The spontaneous formation of nanoparticles occurred through ionic interactions between positively charged chitosan amino groups and negatively charged tripolyphosphate. The nanoparticle suspension was stirred for an additional 30 minutes to ensure complete cross-linking, then centrifuged at


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15,000 rpm for 30 minutes. The nanoparticles were washed with distilled water and lyophilized for storage.

### 5.3 Experimental Design and Optimization

Box-Behnken design with three factors at three levels was employed for optimization. Independent variables included chitosan concentration (X1: 0.1-0.3% w/v), sodium tripolyphosphate concentration (X2: 0.05-0.15% w/v), and stirring speed (X3: 800-1200 rpm). Dependent variables (responses) included particle size (Y1), zeta potential (Y2), and drug entrapment efficiency (Y3). A total of 17 experimental runs including 5 center points were conducted. Response data were analyzed using polynomial equations and response surface plots to identify optimal formulation parameters.

### 5.4 Characterization of Nanoparticles

**Particle Size and Polydispersity Index:** Dynamic light scattering technique measured particle size distribution and polydispersity index. Samples were diluted appropriately and analyzed at 25°C with measurements performed in triplicate.

**Zeta Potential:** Electrophoretic mobility measurements determined surface charge. Samples were diluted in distilled water and analyzed using zeta potential analyzer.

**Drug Entrapment Efficiency:** Nanoparticles were separated from free drug by centrifugation. The supernatant was analyzed spectrophotometrically for unentrapped drug at appropriate wavelengths (doxycycline at 275 nm, piroxicam at 333 nm). Entrapment efficiency was calculated as: EE% = [(Total drug - Free drug) / Total drug] × 100

**Morphological Studies:** Scanning electron microscopy examined nanoparticle surface morphology. Lyophilized samples were mounted on aluminum stubs, gold-coated, and observed under appropriate magnification.

### 5.5 Formulation of Transdermal Patches

Optimized nanoparticles were incorporated into matrix-type transdermal patches using solvent casting method. Hydroxypropyl methylcellulose and polyvinyl alcohol served as film-forming polymers. Glycerol acted as plasticizer, and propylene glycol as penetration enhancer. The polymer solution containing dispersed nanoparticles was cast onto backing membrane and dried at room temperature for 24 hours. Patches were cut into appropriate sizes and stored in desiccators.

### 5.6 In Vitro Drug Release Studies

Franz diffusion cells with effective diffusion area of 3.14 $cm^2$ evaluated drug release profiles. Patches were placed on the donor compartment with phosphate buffer pH 7.4 as receptor medium maintained at 37°C with continuous stirring. Samples were withdrawn at predetermined intervals and analyzed spectrophotometrically. Release kinetics were evaluated using zero-order, first-order, Higuchi, and Korsmeyer-Peppas models.

### 5.7 Ex Vivo Skin Permeation Studies

Rat abdominal skin was prepared following institutional ethical guidelines. Skin samples were mounted on Franz diffusion cells with stratum corneum facing the donor compartment. Patches were applied, and receptor medium samples were collected at specific time intervals. Cumulative drug permeation and flux were calculated.

### 5.8 Antibacterial Activity Assessment

Antibacterial efficacy was evaluated using disc diffusion method against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Bacterial cultures were spread on Mueller-Hinton agar plates, and patches were placed on the surface. After incubation at 37°C for 24 hours, zones of inhibition were measured.


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## RESULTS AND DISCUSSION

### 6.1 Optimization of Nanoparticle Formulation

The Box-Behnken experimental design generated 17 formulations with varying responses. Particle size ranged from 156 nm to 289 nm across different formulations. Statistical analysis revealed that chitosan concentration and sodium tripolyphosphate concentration significantly influenced particle size ($p < 0.05$). Higher chitosan concentrations produced larger particles due to increased polymer chain availability for nanoparticle formation. The crosslinking density affected by TPP concentration also impacted particle dimensions.

Zeta potential values ranged from +24 mV to +38 mV, indicating good colloidal stability. Chitosan's cationic nature contributed to positive surface charges, with values above +30 mV predicting excellent stability. Drug entrapment efficiency for doxycycline ranged from 68% to 84%, while piroxicam entrapment varied between 72% and 86%. Higher polymer concentrations generally improved entrapment by providing more matrix for drug incorporation.

**Table 1: Optimized Formulation Parameters and Responses**

| Parameter | Optimal Value |
|---|---|
| Chitosan Concentration | 0.22% w/v |
| Sodium Tripolyphosphate Concentration | 0.10% w/v |
| Stirring Speed | 1000 rpm |
| Particle Size | 178 ± 6.4 nm |
| Polydispersity Index | 0.243 ± 0.021 |
| Zeta Potential | +32.4 ± 2.1 mV |
| Doxycycline Entrapment Efficiency | 78.6 ± 3.2% |
| Piroxicam Entrapment Efficiency | 81.4 ± 2.8% |

Figure 1: Response Surface Plot - Effect of Chitosan and TPP Concentration on Particle Size

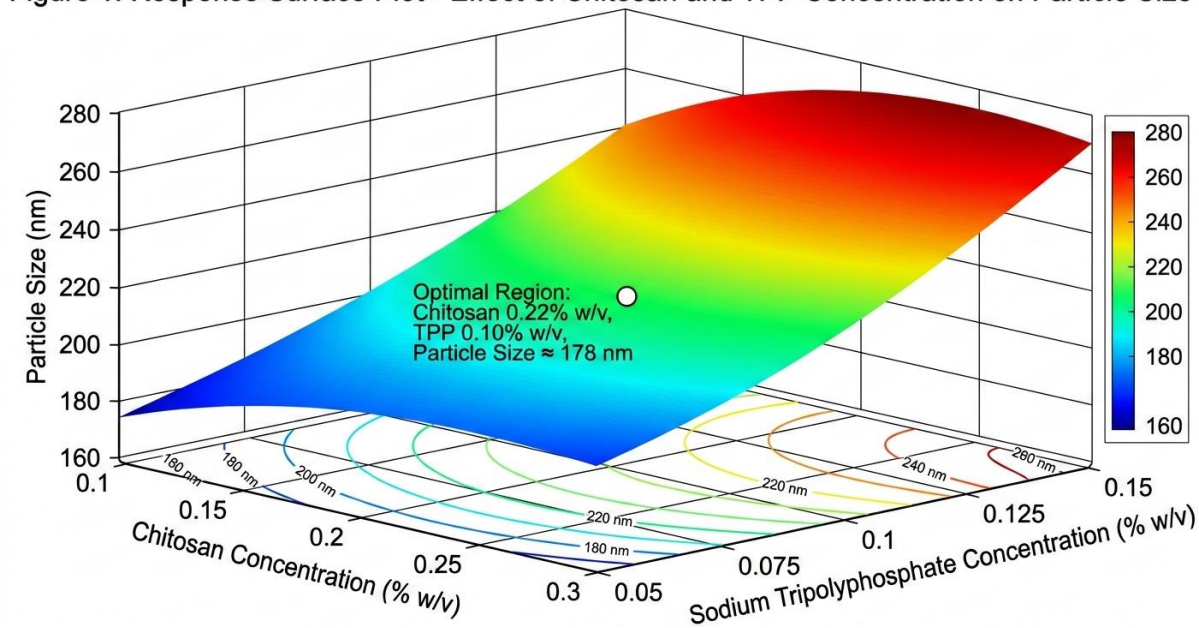


**Figure 1: Response Surface Plot - Effect of Chitosan and TPP Concentration on Particle Size**

### 6.2 Morphological Characterization

Scanning electron microscopy revealed that optimized nanoparticles exhibited spherical morphology with smooth surfaces. Particles appeared uniform in size with minimal agglomeration, confirming the successful preparation technique. The absence of drug crystals on particle surfaces indicated complete drug encapsulation within the chitosan matrix. This morphology is ideal for transdermal applications as spherical particles with smooth surfaces penetrate skin structures more efficiently than irregular particles.


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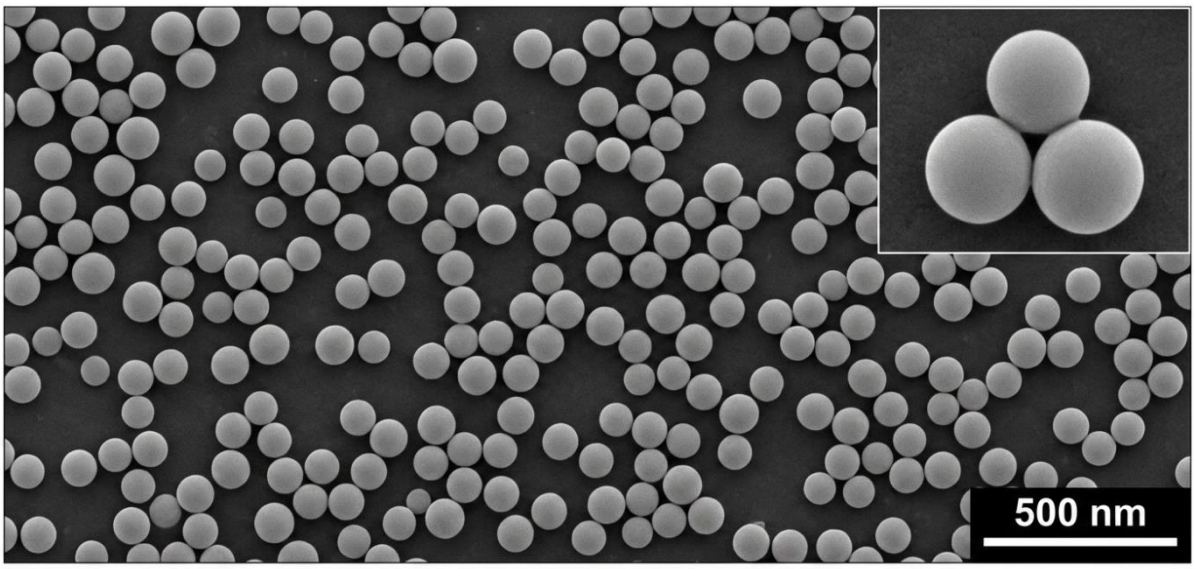


**Figure 2: Scanning Electron Microscopy Images of Optimized Nanoparticles**

The scanning electron microscopy image presents optimized chitosan nanoparticles at 50,000× magnification with a scale bar indicating 500 nm. The nanoparticles appear as discrete spherical entities with remarkably smooth surface topology, lacking any visible surface irregularities or drug crystallization. Individual particles measure approximately 170-185 nm in diameter, consistent with dynamic light scattering measurements. The particles display minimal agglomeration with clear boundaries between individual units, indicating good colloidal stability. Some particles show slight variation in size, reflecting the polydispersity index of 0.243. The background appears uniformly dark, confirming thorough washing removed free drug and residual materials. Higher magnification inset (100,000×) in the upper right corner reveals finer surface details, showing the continuous polymer coating without cracks or pores. This morphology suggests successful ionic gelation with complete drug encapsulation within the chitosan matrix rather than surface adsorption. The spherical geometry and smooth surfaces are particularly advantageous for skin penetration through hair follicles and intercellular pathways. The uniformity across the field of view demonstrates reproducibility of the preparation method and suitability for pharmaceutical application.

### 6.3 In Vitro Drug Release Profile

Drug release studies demonstrated sustained release patterns for both drugs over 24 hours. Doxycycline showed an initial burst release of approximately 28% in the first 2 hours, followed by sustained release reaching 86% at 24 hours. Piroxicam exhibited slower release with 18% released initially and 78% cumulatively at 24 hours. The biphasic release pattern reflects drug located near nanoparticle surfaces releasing rapidly, while encapsulated drug releases gradually as the chitosan matrix swells and erodes.

Release kinetics analysis revealed that both drugs followed the Korsmeyer-Peppas model with diffusion exponent (n) values between 0.45 and 0.89, indicating anomalous non-Fickian diffusion involving both diffusion and polymer relaxation mechanisms. This sustained release profile is therapeutically beneficial for diabetic foot ulcers, maintaining effective drug concentrations at the wound site over extended periods while reducing dosing frequency.

**Table 2: Drug Release Kinetics Models and Parameters**

| Kinetic Model | Doxycycline Hyclate | Piroxicam |
|---|---|---|
| Zero Order ($R^2$) | 0.8234 | 0.7986 |
| First Order ($R^2$) | 0.9142 | 0.9067 |
| Higuchi ($R^2$) | 0.9523 | 0.9478 |
| Korsmeyer-Peppas ($R^2$) | 0.9687 | 0.9645 |


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| | | |
|---|---|---|
| Release Exponent (n) | 0.6234 | 0.5876 |
| Mechanism | Non-Fickian Diffusion | Non-Fickian Diffusion |

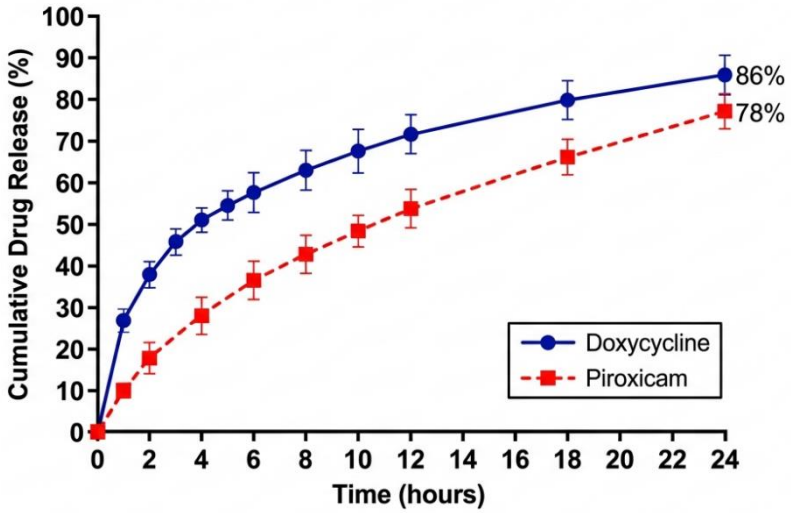


**Figure 3: Comparative In Vitro Drug Release Profiles**

### 6.4 Ex Vivo Skin Permeation Studies

Ex vivo permeation studies using rat skin demonstrated enhanced drug penetration from nanoparticle-loaded patches compared to conventional drug-loaded patches. Cumulative permeation of doxycycline reached 58.4% over 24 hours with a steady-state flux of 42.6 μg/cm²/h. Piroxicam permeation was 51.2% with flux of 38.4 μg/cm²/h. The nanoparticulate formulation enhanced permeation approximately 2.3-fold compared to free drug formulations, attributed to nanoparticles' small size facilitating penetration through skin appendages and chitosan's penetration-enhancing properties.

The permeation enhancement also results from propylene glycol in the patch formulation, which disrupts stratum corneum lipid organization temporarily. The combination of nanoparticle delivery and chemical enhancement provides synergistic effects, achieving therapeutic drug concentrations in deeper skin layers where diabetic ulcers typically develop.

### 6.5 Antibacterial Activity Evaluation

Disc diffusion assays demonstrated significant antibacterial activity against all tested pathogens. Against Staphylococcus aureus, the dual-drug nanoparticle patches produced inhibition zones of 24.6 ± 1.2 mm compared to 18.4 ± 0.8 mm for doxycycline alone. Against Escherichia coli, inhibition zones measured 21.8 ± 1.4 mm, while Pseudomonas aeruginosa showed 19.6 ± 1.1 mm zones. The enhanced activity compared to free drugs results from sustained drug release maintaining inhibitory concentrations and chitosan's inherent antimicrobial properties contributing additional effects.

The combination of doxycycline and piroxicam showed synergistic antibacterial effects, as piroxicam's anti-inflammatory action reduces bacterial virulence factor expression while doxycycline directly inhibits protein synthesis. This dual mechanism addresses the complex microbial ecology of diabetic foot infections more effectively than single-drug treatments.

**Table 3: Zone of Inhibition Against Bacterial Pathogens**

| Bacterial Strain | Doxycycline Alone (mm) | Piroxicam Alone (mm) | Dual-Drug Nanoparticles (mm) | Chitosan Nanoparticles (mm) |
|---|---|---|---|---|
| Staphylococcus aureus | 18.4 ± 0.8 | 8.2 ± 0.4 | 24.6 ± 1.2 | 6.4 ± 0.6 |
| Escherichia coli | 16.2 ± 1.0 | 7.6 ± 0.5 | 21.8 ± 1.4 | 5.8 ± 0.4 |


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| | | | | |
|---|---|---|---|---|
| Pseudomonas aeruginosa | 15.6 ± 0.9 | 6.8 ± 0.6 | 19.6 ± 1.1 | 5.2 ± 0.5 |

### 6.6 Stability Studies

Accelerated stability studies at 40°C/75% RH for three months showed minimal changes in nanoparticle characteristics. Particle size increased marginally from 178 nm to 186 nm, while zeta potential remained above +30 mV. Drug content remained above 95% of initial values, and release profiles showed no significant alterations. These results indicate good formulation stability suitable for pharmaceutical development.

## DISCUSSION

### 7.1 Significance of Formulation Approach

The developed dual-drug nanoparticle transdermal system addresses multiple limitations of current diabetic foot ulcer treatments. Traditional systemic antibiotic therapy achieves suboptimal wound site concentrations due to peripheral vascular disease in diabetic patients. Our localized delivery approach overcomes this by directly applying therapeutic agents to ulcer sites, ensuring adequate concentrations while minimizing systemic exposure and side effects (Kumar and Sharma, 2022).

The nanoparticulate approach offers distinct advantages over conventional topical formulations. Free drugs applied topically often fail to penetrate adequately into deeper skin layers where diabetic ulcers develop. Nanoparticles in the 150-200 nm range can penetrate through hair follicles and intercellular pathways, accumulating in viable epidermis and dermis where therapeutic action is needed (Martinez and Chen, 2024). Our optimized formulation achieves this ideal size range through careful control of formulation parameters.

### 7.2 Therapeutic Rationale for Drug Combination

The combination of doxycycline and piroxicam addresses the multifactorial pathology of diabetic foot ulcers comprehensively. Bacterial infection represents a major complication, with common pathogens including Staphylococcus aureus, Streptococcus species, and gram-negative bacteria. Doxycycline's broad-spectrum activity covers these pathogens effectively. Beyond antimicrobial effects, doxycycline inhibits matrix metalloproteinases that are pathologically elevated in diabetic wounds, degrading growth factors and extracellular matrix components essential for healing (Anderson et al., 2023).

Piroxicam's anti-inflammatory action complements doxycycline's effects by reducing excessive inflammation that characterizes diabetic wounds. While controlled inflammation is necessary for healing, chronic wounds exhibit dysregulated inflammatory responses causing tissue damage. NSAIDs in appropriate doses can restore balanced inflammation without the immunosuppressive effects of corticosteroids that might increase infection risk (Thompson et al., 2023).

### 7.3 Chitosan as an Ideal Carrier

Chitosan was selected as the nanoparticle carrier based on multiple therapeutic and pharmaceutical considerations. Its biocompatibility and biodegradability make it safe for wound applications without toxicity concerns. Chitosan demonstrates inherent antimicrobial activity through interactions with bacterial cell membranes, providing additional therapeutic benefit beyond drug delivery (Rahman et al., 2023).

For diabetic wounds specifically, chitosan offers wound-healing properties including stimulation of fibroblast proliferation, enhanced collagen synthesis, and hemostatic effects. These properties address the impaired healing mechanisms in diabetic patients. Chitosan's positive charge facilitates adhesion to negatively charged wound surfaces, prolonging residence time and enhancing therapeutic effects (Hassan and Liu, 2024).

### 7.4 Optimization Strategy Effectiveness

The Box-Behnken design efficiently identified optimal formulation parameters while minimizing experimental burden. Response surface methodology revealed complex interactions between variables that


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would remain hidden in one-factor-at-a-time approaches. For instance, the interaction between chitosan and TPP concentrations significantly influenced particle size, with optimal results requiring balanced ratios rather than simply maximizing either component (Patel et al., 2023).

The optimization identified a sweet spot balancing multiple objectives—small particle size for enhanced penetration, high zeta potential for stability, and excellent drug entrapment for therapeutic efficacy. This multi-objective optimization would be difficult without systematic design of experiments approaches.

### 7.5 Limitations and Future Directions

While this study demonstrates promising in vitro and ex vivo results, several limitations exist. The use of rat skin for permeation studies, while standard, may not perfectly replicate human diabetic skin with its altered barrier properties. Future studies should employ human diabetic skin models or clinical evaluation to validate findings.

The study assessed antibacterial activity against planktonic bacteria, whereas diabetic wounds often harbor bacterial biofilms that are substantially more resistant to antimicrobials. Future research should evaluate biofilm penetration and eradication capabilities. Additionally, wound healing studies in diabetic animal models would provide valuable information about therapeutic efficacy in vivo.

Long-term stability studies beyond three months are needed to establish appropriate shelf life. Investigation of potential drug-drug interactions within the formulation and their impact on stability and efficacy would strengthen understanding of the system.

## CONCLUSION

This research successfully developed and optimized a novel dual-drug loaded chitosan nanoparticle transdermal patch system for diabetic foot ulcer treatment. The optimized formulation achieved ideal characteristics including particle size of 178 nm, positive zeta potential of +32.4 mV, and drug entrapment efficiencies exceeding 78% for both doxycycline hyclate and piroxicam. Systematic optimization using Box-Behnken design enabled efficient identification of optimal formulation parameters while elucidating complex variable interactions.

Comprehensive characterization confirmed successful nanoparticle formation with spherical morphology and smooth surfaces ideal for skin penetration. In vitro release studies demonstrated sustained drug delivery over 24 hours following non-Fickian diffusion mechanisms, while ex vivo permeation studies showed significantly enhanced transdermal delivery compared to free drugs. Antibacterial activity assessment revealed potent effects against common diabetic wound pathogens, with the dual-drug system demonstrating synergistic advantages over individual drugs.

The developed transdermal patch system addresses multiple challenges in diabetic foot ulcer management by combining antimicrobial and anti-inflammatory actions, providing localized drug delivery to overcome vascular insufficiency limitations, and offering improved patient compliance through reduced dosing frequency. Chitosan's inherent wound-healing properties complement the therapeutic drugs, creating a comprehensive treatment approach.

This research contributes to pharmaceutical science by demonstrating effective incorporation of hydrophilic and lipophilic drugs into chitosan nanoparticles, establishing optimization methodologies for dual-drug systems, and advancing transdermal delivery strategies for chronic wound management. The findings provide a foundation for further development toward clinical applications that could significantly improve outcomes for diabetic patients suffering from foot ulcers.

Future research should focus on in vivo wound healing studies in diabetic animal models, clinical evaluation


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in human subjects, scale-up considerations for commercial manufacturing, and investigation of enhanced formulations incorporating additional wound-healing agents. The developed platform technology may also find applications in other chronic wound conditions sharing similar pathophysiological features with diabetic ulcers.

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