Chitosan Nanoparticle Based Mucoadhesive and Mucopenetrating Vaginal Drug Delivery Systems

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ABSTRACT

Background

The female reproductive tract (FRT) presents a restrictive landscape for conventional therapies, which typically suffer a 90% dose loss due to the "Leakage and Clearance" paradox. This review synthesizes current advancements in chitosan-nanoparticle (CS-NP) systems, which leverage a pH-responsive apparent pKa of approximately 6.5 to ensure stability in the acidic vaginal lumen (pH 3.8-4.5).

Materials and Methods

To optimize therapeutic outcomes, researchers have engineered adaptive surfaces that navigate the 340 ± 70 nm cervicovaginal mucus (CVM) mesh. Technical comparisons demonstrate that thiolated "thiomers" (TCS) utilize covalent disulfide exchange (Polymer-SH + Mucin-SH ⇌ Polymer-S-S-Mucin + $2H^+$ + $2e^-$) to achieve a 140-fold increase in mucosal retention. Conversely, mucus-penetrating particles (MPPs) functionalized with dense polyethylene glycol (PEG) shield surface charges to facilitate deep epithelial access.

Results

State-of-the-art (SOTA) applications include Tenofovir-loaded multiunit carriers for HIV prevention, which achieve entrapment efficiencies of 68.9%, and hybrid lipid-chitosan nanoparticles that enhance steroid flux (J) for hormone replacement. The narrative identifies the integration of Artificial Intelligence (AI) in rational design—achieving test-set predictive accuracies of $R^2 = 0.9943$—and the emergence of Microbiome-Active Drug Delivery Systems (MADDS) that utilize microbial metabolites as release triggers.

Conclusion

Finally, the review addresses the 2025 regulatory updates from the FDA and EMA, emphasizing the need for standardized characterization to overcome translational bottlenecks. This comprehensive analysis positions CS-NPs as a high-precision platform for treating infectious, oncogenic, and endocrine disorders of the FRT.

Keywords: Chitosan nanoparticle; vaginal drug delivery systems; mucoadhesive; mucopenetration.

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I. Introduction

1.1 The Global Landscape of Vaginal Health

The epidemiological burden of female reproductive tract (FRT) disorders necessitates a transition from conventional topical therapies toward advanced nanomedicines (Bassey & Ilomuanya, 2025). In 2021, global surveillance documented over 722 million incident cases of sexually transmitted infections (STIs), emphasizing a stagnant age-standardized incidence rate (ASIR) that requires high-precision intervention (Louisa et al., 2022). Concurrently, the population living with Human Immunodeficiency Virus (HIV) reached approximately 40.8 million by 2024, with women and girls accounting for 53% of these cases and 45% of all new infections (Haider et al., 2025). Beyond viral pathogens, vulvovaginal candidiasis (VVC) represents a ubiquitous clinical challenge, with 70-75% of women experiencing at least one symptomatic episode in their lifetime (Prabhat, 2021). Recent data from South Africa and Greece highlight a significant syndemic interaction between Candida albicans and STIs such as Chlamydia trachomatis, which further exacerbates mucosal dysbiosis and increases the risk of oncogenic progression in the cervical transformation zone (Kroustali et al., 2025; Szymańska et al., 2025). Conventional "Gold Standard" therapies, including azole-based creams and silicone rings, frequently fail to maintain therapeutic concentrations due to poor aqueous solubility of the active pharmaceutical ingredients (APIs) and the lack of bioadhesive depth required to resist physiological clearance (Potaś et al., 2021).

1.2 Biological Impediments to Drug Bioavailability

Effective transmucosal drug delivery is primarily hindered by the "Leakage and Clearance" paradox, wherein approximately 90% of the administered dose is cleared within hours of application (Kadam, 2026). This rapid depletion is driven by the periodic renewal of the

vaginal mucus layer and the continuous peristaltic movements of the vaginal wall (Pei et al., 2025). The vaginal mucus gel is a complex viscoelastic barrier composed of mucin glycoproteins (500 kDa to 20 MDa) characterized by high sialic acid and ester sulfate content (Sogias et al., 2008). These functionalities impart a net negative charge to the mucosal surface, as they are fully ionized at pH > 2.6 (Sogias et al., 2008).

Furthermore, the healthy vaginal pH (typically 3.8-4.5) creates a restrictive environment for conventional formulations (Dinu-Pîrvu et al., 2008). While the acidity protects against pathogens, it also dictates the ionization state and solubility of delivery vehicles (Bassey & Ilomuanya, 2025). Conventional gels and suppositories exhibit non-specific distribution and inadequate retention, leading to sub-optimal plasma drug concentrations and the necessity for high, frequent dosing that can induce local irritation and disrupt commensal Lactobacillus species (Prabhat, 2021). Consequently, there is an exigent need for delivery systems that can navigate the mucus mesh and withstand the dynamic pH fluctuations associated with coitus or hormonal cycles (Ahmed & Aljaeid, 2016).

1.3 The Emergence of Chitosan Nanocarriers

Chitosan, a linear β-(1→4)-linked D-glucosamine copolymer, has emerged as a primary candidate for vaginal delivery due to its unique polycationic character and pH-responsive solubility (Ahmed & Aljaeid, 2016). The primary amino groups of chitosan possess an apparent pKa of approximately 6.5, ensuring protonation ($-NH_3^+$) and subsequent water solubility in the acidic vaginal lumen (Sogias et al., 2008). This cationic charge facilitates strong electrostatic interactions with the anionic sialic acid residues of mucin, promoting a bioadhesive interface (Sogias et al., 2008).

Critical Synthesis: Trimethyl Chitosan (TMC) versus Thiolated Chitosan (TCS)

In engineering mucoadhesive systems, a technical comparison must be drawn between quaternized derivatives and thiolated variants. Trimethyl chitosan (TMC) provides a permanent positive charge independent of pH, extending its solubility into neutral environments (Dinu-Pîrvu et al., 2008). However, high degrees of quaternization can induce conformational changes that sterically hinder the polymer's ability to interpenetrate the mucin mesh, potentially reducing its effective mucoadhesive strength compared to native chitosan at pH 4.5 (Sogias et al., 2008).

In contrast, thiolated chitosan (TCS) introduces a chemisorption mechanism through the covalent attachment of thiol-bearing ligands such as L-cysteine (Bernkop-Schnürch, 2021). While TMC relies solely on ionic interactions, TCS facilitates the formation of disulfide bonds with the cysteine-rich sub-domains of mucin glycoproteins (Dinu-Pîrvu et al., 2008). The chemical reaction follows a thiol/disulfide exchange:

Polymer-SH + Mucin-SH □ Polymer-S-S-Mucin + $2H^+$ + $2e^-$

Under vaginal conditions of pH 3.8-4.5, TCS demonstrates a 20- to 50-fold increase in mucoadhesion compared to unmodified polymers, effectively surpassing the retention limitations of both TMC and native chitosan (Dinu-Pîrvu et al., 2008). Furthermore, "S-protected" thiomers allow for a dual-action synergism: the particles can diffuse through the superficial "fast" mucus layers before the disulfide exchange occurs, ensuring immobilization within the deeper, stable mucus proximal to the epithelium (Bernkop-Schnürch, 2021).

Table 1: Comparative Analysis of Chitosan Modifications on Zeta Potential and Mucoperfusion Rates

Modification Type	Zeta Potential (mV)	Mucoperfusion Rate (Deff/Dw)	Mucoadhesive Mechanism
Native Chitosan	+25 to +40	< 0.01	Electrostatic (Sogias et al., 2008)
Trimethyl Chitosan (TMC)	+15 to +30	< 0.05	pH-Independent Cationic (Dinu-Pîrvu et al., 2008)
Thiolated Chitosan (TCS)	+10 to +25	< 0.005	Disulfide Bonding (Bernkop-Schnürch, 2021)
PEGylated Chitosan (PEG-CS)	-5 to +5	0.1 to 0.8	Steric Hindrance Shielding (Ensign et al., 2012)

The scope of this review extends beyond mere mucoadhesion to explore active penetration strategies. While TCS provides superior retention, recent advancements in PEGylation have enabled the development of mucus-penetrating particles (MPPs) (Ensign et al., 2012). These systems utilize a neutral, hydrophilic coating to minimize electrostatic "trapping" by mucin, allowing the nanocarriers to distribute uniformly across the rugae and reach the underlying squamous epithelium for improved pharmacodistribution (Ensign et al., 2012; Almeida et al., 2024).

II. Anatomical and Physiological Barriers

The female reproductive tract (FRT) presents a dynamic physiological environment where successful drug delivery is predicated on navigating a hierarchical series

of biological obstacles. For the senior researcher, the vaginal tract must be viewed not as a static conduit, but as a hormonally regulated system characterized by high mucus turnover, variable pH gradients, and a complex stratified cellular architecture (Kadam, 2026; Potaś et al., 2021).

2.1 The Vaginal Mucus Gel Layer

The primary chemical defense of the FRT is the cervicovaginal mucus (CVM), a complex viscoelastic hydrogel that coats the vaginal wall to a thickness of approximately 50 µm (Ensign et al., 2012). CVM is composed of 95% water and a 1-5% matrix of high-molecular-weight mucin glycoproteins (500 kDa to 40 MDa), predominantly MUC5B and MUC4 (Bansil & Turner, 2006; Samprasilt et al., 2020).

2.1.1 Biochemical Composition and Charge Density

The functional properties of CVM are dictated by the dense O-linked glycans on the protein backbone, which comprise more than 70% of the mucin mass (Sogias et al., 2008). These glycan chains are rich in sialic acid and ester sulfates, which are fully ionized at physiological pH levels above 2.6, imparting a strong net negative charge to the mucus mesh (Sogias et al., 2008; Dinu-Pîrvu et al., 2008). This polyanionic character creates a significant barrier to cationic nanoparticles, such as native chitosan-tripolyphosphate (CS-TPP) systems, which are effectively "trapped" in the superficial mucus layers through strong electrostatic attraction, preventing deeper tissue penetration (Ensign et al., 2012; Samprasilt et al., 2020).

2.1.2 Micro-rheological Mesh Size and Steric Hindrance

At the nanoscale, CVM forms a heterogeneous mesh network. High-resolution single-particle tracking has demonstrated that non-ovulatory CVM possesses an average pore size of 340 ± 70 nm, with a wide distribution ranging from 50 nm to 1800 nm (Ensign et al., 2012; Pei et al., 2025). Particles exceeding these dimensions face steric exclusion, while smaller particles (approx. 100 nm) may paradoxically exhibit slower diffusion rates if they become immobilized within smaller mucin pockets or pockets of high adhesive potential (Ensign et al., 2012; Ahmed & Aljaeid, 2016).

2.2 Epithelial Architecture and Junctional Integrity

The vaginal epithelium is a non-keratinized, multilayered stratified squamous structure that undergoes cyclical changes in thickness (typically 200-300 µm) and glycogen content under the influence of estrogen (Bassey & Ilomuanya, 2025; Szymańska et al., 2025).

2.2.1 Stratified Squamous vs. Columnar Transformation Zones

The ectocervix and vagina are lined with robust stratified squamous layers, whereas the endocervix features a single layer of simple columnar epithelium (Acharya & Behera, 2024). The transition between these regions-the "transformation zone"-is a critical area for drug targeting, as it is highly susceptible to pathogenic invasion and oncogenic transformation (Kroustali et al., 2025). The squamous architecture prevents the paracellular transport of large molecules and nanoparticles, requiring active modulation for systemic or sub-epithelial delivery (Bassey & Ilomuanya, 2025).

2.2.2 The Role of Tight Junctions (Zonula Occludens)

Barrier integrity is maintained by intercellular junctional complexes, including tight junctions (TJs), adherens junctions, and desmosomes. TJs (Zonula Occludens) are composed of transmembrane proteins such as occludin, claudins, and junctional adhesion molecules (JAMs), which are linked to the actin cytoskeleton via adapter proteins like ZO-1 and ZO-2 (Sogias et al., 2008; Ahmed & Aljaeid, 2016). Research indicates that while apical layers are relatively permeable to large mediators like IgG, the suprabasal and basal layers contain exclusionary junctions that restrict paracellular flux (Sogias et al., 2008). Chitosan-based systems are uniquely engineered to reversibly open these TJs by inducing a redistribution of F-actin, thereby facilitating the translocation of nanoparticles to the submucosa and draining lymph nodes (dLNs) (Ahmed & Aljaeid, 2016; Bernkop-Schnürch, 2021).

2.3 The Dynamic pH and Microbiome Interface

The vaginal environment is characterized by a low homeostatic pH (3.8-4.2), maintained by commensal Lactobacillus species (e.g., L. crispatus and L. acidophilus) that metabolize epithelial glycogen into lactic acid (Szymańska et al., 2025; Kroustali et al., 2025).

2.3.1 pH Shifts and Stability

This acidic environment is critical for the performance of pH-responsive nanocarriers. Below the pKa of chitosan (approx. 6.5), the primary amino groups undergo protonation ($-NH_3^+$), resulting in polymer swelling and increased bioadhesion (Ahmed & Aljaeid, 2016; Samprasilt et al., 2020). However, this environment is neutralized by semen (pH 7.0-8.0) during coitus or by menstrual blood, which can trigger premature drug release or deprotonation of the carrier, leading to a loss of mucoadhesive strength (Potaś et al., 2021; Dinu-Pîrvu et al., 2008).

2.3.2 Critical Synthesis: Trimethyl Chitosan (TMC) vs. Thiolated Chitosan (TCS)

In designing systems to withstand these fluctuations, researchers must weigh the advantages of quaternization versus thiolation. Trimethyl chitosan (TMC) provides a permanent positive charge independent of pH, maintaining solubility even in the neutralized post-coital tract (Dinu-Pîrvu et al., 2008). However, high degrees of quaternization can sterically hinder chain interpenetration into the mucin mesh, potentially

reducing adhesion compared to native chitosan (Sogias et al., 2008). In contrast, thiolated chitosan (TCS) forms covalent disulfide bonds with the cysteine-rich sub-domains of mucins via a thiol/disulfide exchange:

Polymer-SH + Mucin-SH □ Polymer-S-S-Mucin + $2H^+$ + $2e^-$

Under vaginal conditions (pH 3.8-4.5), TCS exhibits a 20- to 140-fold improvement in mucoadhesive strength compared to unmodified polymers, effectively solving the "Leakage and Clearance" paradox where 90% of a conventional dose is lost within hours (Bernkop-Schnürch, 2021; Dinu-Pîrvu et al., 2008).

Table 2: Comparison of physiological parameters across different life stages and their impact on NP behavior

Parameter	Pre-pubertal	Reproductive	Post-menopausal	Impact on CS-NP
Vaginal pH	6.5–7.5	3.8–4.5	6.0–7.5	Acidic pH favors -NH_3^+ protonation and adhesion (Ahmed & Aljaeid, 2016).
Epithelial Thickness	Thin (Atrophic)	Thick (200–300 µm)	Thin (Atrophic)	Thicker tissue requires TJ modulation for paracellular flux (Sogias et al., 2008).
Microbiota	Diverse/Enteric	Lactobacillus-dominant	Low Lactobacillus	Lactobacillus-produced lactic acid maintains pH for NP stability (Kroustali et al., 2025).
Mucus Volume	Low	High (Cyclic)	Very Low	High volume leads to rapid NP clearance/dilution (Ensign et al., 2012).
Glycogen Content	Trace	High	Low	High glycogen supports microbial pH stabilization (Szymańska et al., 2025).

Table 3: Comparative Analysis of Chitosan Modifications on Zeta Potential and Mucoperfusion Rates

Modification	Zeta Potential (mV)	Mucoperfusion (Deff/Dw)	Adhesive Depth
Native CS	+25 to +40	< 0.01	Superficial (Trapped) (Ensign et al., 2012)
TMC (Quaternized)	+15 to +30	< 0.05	pH-Independent Surface Binding (Dinu-Pîrvu et al., 2008)
TCS (Thiolated)	+10 to +25	< 0.005	Covalent Covalent (Tethered) (Bernkop-Schnürch, 2021)
PEG-CS (PEGylated)	-5 to +5	0.1 to 0.8	Deep (Trans-mucosal) (Pei et al., 2025)

III. Chitosan: Fundamental Material Science

The clinical utility of chitosan in the vaginal tract is fundamentally derived from its molecular structure, which facilitates a pH-responsive transition from a hydrophobic solid to a polycationic hydrogel. This section correlates the polymer's chemical architecture with the physiological requirements of the female reproductive tract (FRT), establishing the material science foundation for subsequent engineering strategies (Ahmed & Aljaeid, 2016; Dinu-Pîrvu et al., 2008).

3.1 Chemistry and Protonation Kinetics

Chitosan is a linear polysaccharide composed of β-(1→4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) (Ahmed & Aljaeid, 2016). The presence of primary amino groups

($-NH_2$) at the C-2 position of the glucosamine units dictates the polymer's unique physicochemical profile (Sogias et al., 2008).

3.1.1 Deacetylation Degree (DD) and Apparent pKa

The apparent pKa of chitosan is approximately 6.5, representing the critical threshold for ionization (Ahmed & Aljaeid, 2016; Sogias et al., 2008). In the acidic environment of the healthy vagina (pH 3.8-4.5), these amino groups undergo protonation to form positively charged $-NH_3^+$ moieties (Ahmed & Aljaeid, 2016; Sogias et al., 2008). The DD significantly influences this transition; studies utilizing modified Henderson-Hasselbalch models demonstrate that pKa values increase from 6.17 to 6.51 as the DD decreases from 94.6% to 73.3%, indicating that less deacetylated polymers maintain higher charge densities at near-neutral pH (Dinu-Pîrvu et al., 2008).

3.1.2 pH-Responsive Phase Transitions

In environments where pH < pKa, the electrostatic repulsion between $-NH_3^+$ sites forces the polymer chains apart, allowing for rapid water infiltration and significant swelling of the nanoparticle matrix (Ahmed & Aljaeid, 2016). Conversely, a rise in pH toward neutrality (e.g., during coitus or menses) induces deprotonation, causing the network to collapse as electrostatic forces weaken (Ahmed & Aljaeid, 2016). This transition is vital for controlled release, as it modulates the internal mesh size and diffusivity of the encapsulated therapeutic payload (Potaś et al., 2021).

3.2 Biological Interactions and Degradation

Chitosan is not merely an inert carrier but a bioactive polymer that undergoes specific enzymatic degradation in mucosal environments (Ahmed & Aljaeid, 2016; Haider et al., 2025).

3.2.1 Enzymatic Degradation by Lysozyme

In vivo, chitosan is primarily degraded by lysozyme, a non-specific endo-carbohydrase present in vaginal secretions (Bernkop-Schnürch, 2021; Haider et al., 2025). Lysozyme targets the acetylated residues, cleaving the β-(1→4) glycosidic linkages between monomers (Bernkop-Schnürch, 2021). The degradation rate is inversely proportional to the DD and crystallinity of the polymer (Haider et al., 2025). The resulting metabolic products are non-toxic oligosaccharides and glucosamine, which are readily integrated into natural glycoprotein synthesis or excreted, ensuring zero systemic accumulation (Haider et al., 2025; Bernkop-Schnürch, 2021).

3.2.2 Intrinsic Antimicrobial and Anti-inflammatory Properties

The polycationic backbone of native chitosan exerts intrinsic microbicidal effects by interacting with the negatively charged components of microbial membranes, such as lipopolysaccharides (Ahmed & Aljaeid, 2016; Prabhat, 2021). Furthermore, chitosan inhibits the expression of pro-inflammatory cytokines like TNF-α and IL-1β in vaginal epithelial cells, making it an ideal candidate for managing conditions characterized by chronic inflammation or dysbiosis (Kroustali et al., 2025; Szymańska et al., 2025).

3.3 Sourcing and Purity Standards

The transition from bench-scale synthesis to clinical application requires strict standardization of chitosan's origin and purity to minimize immunogenic risks (Sogias et al., 2008; Haider et al., 2025).

3.3.1 Crustacean-derived vs. Fungal-derived Chitosan

Historically, commercial chitosan (ADC) has been sourced from crustacean shells, which entails significant mineral content (30-60%) and risks of contamination with allergenic proteins (Dinu-Pîrvu et al., 2008; Sogias et al., 2008). Recently, fungal-derived chitosan (MDC) has emerged as a high-purity alternative (Dinu-Pîrvu et al., 2008). MDC exhibits a lower mineral content (2.5-7%) and consistent physicochemical properties, as fungi are cultivated under controlled conditions rather than being subject to seasonal maritime variations (Dinu-Pîrvu et al., 2008; Sogias et al., 2008). Crucially, fungal sources lack the allergenic risk associated with shellfish, facilitating smoother regulatory approval for mucosal applications (Haider et al., 2025).

3.3.2 Standardization of Molecular Weight (MW) Distribution

Pharmaceutical-grade chitosan must adhere to ASTM F2103 standards, which require detailed characterization of MW distribution using gel permeation chromatography (GPC) or intrinsic viscosity measurements (Dinu-Pîrvu et al., 2008). MW typically ranges from 50 to 1000 kDa and directly influences the mechanical strength of formed nanoparticles and the depth of mucoadhesive penetration (Pei et al., 2025). High MW variants offer superior chain entanglement within the mucin mesh but may restrict initial paracellular transport (Pei et al., 2025; Sogias et al., 2008).

IV. Engineering and Synthesis Protocols

The evolution from raw material science to the fabrication of therapeutic delivery vehicles requires precise control over the physical and chemical assembly of chitosan chains. While Section III established the polycationic nature of the polymer, this section delineates the specific engineering protocols required to transform these macromolecules into stable, functional nanoparticles (CS-NPs) capable of navigating the vaginal mucosal barrier (Ahmed & Aljaeid, 2016; Gutiérrez-Ruíz et al., 2024).

4.1 Ionic Gelation: The Benchmark Technique

Ionic gelation remains the predominant strategy for fabricating CS-NPs due to its aqueous-based, "green" synthesis conditions which preserve the bioactivity of encapsulated proteins and sensitive small molecules

(Calvo et al., 1997; Louisa et al., 2022).

4.1.1 Mechanism of CS-TPP Electrostatic Cross-linking

The synthesis is fundamentally driven by ionotropic gelation, a process of physical cross-linking mediated by the electrostatic attraction between the protonated amino groups ($-NH_3^+$) of chitosan and the multivalent anions of sodium tripolyphosphate (TPP) (Ahmed & Aljaeid, 2016; Dinu-Pîrvu et al., 2008). Unlike chemical cross-linking with glutaraldehyde, which involves the formation of Schiff bases, ionic gelation relies on a reversible physical transition (Sogias et al., 2008). In an acidic environment (pH 3.8-5.5), the CS chains adopt an expanded conformation due to intra-chain repulsion; the introduction of TPP induces a rapid phase transition from a liquid sol to a solid nanoparticle matrix (Gutiérrez-Ruíz et al., 2024; Potaś et al., 2021).

4.1.2 Critical Process Parameters (CPPs)

The morphology and polydispersity index (PDI) of CS-NPs are highly sensitive to processing variables. A stirring speed of 500 rpm is generally required to maintain a stable vortex (approx. 1 cm depth), ensuring immediate dispersion of TPP droplets to prevent localized over-cross-linking and subsequent macro-aggregation (Gutiérrez-Ruíz et al., 2024; Ahmed & Aljaeid, 2016). Furthermore, the CS:TPP mass ratio (optimally between 3:1 and 5:1) dictates the final zeta potential; excess TPP can neutralize the surface charge, reducing the electrostatic stability and mucoadhesive potential of the system (Calvo et al., 1997; Gutiérrez-Ruíz et al., 2024).

4.2 Advanced Fabrication: Internal Gelation and Spray Drying

To overcome the limited drug loading of hydrophilic agents and the lack of industrial scalability in traditional dropwise methods, alternative fabrication routes have been developed (Almeida et al., 2024; Samprasilt et al., 2020).

4.2.1 Emulsification-Internal Gelation

This technique is specifically suited for high-efficiency loading of hydrophobic or poorly soluble drugs. The drug-loaded chitosan phase is dispersed in an oil phase (e.g., light liquid paraffin) containing surfactants like Span 80 to form a water-in-oil (w/o) emulsion (Samprasilt et al., 2020). Cross-linking is triggered internally, often by the addition of acid-sensitive calcium complexes or TPP into the dispersed droplets, resulting in rigid, discrete particles with entrapment efficiencies exceeding 68% for microbicide payloads like Tenofovir (Samprasilt et al., 2020; Potaś et al., 2021).

4.2.2 Scalability and Morphological Control in Spray Drying

Spray drying represents a high-throughput, one-step continuous process that transforms liquid suspensions into stable, dry powders (Ahmed & Aljaeid, 2016; Samprasilt et al., 2020). Technical data indicates that process parameters-specifically an inlet temperature of 120-160 °C and an outlet temperature of 40-100 °C-are critical to preventing the degradation of the chitosan backbone (Almeida et al., 2024). While laboratory-scale spray drying typically yields 50% recovery, industrial-scale implementation utilizing cyclone separators can achieve yields of up to 99%, making it the preferred method for producing vaginal microbicide tablets and films (Samprasilt et al., 2020; Dinu-Pîrvu et al., 2008).

4.3 Purification and Stabilization Strategies

The long-term clinical viability of CS-NPs depends on the removal of unreacted reagents and the establishment of a shelf-stable physical state (Gutiérrez-Ruíz et al., 2024; Umerska et al., 2023).

4.3.1 Centrifugation versus Dialysis

A critical critique of post-synthesis processing reveals that centrifugation, while rapid, often induces irreversible particle fusion. Forces exceeding 100,000 RCF can compress the hydration layer, resulting in a rigid pellet that resists redispersion and loses its spherical uniformity (Gutiérrez-Ruíz et al., 2024; Umerska et al., 2023). Conversely, dialysis utilizing a 50 kDa molecular weight cut-off (MWCO) is a "mild" purification alternative. While slower (typically 21 h), dialysis maintains a consistent PDI and preserves the surface charge density necessary for mucoadhesion (Gutiérrez-Ruíz et al., 2024; Umerska et al., 2023).

4.3.2 Lyophilization Kinetics and Cryoprotection

Freeze-drying (lyophilization) is required to prevent the hydrolytic degradation of chitosan during storage (Ball et al., 2017; Cholakova et al., 2020). However, the stresses of ice crystal formation and cryo-concentration can cause structural collapse. The incorporation of cryoprotectants at 5-10% (w/v) is essential (Ball et al., 2017). Trehalose is technically superior to sucrose in this application due to its higher glass transition temperature ($T_g \approx 117$ °C) and its ability to form a more robust hydrogen-bonded "cocoon" around the nanoparticles, ensuring complete redispersion into simulated vaginal fluid (Ball et al., 2017; Sreenivas & Sharma, 2011).

Suggested Figure 2: Procedural Flowchart showing the different synthesis routes (Ionic vs. Emulsion) and their respective nanoparticle morphologies.

V. Mechanics of Mucoadhesion

The engineering protocols detailed in Section IV establish the physical framework of chitosan-nanoparticles (CS-NPs), yet their therapeutic efficacy is ultimately governed by the interfacial phenomena occurring at the mucosal boundary. The transition from a discrete nanoparticle suspension to a retained drug

depot requires a multi-modal interaction with the cervicovaginal mucus (CVM), involving electrostatic attraction, hydrogen bonding, and covalent tethering (Ahmed & Aljaeid, 2016; Bernkop-Schnürch, 2021).

5.1 Electrostatic and Hydrogen Bonding Forces

Mucoadhesion is initiated by the contact and subsequent interpenetration of polymer chains into the mucin mesh. This process is driven by the physicochemical affinity between the polycationic chitosan backbone and the polyanionic mucin glycoproteins (Dinu-Pîrvu et al., 2008; Sogias et al., 2008).

5.1.1 Interactions Between -NH_3^+ and Sialic Acid Residues

At the homeostatic vaginal pH of 3.8-4.5, the primary amino groups of chitosan are protonated to form -NH_3^+ moieties (Ahmed & Aljaeid, 2016). These cationic sites engage in robust electrostatic attraction with the negatively charged sialic acid and ester sulfate residues of the mucin glycoproteins, which remain fully ionized at pH > 2.6 (Sogias et al., 2008; Bansil & Turner, 2006). This ionic coupling creates an immediate bioadhesive interface, providing the initial "anchoring" necessary to resist the rapid clearance associated with vaginal self-cleaning (Kadam, 2026; Potaś et al., 2021).

5.1.2 Chain Entanglement and Interpenetration

Beyond surface adsorption, mucoadhesion involves the diffusion of hydrated chitosan chains into the CVM mesh network. This mesh is composed of high-molecular-weight mucins characterized by dense O-linked glycans and "PTS" domains rich in Proline, Threonine, and Serine (Bansil & Turner, 2006; Pei et al., 2025). Hydrogen bonding between the hydroxyl (-OH) and amino (-NH_2) groups of chitosan and the sugar units of mucin facilitates this interpenetration (Sogias et al., 2008). The depth of entanglement is dictated by the polymer's molecular weight and the micro-rheological pore size of the mucus, which averages 340 ± 70 nm in non-ovulatory CVM (Ensign et al., 2012).

5.2 Quantitative Assessment of Adhesion

The characterization of mucoadhesive strength requires standardized methodologies to predict in vivo performance and residence times (Bassey & Ilomuanya, 2025).

5.2.1 Mechanical and Rheological Synergism

The "Work of Adhesion" is typically quantified using tensile strength testing, which measures the maximum force required to detach a formulation from a mucosal substrate (Bonferoni et al., 2006; Samprasilt et al., 2020). Rheological synergism is an alternative analytical approach, where the viscosity of a chitosan-mucin mixture is found to be significantly higher than the sum of the individual components, indicating a high degree of macromolecular interaction (Bonferoni et al., 2006). Furthermore, zeta potential shifts-moving from the negative charge of native mucin toward a neutralized or positive value-provide real-time evidence of electrostatic complexation (Samprasilt et al., 2020).

5.2.2 Ex Vivo Models Using Porcine and Bovine Mucosa

Due to its anatomical and histological similarity to human tissue, porcine vaginal mucosa is the gold standard for ex vivo mucoadhesion studies (Ahmed & Aljaeid, 2016; Bonferoni et al., 2006). Studies utilizing high-resolution fluorescence imaging on bovine vaginal mucosa have confirmed that CS-NPs distribute more uniformly across the folded epithelium (rugae) compared to bulk gels, which are prone to localized accumulation and premature expulsion (Ensign et al., 2012; Samprasilt et al., 2020).

5.3 Factors Influencing Residence Time

The longevity of a delivery system within the vaginal tract is a function of its chemical stability and resistance to physiological turnover (Kadam, 2026).

5.3.1 Cross-linking Density versus Chain Flexibility

A critical paradox in nanoparticle design is the relationship between stability and adhesion. High cross-linking density (e.g., high TPP concentration) improves nanoparticle integrity but restricts the flexibility of the chitosan chains, thereby reducing their ability to interpenetrate the mucin mesh (Ahmed & Aljaeid, 2016; Dinu-Pîrvu et al., 2008). Optimized systems must balance a CS:TPP ratio (approx. 4:1) that ensures structural stability while maintaining enough free amino groups for biointerfacial interaction (Dinu-Pîrvu et al., 2008).

5.3.2 Impact of Vaginal Fluid Simulant (VFS) and pH Fluctuations

The adhesive bond is susceptible to degradation by vaginal secretions. Exposure to VFS can induce a dilution effect, potentially reducing the concentration of the bioadhesive layer (Bonferoni et al., 2006). More critically, shifts toward neutral pH (e.g., pH 7.0 post-coitus) trigger the deprotonation of -NH_3^+ groups to -NH_2, leading to a dramatic loss of electrostatic mucoadhesiveness (Ahmed & Aljaeid, 2016; Sogias et al., 2008).

VI. Evolution of Mucopenetrating Strategies

The historical emphasis on mucoadhesion in vaginal drug delivery has encountered a significant technical bottleneck known as the "Mucoadhesive Paradox." While the polycationic nature of chitosan ensures prolonged retention on the superficial mucosal surface, this same attribute restricts the translocation of nanocarriers to deeper tissue targets. This section details the transition from high-retention mucoadhesive systems to the development of mucus-penetrating particles (MPPs) designed for uniform distribution and deep epithelial access (Ensign et al., 2012; Pei et al., 2025).

6.1 The "Trapping" Limitation of Conventional Chitosan Nanoparticles

Conventional chitosan-nanoparticles (CS-NPs) are fundamentally limited by their high affinity for the polyanionic constituents of cervicovaginal mucus (CVM). This affinity, while beneficial for surface-level pathologies, constitutes a primary barrier for therapies requiring systemic absorption or targeting of the basal epithelium (Sogias et al., 2008; Dinu-Pîrvu et al., 2008).

6.1.1 Agglomeration Kinetics and Superficial Clearance

The primary mechanism of immobilization involves electrostatic attraction between the protonated amino groups ($-NH_3^+$) of the chitosan backbone and the negatively charged sialic acid and ester sulfate residues on mucin glycoproteins (Ahmed & Aljaeid, 2016; Bansil & Turner, 2006). At a healthy vaginal pH of 3.8-4.5, this interaction occurs rapidly, leading to the formation of macro-aggregates within the CVM mesh (Sogias et al., 2008). Because the superficial mucus layer undergoes continuous physiological turnover-with a thickness of approximately 50 µm and a clearance rate of several millimeters per minute-these "trapped" nanoparticles are effectively removed from the vaginal vault within 2-6 hours, severely limiting the window for drug diffusion (Ensign et al., 2012; Potaś et al., 2021).

6.1.2 Spatial Constraints for Deep Tissue Targeting

Targeting deep tissue or immune sanctuaries requires nanoparticles to navigate the heterogeneous mesh network of the CVM, which possesses an average pore size of 340 ± 70 nm (Ensign et al., 2012). Conventional CS-NPs (typically 150-400 nm) face both steric hindrance and adhesive trapping. High-resolution single-particle tracking has confirmed that mucoadhesive CS-NPs exhibit speeds at least 8,000-fold lower in CVM than in water, whereas "stealth" modified particles can rapidly traverse the same medium (Ensign et al., 2012; Yang et al., 2014).

6.2 PEGylation: The "Stealth" Engineering Approach

To overcome adhesive trapping, researchers have adopted PEGylation-the covalent attachment of polyethylene glycol (PEG) chains-to create a neutral, hydrophilic surface that minimizes mucin-nanoparticle interactions (Davis & Abuchowski, 1977; Lai et al., 2009).

6.2.1 Shielding Mechanisms: Steric and Electrostatic Neutralization

The introduction of a dense PEG layer shields the polycationic chitosan core, effectively "neutralizing" the zeta potential from > +25 mV to a near-neutral range of -5 to +5 mV (Ahmed & Aljaeid, 2016; Ensign et al., 2012). This prevents the formation of ionic bonds and reduces hydrophobic associations with the non-glycosylated cysteine-rich domains of mucin (Bansil & Turner, 2006; Samprasilt et al., 2020). Consequently, PEGylated CS-NPs do not aggregate and are capable of diffusing through the watery interstitial spaces of the mucus mesh at speeds only 4- to 10-fold slower than their diffusion in pure water (Ensign et al., 2012; Yang et al., 2014).

6.2.2 Critical PEG Density and Molecular Weight Constraints

Technical optimization requires a precise balance of PEG molecular weight and surface density. Research utilizing 1H NMR quantification indicates that a minimum surface density of 5% PEG (using 5 kDa chains) is required to effectively shield the core from mucin binding (Ensign et al., 2012). Insufficient PEG density leads to the exposure of "adhesive patches" on the chitosan core, resulting in immediate immobilization (Ensign et al., 2012). Conversely, excessive PEG molecular weights can lead to chain entanglement with the mucin fibers themselves, paradoxically increasing mucoadhesion (Popov, 2020; Lai et al., 2009).

6.3 Non-PEG Alternatives and Advective Transport Mechanisms

While PEG remains the gold standard, its potential for inducing anti-PEG immune responses has spurred the investigation of alternative non-ionic hydrophilic polymers (Ahmed & Aljaeid, 2016; Pei et al., 2025).

6.3.1 POZylation and PVP-Grafting: The Next Generation

Recent studies have grafted poly(2-ethyl-2-oxazoline) (POZ) and poly(N-vinyl pyrrolidone) (PVP) onto chitosan backbones to create mucopenetrating architectures (Ahmed & Aljaeid, 2016; Samprasilt et al., 2020). Small-angle neutron scattering (SANS) data reveals that these POZylated nanogels maintain a highly hydrated, low-density internal structure which facilitates rapid diffusivity through sheep nasal and bovine vaginal mucosa (Ahmed & Aljaeid, 2016). Unlike thiolated chitosan (TCS), which forms covalent disulfide bonds (Polymer-S-S-Mucin) via thiol/disulfide exchange, these non-ionic derivatives rely on "stealth" kinetics to reach the epithelial surface (Bernkop-Schnürch, 2021; Schmitz et al., 2008).

6.3.2 Hypotonic Delivery and the Rationale for Advective Flux

The transport of MPPs can be further enhanced by manipulating the osmolarity of the delivery vehicle. Isotonic formulations rely purely on diffusive transport, which is governed by the concentration gradient (Fick's Law). In contrast, hypotonic formulations induce an osmotically driven fluid absorption by the vaginal epithelium (Ensign et al., 2012). This absorption creates a convective (advective) flow that "drags" the MPPs deep into the vaginal rugae and epithelial folds within minutes (Ensign et al., 2012; Xu et al., 2013). Studies in murine models demonstrate that MPPs delivered

hypotonically provide a 10-fold improvement in uniform coating of the vaginal surface compared to isotonic counterparts (Ensign et al., 2012; Xu et al., 2013).

VII. Advanced Chemical Functionalization

The engineering of second-generation chitosan derivatives enables a transition from simple bioadhesion to "smart," stimuli-responsive nanocarriers. By exploiting the reactive primary amino and hydroxyl groups of the chitosan backbone, researchers can tune the polymer's hydrophilic-lipophilic balance (HLB), covalent bonding potential, and degradation kinetics to match the physiological dynamics of the female reproductive tract (Ahmed & Aljaeid, 2016; Haider et al., 2025).

7.1 Thiolation (Thiomers): Covalent Mucoadhesion

Thiolation involves the covalent immobilization of sulfhydryl-bearing ligands (e.g., L-cysteine, thioglycolic acid, or glutathione) onto the chitosan backbone (Bernkop-Schnürch, 2021). This modification introduces a chemisorption mechanism that fundamentally solves the "Leakage and Clearance" paradox (Mfoafo, 2023).

7.1.1 Disulfide Bond Formation with Mucin Domains

Unlike native chitosan, which relies on reversible ionic attraction, thiolated chitosan (TCS) forms permanent covalent anchors through a thiol/disulfide exchange reaction with the cysteine-rich sub-domains of mucin glycoproteins (Dinu-Pîrvu et al., 2008). The molecular interaction follows the reaction:

Polymer-SH + Mucin-SH □ Polymer-S-S-Mucin + $2H^+$ + $2e^-$

Technical quantification using Ellman's reagent confirms that optimal mucoadhesion is achieved when the degree of thiolation ranges between 25 and 250 µmol of thiol groups per gram of polymer (Bernkop-Schnürch, 2021). At a vaginal pH of 3.8-4.5, this covalent "tethering" provides a 50- to 140-fold increase in retention time compared to unmodified polymers, as the disulfide bridges resist the mechanical shear forces of the vaginal wall (Bernkop-Schnürch & Steininger, 2000; Meresman, 2021).

7.1.2 S-protection Strategies for Deep Mucosal Penetration

A critical advancement in thiomer technology is the development of "S-protected" derivatives, where the thiol groups are pre-activated with ligands such as 6-mercaptonicotinic acid (6-MNA) (Griesser, 2022). These protected thiomers exhibit a 2-fold increase in mucoadhesion compared to unprotected TCS and a 14-fold increase over native chitosan (Patel, 2023). The protection mechanism allows nanoparticles to diffuse through the superficial "fast" mucus layer before the disulfide exchange occurs, ensuring that the system is immobilized only within the deeper, more stable mucus layers proximal to the squamous epithelium (Bernkop-Schnürch, 2021).

7.2 Acylation and Amphiphilic Derivatives

Acylation involves reacting the amino groups of chitosan with organic acids, anhydrides, or acyl chlorides to incorporate aliphatic or aromatic chains (Almeida et al., 2024).

7.2.1 Self-assembling Micelles for Hydrophobic Payloads

The introduction of hydrophobic acyl groups transforms chitosan into an amphiphilic copolymer capable of spontaneous self-assembly into core-shell micelles in aqueous media (Almeida et al., 2024). These micelles feature a hydrophobic core suited for the encapsulation of poorly soluble drugs (e.g., progesterone or antiretrovirals) and a polycationic shell that ensures mucoadhesion (Almeida et al., 2024). Technical data indicates that N-hexanoylation and N-octanoylation improve the stability and hydrophobic interactions of the carrier, reducing the critical micelle concentration (CMC) and preventing premature cargo leakage (Almeida et al., 2024).

7.2.2 Enhancement of Thermogelling Properties

Acylated derivatives, such as acetylated glycol chitosan, have been utilized to engineer in situ gelling systems (Haider et al., 2025). These formulations transition from a liquid state at room temperature (25 °C) to a mucoadhesive hydrogel at physiological temperature (37 °C) (Haider et al., 2025). This phase transition is critical for vaginal delivery, as it prevents the immediate expulsion (leakage) of the dose following application, maintaining close contact with the mucosal rugae for over 24 hours (Potaś et al., 2021).

7.3 pH-Responsive and Stimuli-Triggered Systems

The vaginal tract is characterized by dramatic pH shifts, particularly the neutralization induced by semen (pH shift from 4.0 to 7.0-8.0), which can be leveraged as a "master switch" for drug release (Potaś et al., 2021; Samprasilt et al., 2020).

7.3.1 Semen-Induced Smart Release

pH-responsive nanocarriers utilize the apparent pKa of chitosan (~6.5) to control release kinetics (Ahmed & Aljaeid, 2016). In the acidic environment of a healthy vagina, the nanoparticles remain stable and swollen due to the protonation of amino groups ($-NH_3^+$) (Ahmed & Aljaeid, 2016). However, the introduction of alkaline seminal fluid triggers deprotonation, causing the polymer network to shrink and collapse, thereby "squeezing" the therapeutic payload into the lumen exactly when microbicidal protection is required (Potaś et al., 2021; Samprasilt et al., 2020).

7.3.2 Enzyme-Responsive Degradation

Chitosan nanocarriers are specifically sensitive to lysozyme, an endo-carbohydrase present in vaginal secretions that cleaves the β-(1→4) glycosidic linkages

(Haider et al., 2025). By adjusting the degree of deacetylation (DD), researchers can precisely tune the degradation rate (Haider et al., 2025). Systems with lower DD values (higher acetylation) degrade more rapidly, providing a mechanism for "programmed" release where the carrier itself is enzymatically metabolized into non-toxic glucosamine monomers (Bernkop-Schnürch, 2021; Haider et al., 2025).

VIII. Hybrid and Multi-compartment Nanosystems

The design of advanced vaginal delivery platforms has transitioned from monolithic polymer matrices to hybrid architectures that integrate the mechanical robustness of polymers with the solubilizing capacity of lipids and the administrative convenience of in situ gelling hydrogels. This multi-compartment approach addresses the inherent limitations of native chitosan, specifically its restricted solubility at physiological pH and its sub-optimal loading efficiency for highly lipophilic steroids and antiretrovirals (Ahmed & Aljaeid, 2016; Almeida et al., 2024).

8.1 Chitosan-Coated Solid Lipid Nanoparticles (SLPs)

Solid lipid nanoparticles (SLPs) have emerged as a superior alternative to traditional liposomes, offering enhanced chemical stability and a solid matrix that provides controlled release of hydrophobic therapeutic agents (Martins et al., 2007).

8.1.1 Structural Integration and Steroid Loading

In hybrid SLP systems, a core composed of biocompatible lipids, such as Compritol ATO 888 or Precirol ATO 5, serves as a reservoir for lipophilic hormones like estradiol and progesterone (Kadam, 2026; Potaś et al., 2021). The addition of a chitosan coating is achieved through electrostatic adsorption, where the positively charged $-NH_3^+$ groups of the chitosan backbone interact with the negatively charged surface of the lipid matrix, often stabilized by surfactants like Tween 80 (Samprasilt et al., 2020). Technical characterization indicates that this coating transforms the SLP from a non-adhesive carrier into a mucoadhesive vehicle capable of resisting the rapid "Leakage and Clearance" associated with the vaginal tract (Potaś et al., 2021).

8.1.2 Nanonization Effects and Flux Parameters

The "nanonization effect" is a critical mechanistic driver in hybrid SLPs. By reducing the particle size to a range of 450-850 nm, the total surface area available for drug dissolution is exponentially increased (Ahmed & Aljaeid, 2016). Comparative studies demonstrate that chitosan-coated SLPs containing estradiol achieve a cumulative release of 51% to 83% over 6 hours, representing a 4.4-fold increase over conventional drug suspensions (Dinu-Pîrvu et al., 2008). Furthermore, the ex vivo flux (J) across porcine vaginal mucosa is increased by approximately 2.2-fold, facilitating the "first uterine pass effect" where high local tissue concentrations are achieved despite low systemic plasma levels (Martins et al., 2007).

8.2 In situ Gelling Nanocomposites

To solve the clinical challenge of dose expulsion, researchers have merged CS-NPs with thermoresponsive polymers to create "Liquid-to-Gel" transition systems (Bassey & Ilomuanya, 2025; Haider et al., 2025).

8.2.1 Thermoresponsive Synergism with Pluronic F127

The integration of Pluronic F127-a poly(ethyleneol)-block-poly(propyleneol)-block-poly(ethyleneol) copolymer-allows the formulation to remain a low-viscosity liquid at room temperature (25 °C), facilitating easy application via standard vaginal applicators (Potaś et al., 2021; Samprasilt et al., 2020). Upon administration, the physiological temperature of 37 °C triggers the formation of a dense, three-dimensional micellar network (Potaś et al., 2021). Chitosan acts as a mucoadhesive "anchor" within this network, with its protonated amino groups engaging in hydrogen bonding and electrostatic attraction with the mucin glycoproteins of the vaginal wall (Ahmed & Aljaeid, 2016; Bonferoni et al., 2006).

8.2.2 Vaginal Retention and Spreadability

These nanocomposites exhibit "rheological synergism," where the viscosity of the CS-Pluronic-mucin complex is significantly higher than that of its individual components (Bonferoni et al., 2006). This increased viscosity ensures that the drug depot is retained within the highly folded vaginal rugae for durations exceeding 24 hours, effectively bypassing the 90% dose loss typically observed with conventional gels (Kadam, 2026; Pei et al., 2025).

8.3 Chitosomes: Chitosan-Liposome Hybrids

Chitosomes represent a generational leap in vesicular delivery, combining the biomimetic properties of phospholipids with the bioadhesion of chitosan (Alukda et al., 2011; Almeida et al., 2024).

8.3.1 Surface-Available vs. Embedded Architectures

In the engineering of chitosomes, the chitosan polymer can be either adsorbed as a surface coating or embedded within the aqueous compartments of the liposome (Almeida et al., 2024; Samprasilt et al., 2020). Provenance data suggests that chitosomes where the polymer is both surface-available and embedded provide a "dual-action" release profile (Samprasilt et al., 2020). For the delivery of metronidazole in the treatment of bacterial vaginosis and vulvovaginal candidiasis (VVC) co-infections, the surface-available chitosan provides immediate antifungal action by disrupting yeast cell membranes, while the embedded drug is released slowly to maintain antibacterial potency (Haider et al., 2025).

8.3.2 Covalent Interaction and Redox Responsiveness

The most technical variants utilize thiolated chitosan (TCS) to form covalent chitosomes. These systems exploit the formation of disulfide bonds (Polymer-S-S-Mucin) with the cysteine-rich sub-domains of mucins via a thiol/disulfide exchange:

Polymer-SH + Mucin-SH □ Polymer-S-S-Mucin + $2H^+$ + $2e^-$

Under vaginal pH conditions (3.8-4.5), these covalent linkages provide a work of adhesion up to 140 times stronger than the purely ionic interactions of native liposomes (Bernkop-Schnürch, 2021; Dinu-Pîrvu et al., 2008). This ensures that the vesicles remain "tethered" to the epithelium, providing a sustained concentration gradient that facilitates deep tissue penetration (Ensign et al., 2012; Pei et al., 2025).

IX. Therapeutic Applications: Bench to SOTA

The translational potential of chitosan-nanoparticle (CS-NP) systems is evidenced by their high-precision performance across infectious, oncogenic, and endocrine pathologies of the female reproductive tract (FRT). By leveraging the "3Ms" paradigm-mucoadhesion, microbicidal activity, and muco-retention-these systems provide a technical solution to the bioavailability failures of conventional gels and rings (Potaś et al., 2021; Kadam, 2026).

9.1 HIV/STI Microbicide Delivery (2023-2025 SOTA)

The development of vaginal microbicides is currently focused on overcoming the adherence challenges observed in early Phase IIb/III clinical trials, such as CAPRISA 004, where inconsistent use of 1% tenofovir gels limited efficacy (Samprasilt et al., 2020; Potaś et al., 2021).

9.1.1 Tenofovir-loaded Systems and the 3Ms Concept

State-of-the-art (SOTA) research utilizes Tenofovir disoproxil fumarate (TDF) loaded into CS-nanoparticles to ensure sustained inhibitory concentrations (Samprasilt et al., 2020). Batch ECH-4, synthesized via emulsification internal gelation, has demonstrated an entrapment efficiency of 68.93 ± 1.76% (Samprasilt et al., 2020). These systems facilitate the 3Ms concept: the polycationic charge ensures mucoadhesion to the polyanionic mucin mesh, the encapsulated TDF provides microbicidal reverse transcriptase inhibition, and the hydrogel-matrix expansion ensures muco-retention for over 24 hours (Samprasilt et al., 2020; Ahmed & Aljaeid, 2016).

9.1.2 Multiunit Carriers: Dispersible Tablets and Microparticles

To improve patient compliance, researchers have engineered dispersible vaginal tablets (DT-TCM) that readily disintegrate into constituent bioadhesive microparticles upon contact with vaginal fluid (Samprasilt et al., 2020). These microparticles exhibit a surface-mean diameter of approximately 12.41 µm and achieve a sustained release of 89.98 ± 1.61% TDF at 24 h in simulated vaginal fluid at pH 4.5 (Samprasilt et al., 2020). Furthermore, CS acts as an antiviral adjunct; studies using zidovudine (ZVD)-loaded chitosan microparticles found that the polymer itself hindered Herpes Simplex Virus-2 (HSV-2) attachment to keratinocytes and vaginal epithelial cells (Potaś et al., 2021).

9.2 Management of VVC and Co-Infections

Vulvovaginal candidiasis (VVC) affects over 70% of women, often recurring due to biofilm formation and azole resistance (Prabhat, 2021; Almeida et al., 2024).

9.2.1 Synergistic Antimicrobial Action

SOTA formulations integrate chitosan with essential oils to exploit multimodal mechanisms of action. Chitosan-oleic acid nanoparticles loaded with lemon peel essential oil (LPEO) exhibit potent antifungal activity by disrupting Candida albicans cell membrane integrity (Almeida et al., 2024). In rat models of VVC, these formulations significantly reduced pro-inflammatory markers, including Interleukin-1β (IL-1β) and myeloperoxidase (MPO), while increasing glutathione (GSH) activity (Almeida et al., 2024).

9.2.2 Biofilm Disruption and Mixed Infection Control

Hybrid chitosomes (chitosan-liposome hybrids) have been engineered to deliver metronidazole for bacterial vaginosis while the chitosan coating provides intrinsic antifungal action (Haider et al., 2025; Ahmed & Aljaeid, 2016). This dual-action approach is critical for treating mixed infections where C. albicans and Gram-negative bacteria co-occur (Prabhat, 2021). The nanoparticles disrupt established fungal biofilms, allowing the antibacterial payload to reach the underlying epithelial surface (Almeida et al., 2024).

9.3 Cancer Therapy and Ovarian Malignancies

The application of nanomedicine in gynecological oncology has transitioned toward multifunctional delivery systems that combine hygiene with therapeutic potency (Ahmed & Aljaeid, 2016).

9.3.1 Targeted Cytotoxicity in the SKOV3 Cell Line

A multifunctional vaginal wash incorporating chitosan nanoparticles (VW+Chit) with a particle size of ~41 nm has been characterized for its anticancer efficacy (Ahmed & Aljaeid, 2016). Against the SKOV3 ovarian cancer cell line, this formulation exerted a dose-dependent cytotoxic effect with an IC_{50} of 147.3 µg/mL (Ahmed & Aljaeid, 2016).

9.3.2 Induction of Apoptosis and G1 Phase Arrest

The mechanism of action involves the induction of cell cycle arrest. VW+Chit treatment leads to a significant increase in G1 phase arrest (60.92% vs. 53.60% in control) and a shift into the G0 resting phase, preventing the rapid proliferation characteristic of high-grade serous ovarian carcinomas (Ahmed & Aljaeid, 2016).

Additionally, the formulation demonstrated high anti-inflammatory efficiency, inhibiting COX-1 (92.9%) and COX-2 (94.3%) enzymes (Ahmed & Aljaeid, 2016).

9.4 Hormone Replacement and Pregnancy Support

The vaginal route provides high local concentrations in uterine tissues through the "first uterine pass effect," bypassing hepatic first-pass metabolism and reducing systemic side effects (Martins et al., 2007).

9.4.1 Enhanced Flux and Nanonization Effects

Estradiol-loaded solid lipid nanoparticles (SLPs) coated with chitosan have achieved a 4.4-fold increase in drug release rates compared to free drug suspensions (Martins et al., 2007). In ex vivo porcine vaginal mucosa models, these hybrid systems showed 1.7- to 2.2-fold increases in flux (J) and apparent permeability (Papp), ensuring therapeutic levels are maintained with lower dosing frequencies (Martins et al., 2007).

9.4.2 Progesterone Support for Pregnancy

Chitosan-based hydrogels are increasingly utilized for the delivery of progesterone (PGT) to support the corpus luteum and reduce the risk of miscarriage (Potaś et al., 2021). These in situ gelling systems utilize the thermodynamic transition from liquid (25 °C) to mucoadhesive gel (37 °C) to ensure the dose is retained within the vaginal folds, effectively preventing the "leakage" phenomenon that typically accounts for 90% of a conventional dose loss (Potaś et al., 2021; Kadam, 2026).

X. Pharmacokinetics and Safety Profiles

The clinical translation of chitosan-nanoparticle (CS-NP) systems is contingent upon a rigorous characterization of their biological fate and toxicological impact. The shift from monolithic polymer matrices to engineered nanocarriers fundamentally alters the absorption, distribution, metabolism, and excretion (ADME) profiles of encapsulated therapeutics, necessitating a granular analysis of pharmacokinetic (PK) modulation and tissue-level biocompatibility.

10.1 Comparative Pharmacokinetic Parameters

The utilization of CS-NPs significantly enhances the bioavailability of active pharmaceutical ingredients (APIs) by protecting them from enzymatic degradation and extending their mucosal residence time.

10.1.1 Bioavailability Enhancement: AUC and Cmax Dynamics

Comparative PK studies demonstrate that drug loading into CS-nanostructures facilitates sustained-release profiles that modify peak plasma concentrations (Cmax) and total drug exposure (Area Under the Curve, AUC). For instance, curcumin-loaded chitosan nanoparticles exhibit an 11.29-fold increase in AUC_{0-48} compared to conventional drug solutions. Similarly, polymeric nanoparticles loaded with docetaxel achieve a 38-fold increase in AUC and a 5.2-fold prolongation of the elimination half-life. In targeted organ delivery, primaquine-loaded chitosan nanoparticles reduce the plasma Cmax to 0.42 times that of the free drug, which effectively increases target liver concentrations by 3-fold while minimizing off-target systemic toxicity (Louisa et al., 2022; Martins et al., 2007).

10.1.2 First Uterine Pass Effect and Lymphatic Trafficking

Vaginally administered nanocarriers exploit the "first uterine pass effect," a phenomenon characterized by direct transport from the vaginal vasculature to the uterine tissues. This mechanism allows for high local concentrations of steroids, such as estradiol and progesterone, in the reproductive tract while maintaining plasma levels significantly lower than those achieved via oral or transdermal routes. Beyond local vascular transport, chitosan acts as a powerful adjuvant for lymphatic trafficking. Intravaginal pre-treatment with chitosan facilitates the translocation of 200 nm nanoparticles across the multilayered squamous epithelium, enabling their accumulation in the internal iliac (ILLNs) and inguinal lymph nodes (IGLNs) within 24 hours. This translocation occurs via the transient opening of tight junctions, as positively charged chitosan induces a redistribution of cytoskeletal F-actin and tight junction proteins (Martins et al., 2007; Ahmed & Aljaeid, 2016).

10.2 Biocompatibility and Tissue Response

Chitosan's high biocompatibility is attributed to its structural similarity to glycosaminoglycans, which are integral components of the natural extracellular matrix (Haider et al., 2025).

10.2.1 Mucosal Histopathology and Structural Integrity

Histopathological evaluations in animal models have confirmed the safety of chronic exposure to CS-nanocarriers. Studies using female C57BL/6 mice and porcine models revealed that thiolated chitosan (TCS)-coated multilayer microparticles do not induce signs of epithelial damage or significant inflammatory cell infiltrate. Furthermore, histological study confirms that chitosan nanoparticles maintain the structural integrity of mucosal barriers without inducing cellular necrosis, provided the formulations are adjusted to a physiological pH range appropriate for the administration site (Samprasilt et al., 2020; Haider et al., 2025).

10.2.2 Efflux Pump Inhibition: Bypassing P-glycoprotein

A critical mechanism by which CS-NPs improve intracellular drug accumulation is the inhibition of the P-glycoprotein (P-gp) multidrug efflux pump . P-gp on mucosal membranes often limits the absorption of APIs by pumping them back into the lumen. Thiolated chitosan derivatives, such as chitosan-thioglycolic acid (CS-TGA) or glycol chitosan-N-acetylcysteine (GCS-

NAC), inhibit this activity, restoring the intracellular entrance of P-gp substrates like rhodamine-123. This inhibition is likely mediated by the formation of disulfide bonds with the exofacial thiols of transmembrane proteins, following a thiol/disulfide exchange reaction: (Bernkop-Schnürch, 2021; Schmitz et al., 2008).

Polymer-SH + Mucin-SH □ Polymer-S-S-Mucin + $2H^+$ + $2e^-$

10.3 Toxicology of Chitosan Derivatives

While chitosan is recognized as Generally Recognized as Safe (GRAS) by the FDA, its nanoscale derivatives require specific cytotoxicity assessments to account for their high surface-to-area ratios (FDA, 2025; Haider et al., 2025).

10.3.1 In Vitro Cytotoxicity Assays

The cytotoxicity of CS-NPs is dependent on concentration, particle size, and solution pH. In vitro assessments using human vaginal (VK2/E6E7) and keratinocyte (HaCaT) cell lines indicate that CS-NPs are well tolerated . Standardized MTT assays demonstrate cell survival rates exceeding 80% at concentrations up to 0.1%. Notably, smaller nanoparticles (<100 nm) may exhibit higher relative toxicity than larger variants, highlighting the importance of particle size control during the synthesis protocols outlined in Section IV (Ahmed & Aljaeid, 2016; Haider et al., 2025).

10.3.2 Endotoxin Thresholds and Pro-inflammatory Markers

The natural origin of chitosan makes it susceptible to endotoxin contamination, which can elicit erroneous pro-inflammatory conclusions in safety studies. Regulatory compliance for medical devices necessitates a limit of less than 0.5 EU/mL. When properly purified, CS-NPs have been shown to exert anti-inflammatory effects. For example, chitosan-oleic acid nanoparticles loaded with essential oils significantly reduced levels of pro-inflammatory markers, including Interleukin-1β (IL-1β) and myeloperoxidase (MPO), in rat models of vaginal infection (FDA, 2025; Almeida et al., 2024).

XI. Industrial Challenges and Regulatory Landscape

The translation of chitosan-nanoparticle (CS-NP) systems from academic proof-of-concept to commercial vaginal therapeutics necessitates overcoming significant manufacturing heterogeneities and navigating a rapidly evolving global regulatory framework. While the polycationic and bioadhesive advantages of chitosan are technically established, the transition to high-throughput production requires a critical appraisal of material consistency, terminal sterilization protocols, and nanospecific regulatory scrutiny (Ahmed & Aljaeid, 2016; Dinu-Pîrvu et al., 2008).

11.1 Scalability and Batch-to-Batch Consistency

Achieving consistent therapeutic outcomes in the female reproductive tract (FRT) depends on the precision of the nanoparticle's physicochemical attributes, specifically the degree of deacetylation (DD) and molecular weight (MW) distribution.

11.1.1 The Challenge of Natural Material Inconsistency

Chitosan is fundamentally a natural copolymer, and its structural properties vary significantly based on the biological source-crustacean shells versus fungal cell walls-and the seasonal maritime conditions of harvest (Sogias et al., 2008; Haider et al., 2025). Industrial-scale extraction involves harsh alkaline deacetylation, which often results in unpredictable chain scission and a wide polydispersity index (PDI) (Sogias et al., 2008). Such variability in the starting material can minimize the reproducibility of ionic gelation batches by up to 73% (Sreenivas & Sharma, 2011). Consequently, pharmaceutical-grade production requires strict adherence to ASTM F2103 standards to ensure that each batch exhibits a stable assay value and a highly consistent impurity profile (Haider et al., 2025).

11.1.2 Transitioning to Industrial Homogenization

The laboratory benchmark for CS-NP synthesis-dropwise addition of tripolyphosphate (TPP) into a vortex-is inherently unscalable due to localized over-crosslinking at the injection site (Ahmed & Aljaeid, 2016). Industrial manufacturing has transitioned toward high-pressure homogenization (HPH) and spinning disc processing (SDP). These techniques utilize high shear forces and controlled temperature gradients to eliminate API degradation and produce extremely tight particle size distributions in the 100-300 nm range (Louisa et al., 2022; Samprasilt et al., 2020). For the delivery of antiretrovirals like Tenofovir, these homogenization protocols ensure that the drug exists in an amorphous solid-solution state within the polymer matrix, optimizing dissolution kinetics in the acidic vaginal lumen (Samprasilt et al., 2020; Potaś et al., 2021).

11.2 Sterilization Dilemmas

Terminal sterilization is a mandatory requisite for vaginal products, yet conventional methods often compromise the structural and functional integrity of the CS-NP matrix.

11.2.1 Impact of Autoclave, Gamma, and Ozone on NP Integrity

Subjecting CS-TPP nanoparticles to steam autoclaving induces significant hydrolytic degradation and the formation of irreversible macro-aggregates (Ahmed & Aljaeid, 2016). Similarly, gamma irradiation at standard sterility assurance levels (SAL 10^{-6}) causes a measurable decrease in zeta potential and promotes particle agglomeration by compromising the suspension stability (Haider et al., 2025). Ozone sterilization has emerged as a promising low-temperature alternative; however, FTIR spectral data indicates that ozone can induce minor chemical alterations in the glycosidic

linkages, leading to a reduction in mucoadhesive strength (Ahmed & Aljaeid, 2016).

11.2.2 The Role of Sugars as Radioprotectants

To mitigate the stresses of sterilization and subsequent lyophilization, the incorporation of cryoprotectants and radioprotectants is essential (Ball et al., 2017). Saccharides such as trehalose and sucrose at 5% w/v have been shown to shield the nanoparticles during irradiation by forming antioxidant complexes (Ball et al., 2017; Cholakova et al., 2020). These sugars form a hydrogen-bonded "cocoon" that prevents ice crystal-induced mechanical damage and stabilizes the $-NH_3^+$ surface charge, ensuring complete redispersion into simulated vaginal fluid without a significant increase in PDI (Ball et al., 2017).

11.3 Global Regulatory Standards (FDA/EMA 2025 Updates)

Regulatory authorities, including the EMA and U.S. FDA, have updated their frameworks in 2025 to address the unique biological behaviors of nanotechnology-enabled health products (EMA, 2025; FDA, 2025).

11.3.1 GRAS Status vs. Case-by-Case Nanospecific Assessments

While native chitosan is recognized as Generally Recognized as Safe (GRAS) for use as an oral excipient, its application in nanoscale vaginal delivery systems triggers a "case-by-case" risk-based assessment (FDA, 2025; Haider et al., 2025). The FDA's Center for Drug Evaluation and Research (CDER) now requires comprehensive data on the product's size-driven biodistribution and its potential for bioaccumulation in the reproductive tissues (FDA, 2025). Manufacturers must provide detailed Chemistry, Manufacturing, and Controls (CMC) data, particularly regarding surface charge and the presence of endotoxins, which must be maintained below 0.5 EU/mL to prevent pro-inflammatory responses (FDA, 2025; Szymańska et al., 2025).

11.3.2 CE Marking and Medical Device Regulation (MDR 2017/745) Pathways

In the European Union, vaginal rings or applicators incorporating CS-NPs are regulated under the Medical Device Regulation (MDR 2017/745). These products are classified as Class III if they present a high potential for internal mucosal exposure (EMA, 2025). Compliance involves rigorous post-market surveillance and the demonstration of "Level A" in vitro-in vivo correlations (IVIVC) to justify the use of dissolution testing in lieu of repeated clinical bioequivalence studies for new manufacturing batches (Haider et al., 2025; Kroustali et al., 2025).

XII. Future Trajectory and Unsolved Mysteries

The translation of chitosan-nanoparticle (CS-NP) systems from academic proof-of-concept to clinical utility in the female reproductive tract (FRT) is entering a disruptive phase characterized by computational rational design and bio-instructive architectures. While previous sections detailed the engineering of mucoadhesive and mucopenetrating systems, this final chapter identifies the technological convergence required to address remaining clinical gaps and the "unsolved mysteries" of mucosal nanomedicine (Bassey & Ilomuanya, 2025; Haider et al., 2025).

12.1 AI and Machine Learning in Rational NP Design

The multi-dimensional parameter space governing CS-NP synthesis-specifically the interplay between chitosan concentration, degree of deacetylation (DD), and sodium tripolyphosphate (TPP) mass ratios-has historically relied on resource-intensive trial-and-error methodologies. Artificial Intelligence (AI) and Machine Learning (ML) are now redefining these paradigms (Gutiérrez-Ruíz et al., 2024; Haider et al., 2025).

12.1.1 Deep Learning for Physicochemical Optimization

Advanced predictive modeling utilizing Multilayer Perceptron (MLP) networks has demonstrated superior accuracy in predicting CS-NP particle size and polydispersity index (PDI) compared to traditional Box-Behnken designs (Gutiérrez-Ruíz et al., 2024). For instance, MLP models trained on systematically generated batches of CS-TPP have achieved a test-set R^2 of 0.9943, enabling the independent identification of optimal CS:TPP ratios (e.g., 0.30% w/v CS at a 1:5 TPP ratio) that satisfy specific entrapment efficiency and zeta potential requirements for vaginal delivery (Gutiérrez-Ruíz et al., 2024).

12.1.2 Automated Machine Learning (AutoML) and Synergism

The integration of Automated Machine Learning (AutoML) allows for the rapid screening of synergistic drug combinations within chitosan matrices (Gutiérrez-Ruíz et al., 2024). By analyzing high-dimensional datasets containing Raman spectral signatures and time-resolved dissolution data, ensemble learning frameworks can forecast controlled drug release profiles with certainty levels exceeding 90% (Kamath et al., 2025; Haider et al., 2025). This computational speed is critical for developing multi-modal microbicides where the amorphous state of the drug must be maintained within the polymer network to ensure rapid onset of action (Samprasilt et al., 2020; Potaś et al., 2021).

12.2 Microbiome-Active Drug Delivery Systems (MADDS)

A significant frontier in FRT therapeutics is the transition from pH-responsive systems to Microbiome-Active Drug Delivery Systems (MADDS). Unlike conventional carriers that simply coexist with the vaginal flora, MADDS harness resident microbes and their metabolites to trigger drug activation or release

(Kamath et al., 2025; Bassey & Ilomuanya, 2025).

12.2.1 Leveraging Microbial Cues for Controlled Release

The vaginal microbiome, dominated by Lactobacillus species, creates a dynamic microenvironment rich in lactic acid and specific enzymes (Szymańska et al., 2025). MADDS are engineered to exploit these microbial cues. For example, enzyme-responsive designs can utilize the presence of microbial sialidases or hyaluronidases as "biological switches" to trigger the deprotection of S-protected thiomers (Bernkop-Schnürch, 2021; Kamath et al., 2025). This ensures that the therapeutic payload is released only upon the detection of pathogenic overgrowth or dysbiosis-related enzymatic activity (Bernkop-Schnürch, 2021).

12.2.2 Metabolic Calibration and Biofilm Interaction

Emerging research into "metabolic calibration" suggests that nanoparticles can be designed to release specific prebiotics that selectively support Lactobacillus growth while delivering microbicides (Kamath et al., 2025). Furthermore, MADDS can penetrate pathogenic biofilms by utilizing the intrinsic antimicrobial activity of the polycationic chitosan backbone, which disrupts microbial cell membrane integrity through electrostatic interaction with negatively charged lipopolysaccharides (Ahmed & Aljaeid, 2016; Almeida et al., 2024).

12.3 Personalized and 3D-Bioprinted Vaginal Scaffolds

The necessity for patient-specific anatomical matching has led to the development of 3D-bioprinted scaffolds that incorporate CS-NPs for localized, sustained therapy (Bassey & Ilomuanya, 2025; Almeida et al., 2024).

12.3.1 Infusing Nanoparticles into Bio-inks

Native chitosan often exhibits limited mechanical strength and printing quality, justifying the use of hybrid bio-inks. Blending chitosan with agarose or sodium alginate has been shown to improve printing characteristics while maintaining high cell viability (Almeida et al., 2024). These 3D-bioprinted scaffolds can be infused with ZnO or MgO nanoparticles to provide sustained antibacterial protection against pathogens such as S. aureus, effectively preventing implant-related infections during vaginal tissue regeneration (Almeida et al., 2024).

12.3.2 Patient-Specific Reconstruction and Stem Cell Delivery

Biomimetic 3D-printed bilayered scaffolds are currently being investigated for vaginal regeneration and the management of defects (Almeida et al., 2024). By incorporating stem cells and their paracrine factors into mucoadhesive chitosan hydrogels, these systems facilitate extracellular matrix (ECM) remodeling (Almeida et al., 2024). This approach offers a potential solution for post-surgical recovery or treating atrophic conditions by providing a structural template that matches the individual's vaginal rugae and hormonal status (Bassey & Ilomuanya, 2025).

12.4 Final Synthesis: The Path Forward

The "Mucoadhesive Paradox"-the trade-off between superficial retention and deep tissue penetration-remains a central challenge (Ensign et al., 2012). The path forward requires a shift toward mucoadhesive-to-mucopenetrating nanocarriers that exhibit "adaptive" surface properties (Yang et al., 2014).

12.4.1 Critical Synthesis: Trimethyl Chitosan (TMC) vs. Thiolated Chitosan (TCS)

At the acidic vaginal pH of 3.8-4.5, TMC offers pH-independent solubility but may face steric hindrance during chain entanglement (Sogias et al., 2008; Dinu-Pîrvu et al., 2008). In contrast, TCS facilitates covalent tethering through a thiol/disulfide exchange with the cysteine-rich sub-domains of mucin:

Polymer-SH + Mucin-SH □ Polymer-S-S-Mucin + $2H^+$ + $2e^-$

This covalent ligation provides a 140-fold increase in retention time, effectively solving the "Leakage and Clearance" paradox where 90% of a dose is typically lost (Bernkop-Schnürch, 2021; Mfoafo, 2023). Future systems will likely utilize S-protected thiomers to surpass the loose outer mucus layer before forming these permanent anchors (Bernkop-Schnürch, 2021; Griesser, 2022).

12.4.2 Standardized Mucosal Testing Protocols

To achieve reproducibility across global studies, the field exigent requires standardized testing protocols. Single-particle tracking (SPT) has emerged as a powerful tool to quantify Dw/Dm ratios (diffusion in water vs. diffusion in mucus) at high spatiotemporal resolution (Ensign et al., 2012; Yang et al., 2014). The adoption of benchmark materials and standardized reporting of diffusion coefficients is essential to correlate in vitro findings with in vivo outcomes in the dynamic FRT environment (Ensign et al., 2012; Yang et al., 2014).

Conclusion

In conclusion, chitosan-nanoparticle systems represent a transformative solution to the unique biological and physiological barriers characterizing the female reproductive tract. By exploiting the pH-responsive apparent pKa of approximately 6.5, these polycationic platforms effectively surmount the "Leakage and Clearance" paradox, providing a mechanism for sustained drug release that counters the 90% dose loss typical of conventional dosage forms.

The transition from first-generation mucoadhesive matrices to adaptive, "stealth" modified nanocarriers marks a significant milestone in mucosal nanomedicine. The development of mucus-penetrating particles (MPPs) through high-density PEGylation enables Dw/Dm ratios that facilitate rapid diffusion through the

cervicovaginal mucus (CVM), ensuring that therapeutic payloads reach deep epithelial targets and immune sanctuaries. Furthermore, the emergence of thiolated "thiomers" facilitates a chemisorption mechanism via covalent disulfide bonding (Polymer-SH + Mucin-SH □ Polymer-S-S-Mucin + $2H^+ + 2e^-$), providing up to a 140-fold increase in retention time compared to unmodified polymers.

The future trajectory of this field is defined by two primary technological convergences:

1. **Computational Rational Design:** The integration of supervised machine learning and AutoML frameworks has demonstrated predictive accuracies for release kinetics and particle size exceeding $R^2 = 0.96$, enabling the high-precision optimization of CS-TPP mass ratios without the constraints of traditional trial-and-error methods.
2. **Microbiome-Active Systems:** The shift toward Microbiome-Active Drug Delivery Systems (MADDS) allows for site-specific drug activation triggered by microbial enzymes and metabolites, aligning therapeutic delivery with the host's specific microbial health status.

To achieve successful clinical translation, the field must prioritize the standardization of mucosal testing protocols, utilizing high-resolution single-particle tracking (SPT) to correlate ex vivo diffusion coefficients with in vivo therapeutic outcomes. Navigating the 2025 regulatory landscape-including updated FDA guidance on nanomaterial characterization and the European Medical Device Regulation (MDR 2017/745)-remains critical for ensuring the commercial scalability and patient safety of these advanced delivery platforms. Ultimately, the convergence of stimuli-responsive polymer chemistry and AI-driven optimization positions chitosan-nanoparticle systems as a cornerstone of next-generation therapeutic interventions for women's reproductive health.

Conflict of Interest

None. Declared by all authors.

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