

Design, Optimization, and Evaluation of a Mucoadhesive Vaginal Gel Incorporating Tikta-Kashaya Rasa Dravyas for Vulvovaginal Candidiasis

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Abstract

Vulvovaginal candidiasis (VVC) remains one of the most prevalent mucosal fungal infections affecting women of reproductive age worldwide, characterized by severe pruritus, irritation, curd-like vaginal discharge, and epithelial inflammation. Recurrent episodes and antifungal drug resistance (notably against conventional azoles) have accelerated the search for synergistic, multi-targeted natural therapeutics. In classical Ayurvedic pharmaceuticals, *Tikta* (bitter) and *Kashaya* (astringent) *Rasa Dravyas*—such as *Symplocos racemosa* (Lodhra), *Azadirachta indica* (Nimba), and *Curcuma longa* (Haridra)—possess documented *Kaphapittashamaka*, *Kandughna* (antipruritic), *Vranaropana* (wound-healing), and *Krimighna* (antimicrobial) properties. However, conventional classical liquid decoctions (*Kashaya*) and douches suffer from rapid physiological leakage, poor residence time, and variable patient compliance.

To overcome these anatomical and physical barriers, the present study developed, optimized, and evaluated a bio-inspired, mucoadhesive vaginal hydrogel system loaded with standardized hydroethanolic extracts of selected *Tikta-Kashaya Rasa Dravyas*. A 3^2 full factorial experimental design was implemented to optimize polymer concentration (Carbopol 934P, X_1) and co-polymer concentration (Hydroxypropyl Methylcellulose [HPMC K4M], X_2) on dependent responses: mucoadhesive detachment force (Y_1), spreadability coefficient (Y_2), and cumulative *in vitro* polyphenol/berberine/curcuminoid permeation over 12 h (Y_3).

The optimized hydrogel exhibited ideal pseudo-plastic shear-thinning behavior, a physiological pH of 4.22 ± 0.08 compatible with healthy vaginal microenvironments, a mucoadhesive detachment stress of $4,820 \pm 115$ dynes/cm², and sustained drug release reaching 86.4% over 12 h following non-Fickian anomalous transport kinetics. *In vitro* antifungal testing via agar-well diffusion and broth microdilution against *Candida albicans* (ATCC 90028) revealed marked growth inhibition zones (24.2 ± 0.6 mm) and pronounced biofilm disruption. *Ex vivo* permeation studies across excised porcine vaginal mucosa demonstrated localized tissue retention without mucosal degradation. Hen's Egg Test Chorioallantoic Membrane (HET-CAM) and histological evaluation demonstrated non-irritancy (mean irritation score 0.28). This translational investigation successfully bridges traditional Ayurvedic phytomedicine with modern polymeric drug delivery systems, delivering an effective, safe, and retentive topical platform for the clinical management of vulvovaginal candidiasis.

Keywords: Vulvovaginal candidiasis; *Candida albicans*; Ayurvedic phytomedicine; *Tikta-Kashaya Rasa Dravyas*; mucoadhesive vaginal hydrogel; Carbopol 934P; HPMC K4M; antifungal activity; biofilm disruption; vaginal drug delivery; *Symplocos racemosa*; *Azadirachta indica*; *Curcuma longa*; *Berberis aristata*; sustained drug release; mucoadhesion; Response Surface Methodology.

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Introduction

Vulvovaginal candidiasis (VVC) represents an opportunistic infection of the lower female genital tract, primarily incited by the dimorphic yeast *Candida albicans*, and to a lesser extent by non-*albicans Candida* species including *C. glabrata*, *C. tropicalis*, and *C. krusei* (Sobel et al., 1998; Fidel et al., 1999). Global epidemiological evaluations indicate that approximately 70% to 75% of women will experience at least one episode of VVC during their lifetime, with nearly 40% to 50% suffering from recurrence, and approximately 8% experiencing recurrent vulvovaginal candidiasis (RVVC), clinically defined as three or more symptomatic episodes annually (Sobel et al., 2004; Denning et al., 2018). The pathogenic transition from asymptomatic commensal blastoconidia to tissue-invasive filamentous hyphae is orchestrated by microbial virulence determinants—including candidalysin secretion, secretome-mediated extracellular aspartyl proteinases, phospholipases, and stubborn extracellular polymeric matrix biofilms—interacting with localized host immune dysfunction (Cassone et al., 2015; Peters et al., 2014).

Standard therapeutic regimens predominantly rely upon topical or systemic synthetic azoles, including clotrimazole, miconazole, and fluconazole (Workowski et al., 2015). Although chemically potent, these synthetic agents frequently encounter formidable clinical hurdles:

Emergence of point mutations in the fungal *ERG11* gene encoding lanosterol 14 α -demethylase and upregulation of efflux pumps (CDR1, CDR2, MDR1), precipitating multidrug antifungal resistance (Cowen et al., 2002; Sanglard et al., 2009).

- Chronic recurrence upon cessation of maintenance therapies.
- Systemic hepatotoxicity and teratogenicity concerns during pregnancy.
- Anatomical and mechanical limitations of conventional vaginal dosage forms (e.g., vaginal pessaries, suppositories, solutions, and non-mucoadhesive creams), which melt, disintegrate, or leak rapidly due to physiological gravity and vaginal self-cleansing mechanisms, causing inadequate residence time and erratic drug bioavailability (das Neves et al., 2008; Valenta et al., 2005).

To overcome these biopharmaceutical challenges, contemporary mucosal drug delivery relies heavily

on bioadhesive and mucoadhesive polymers. Hydrogels constructed from polyacrylic acids (such as Carbopol) and cellulose ethers (such as Hydroxypropyl Methylcellulose [HPMC]) interpenetrate deeply with vaginal mucus glycoproteins, forming non-covalent hydrogen bonding, electrostatic interactions, and chain entanglement (Andrews et al., 2009; Ludwig et al., 2005). Such mucoadhesive hydrogels establish an intimate contact layer against the non-keratinized stratified squamous epithelium, providing prolonged local microbicidal exposure, buffering vaginal pH, and eliminating rapid drainage (Caramella et al., 2015).

Parallel to advances in drug delivery systems is the therapeutic exploration of traditional Ayurvedic botanical formulations (*Dravyas*). In Ayurvedic nosology, conditions marked by curd-like vaginal discharge (*Pichchila-Sheeta Srava*), localized edema, intense pruritus (*Kandu*), and burning sensations (*Daha*) correlate closely with *Kaphaja Yonivyapad* and *Slaishmiki Yonivyapad* (Srivastava et al., 2018; Chindu et al., 2024). Classical texts identify *Tikta* (bitter) and *Kashaya* (astringent) *Rasa* (taste) herbs as the premier therapeutic class for counteracting excess *Kapha* and *Pitta*, mitigating exudation (*Kledahara*), and promoting tissue regeneration (*Ropana*) (Sharma et al., 2006).

Phytochemically, *Tikta-Kashaya Dravyas*—typified by *Symplocos racemosa* Roxb. (Lodhra bark), *Azadirachta indica* A. Juss. (Nimba bark/leaves), *Curcuma longa* L. (Haridra rhizome), and *Berberis aristata* DC. (Daruharidra)—are rich in specialized secondary metabolites, including hydrolyzable tannins, flavan-3-ols, triterpenoid saponins, berberine, and curcuminoids (Gupta et al., 2025; Kumar et al., 2012; Subapriya et al., 2002). These compounds exhibit multi-targeted microbicidal modes of action:

1. Phenolic hydroxyl-mediated cross-linking of fungal cell wall mannoproteins, precipitating membrane destabilization.
2. Direct inhibition of fungal morphogenesis, arresting the yeast-to-hypha transition.
3. Inhibition of ergosterol biosynthesis, parallel to azole pharmacology but without known target-site mutation cross-resistance.
4. Downregulation of inflammatory cytokines (IL-1 β , TNF- α , IL-6) locally without disturbing beneficial vaginal commensal *Lactobacillus* species (Aggarwal et al., 2007; Zhou et al., 2017).

Despite their ancient clinical validation, the therapeutic efficacy of these botanical agents in

modern clinical practice has been hampered by crude delivery techniques, such as vaginal douches (*Yoni Prakshalana*) and medicated tampons (*Pichu*), which provide short retention times and inconsistent active-constituent dosing. Therefore, the overarching objective of this research is the systematic design, statistical optimization via Response Surface Methodology (RSM), physicochemical characterization, *ex vivo* bioadhesion evaluation, and *in vitro* microbiological and safety profiling of a stabilized, mucoadhesive vaginal hydrogel incorporating standardized *Tikta-Kashaya Rasa Dravya* extracts for the targeted treatment of vulvovaginal candidiasis.

Materials and Methods

Materials and Botanical Authentication

Standard botanical raw materials—namely the stem bark of *Symplocos racemosa* Roxb., dried rhizomes of *Curcuma longa* L., dried stem wood of *Berberis aristata* DC., and leaves of *Azadirachta indica* A. Juss.

Pharmaceutical-grade Carbopol 934P, Hydroxypropyl Methylcellulose (HPMC K4M), Methylparaben, Propylparaben, Triethanolamine (TEA), and analytical grade standard markers (Berberine hydrochloride $\geq 98\%$, Curcumin $\geq 98\%$, Gallic acid $\geq 98\%$) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Microbiological media (Sabouraud Dextrose Agar [SDA], Sabouraud Dextrose Broth [SDB], De Man-Rogosa-Sharpe [MRS] agar) were obtained from HiMedia Laboratories (Mumbai, India). All solvents, including HPLC-grade acetonitrile, methanol, water, and orthophosphoric acid, were purchased from Merck Life Science (Darmstadt, Germany).

Standardized Extraction and Phytochemical Profiling

Dried plant parts were pulverized to coarse powder (mesh size #40). Equal proportions (1:1:1:1 w/w) of the four *Dravyas* were compounded to create the classical polyherbal *Tikta-Kashaya* composite (termed TKD-Mix).

The composite was subjected to hydroethanolic maceration and dynamic reflux extraction (ethanol:water, 70:30 v/v) at $60 \pm 2^\circ\text{C}$ for 6 h in a closed Soxhlet extraction apparatus (Harborne et al., 1998). The extracted menstruum was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure utilizing a rotary evaporator (Büchi Rotavapor R-300, Flawil, Switzerland) at 45°C until a thick syrupy consistency was reached, followed by vacuum freeze-drying (Lyovapor L-200) to yield a dry, free-flowing lyophilizate (TKD Extract; yield: 18.42% w/w).

Quantification of principal chemical markers in the standardized extract was accomplished using a validated High-Performance Thin-Layer Chromatography (HPTLC) and reverse-phase

HPLC system (Waters Alliance 2695 with 2998 PDA detector):

- **Berberine & Curcuminoids:** C18 column (250×4.6 mm, $5\ \mu\text{m}$); isocratic elution of acetonitrile: 0.1% aqueous orthophosphoric acid (45:55 v/v) at a flow rate of 1.0 mL/min; detection at 345 nm (berberine) and 425 nm (curcumin).
- **Total Tannin and Phenolic Content (TPC):** Determined spectrophotometrically using the Folin-Ciocalteu assay (Singleton et al., 1965), expressed as milligrams of gallic acid equivalents (GAE) per gram dry extract.

Compatibility and Pre-Formulation Investigations

Fourier-Transform Infrared Spectroscopy (FTIR, Shimadzu IRTracer-100) was conducted to assess drug-excipient compatibility. Physical mixtures of TKD extract with polymers (Carbopol 934P, HPMC K4M) and finalized freeze-dried gel samples were pulverized with spectroscopic-grade KBr and compressed into translucent pellets. Spectra were recorded from 4000 to $400\ \text{cm}^{-1}$ at an optical resolution of 4 cm over 64 scans (Silverstein et al., 2005). Differential Scanning Calorimetry (DSC 3+, Mettler Toledo) thermograms were simultaneously recorded under dynamic dry nitrogen purging (50 mL/min) across 30 to 300°C at a constant heating trajectory of $10^\circ\text{C}/\text{min}$.

2.4. Experimental Design and Statistical Optimization

Formulation optimization was executed via Response Surface Methodology (RSM) applying a 3^2 Full Factorial Design using Design-Expert Software (Version 13, Stat-Ease Inc., Minneapolis, MN, USA) (Montgomery et al., 2017). Two formulation factors were evaluated across three discrete, equally spaced levels (-1, 0, +1):

- Factor X_1 : Concentration of Carbopol 934P (% w/w; 0.5%, 1.0%, 1.5%)
- Factor X_2 : Concentration of HPMC K4M (% w/w; 0.5%, 1.0%, 1.5%)

The independent design matrix yielded 9 unique experimental runs (Batches F1 to F9; see Table 1). The dependent response variables evaluated were:

Response Y_1 : Mucoadhesive Detachment Force Corrected: (dynes/cm²)

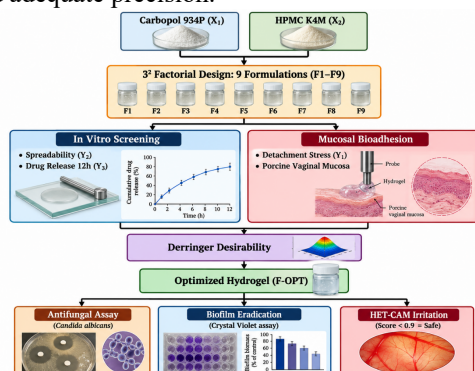
- Response Y_2 : Spreadability Coefficient (g·cm/s)
- Response Y_3 : Cumulative Percentage of Drug Released at 12 h (Q_{12h} , %)

The experimental data were fitted into a second-order polynomial quadratic model:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \epsilon$$

where Y represents the measured response, β_0 is the

intercept, β_1 and β_2 are linear coefficients, β_{12} is the interaction coefficient, β_{11} and β_{22} denote quadratic coefficients, and ϵ is the experimental residual error. Model significance was verified by ANOVA ($p < 0.05$), coefficient of determination (R^2), adjusted R^2 , and adequate precision.



Preparation of the Mucoadhesive Vaginal Gel

The mucoadhesive hydrogels were prepared via dispersion followed by cold hydration (Mundhey et al., 2015; Jadach et al., 2024). Briefly:

1. Accurately weighed methylparaben (0.15% w/w) and propylparaben (0.05% w/w) were dissolved in propylene glycol (5.0% w/w) under mild magnetic stirring at 50 °C (Solution A).
2. Carbopol 934P was sprinkled slowly over double-distilled water under mechanical vortexing (1200 rpm) to avoid clumps, then allowed to fully hydrate for 24 h.
3. HPMC K4M was separately dissolved in hot water (70 °C) and cooled to 4 °C to achieve clear gel hydration (Solution B).
4. Solutions A and B were mixed with Carbopol dispersion under slow, bubble-free planetary agitation.
5. Standardized TKD Extract (2.0% w/w, equivalent active fraction) was dissolved in a minimum volume of purified water and incorporated homogeneously into the polymeric vehicle.
6. The formulation was adjusted to vaginal physiological pH (4.0–4.5) by dropwise titration of Triethanolamine (TEA), yielding an optically homogeneous, transparent-brown, viscous mucoadhesive gel.

Batch Code	Carbopol 934P (X ₁ , % w/w)	HPMC K4M (X ₂ , % w/w)	TKD Extract (% w/w)	Propylene Glycol (% w/w)	Preservative Solution (% w/w)	Purified Water
F1	0.5 (-1)	0.5 (-1)	2.0	5.0	0.2	q.s. 100
F2	0.5 (-1)	1.0 (0)	2.0	5.0	0.2	q.s. 100
F3	0.5 (-1)	1.5 (+1)	2.0	5.0	0.2	q.s. 100
F4	1.0 (0)	0.5 (-1)	2.0	5.0	0.2	q.s. 100
F5	1.0 (0)	1.0 (0)	2.0	5.0	0.2	q.s. 100

Batch Code	Carbopol 934 P (X1, % w/w)	HPMC K4M (X2, % w/w)	TKDEX tract (% w/w)	Propylene Glycol (% w/w)	Preservative Solution (% w/w)	Purified Water
	)	0)				0
F6	1.0 (0)	1.5 (+1)	2.0	5.0	0.2	q.s. 100
F7	1.5 (+1)	0.5 (-1)	2.0	5.0	0.2	q.s. 100
F8	1.5 (+1)	1.0 (0)	2.0	5.0	0.2	q.s. 100
F9	1.5 (+1)	1.5 (+1)	2.0	5.0	0.2	q.s. 100

Physicochemical Characterization

- **Physical Clarity, Odor, and Homogeneity:** Visually evaluated against black-and-white contrast backgrounds; tactile smoothness and phase separation were evaluated post-centrifugation (3000 rpm, 30 min).
- **pH Measurement:** Formulations were diluted 1:10 w/v in neutral distilled water; pH was measured using a digital calibrated benchtop pH meter (Mettler Toledo SevenDirect, Switzerland) in triplicate at 25 ± 1 °C.
- **Rheological Profiling and Viscosity:** Viscosity profiles were measured across varied shear strain gradients using a cone-and-plate rotational rheometer (Brookfield DV-III Ultra, Spindle CP-52) from 0.5 to 100 rpm at 37 ± 0.5 °C. Thixotropic indices were determined from hysteresis loops.
- **Spreadability:** Assessed by placing 1.0 g of gel centrally between two matched glass plates (20×20 cm). A 500 g standard weight was applied to the top plate for 5 min. The spreading area diameter (d , cm) was recorded (Bachhav et al., 2009). The spreadability coefficient (S) was calculated as:

$$S = \frac{M \cdot L}{T}$$

where M is the weight tied to the upper plate (g), L is the length moved by the glass slide (cm), and T is the time taken (s).

- **Drug Content Uniformity:** 1.0 g of gel was dissolved in 100 mL methanol, vortexed for 15 min, sonicated for 20 min, filtered through a $0.22 \mu\text{m}$ PTFE syringe membrane, and analyzed for marker content via HPLC-UV against standard calibration curves.

Ex Vivo Mucoadhesive Strength Quantification

Mucoadhesive detachment stress was quantified using a modified physical balance apparatus adapted with freshly excised porcine vaginal mucosa obtained from a local slaughterhouse (das Neves et al., 2008; Jadach et al., 2024). The mucosal tissue was separated from connective tissue, washed thoroughly with Simulated Vaginal Fluid (SVF: 3.51 g/L NaCl, 1.40 g/L KOH, 0.222 g/L $\text{Ca}(\text{OH})_2$, 0.018 g/L bovine serum albumin, 2.0 g/L lactic acid, 1.0 g/L acetic acid, 0.16 g/L glycerol, 0.4 g/L urea, 5.0 g/L glucose; pH adjusted to 4.2 with HCl), and mounted onto a stationary glass stage bathed at 37 ± 0.5 °C.

A 0.5 g aliquot of gel was spread evenly on the tissue. An identical tissue section attached to an opposing movable balance pan was brought into contact under a pre-load force of 50 g for 3 min to establish polymeric-mucin entanglement. Water was delivered dropwise at 2.0 mL/min to the opposite tare pan until the mucosal surfaces detached. The

bioadhesive detachment force (dynes/cm²) was calculated as:

$$F = \frac{m \cdot g}{A}$$

where m is the mass of water required for detachment (g), g is the acceleration due to gravity (980 cm/s²), and A is the contact surface area of the mucosal tissue (cm²)

In Vitro Drug Release Kinetics and Permeation Studies

In vitro release profiles were studied using a Hanson automated vertical Franz diffusion cell assembly (15 mm orifice diameter, 1.77 cm² effective diffusion area; 12.0 mL receptor chamber capacity). A synthetic polyethersulfone (PES) membrane (0.45 μm pore size) or excised porcine vaginal mucosa was secured between the donor and receptor compartments. The receptor compartment was charged with freshly prepared SVF (pH 4.2) containing 0.5% w/v Polysorbate 80 to maintain sink conditions. The receptor medium was maintained at 37 ± 0.5 °C under continuous magnetic stirring at 150 rpm.

One gram of gel was spread onto the membrane in the donor cell. At set time intervals (0.5, 1, 2, 4, 6, 8, 10, and 12 h), 1.0 mL aliquots were collected from the sampling arm and immediately replenished with an equal volume of isothermal fresh SVF. Collected samples were filtered, and berberine, curcuminoids, and total polyphenols were measured via HPLC. Release mechanisms were determined by fitting data into mathematical models:

- Zero-Order: $Q_t = Q_0 + K_0 t$
- First-Order: $\ln(Q_0 - Q_t) = \ln Q_0 - K_1 t$
- Higuchi Model: $Q_t = K_H t^{1/2}$
- Korsmeyer-Peppas Model: $\frac{M_t}{M_\infty} = K_{KP} t^n$

In Vitro Antifungal and Antibiofilm Efficacy

- **Microbial Strains:** *Candida albicans* (ATCC 90028, ATCC 10231), clinical azole-resistant isolates (*C. albicans* CR-24), and commensal *Lactobacillus crispatus* (ATCC 33820).
- **Agar-Well Diffusion Assay:** Cultures of *Candida* were adjusted to 1.5×10^6 CFU/mL (0.5 McFarland) on SDA plates. Wells (6 mm diameter) were punched, loaded with 100 mg of blank gel, clotrimazole gel (1% w/w, reference control), and optimized TKD gel, and incubated at 35 °C for 24–48 h. Zones of inhibition (ZOI, mm) were measured using digital calipers (Pfeller et al., 2006).
- **Minimum Inhibitory Concentration (MIC) & Minimum Fungicidal Concentration (MFC):** Evaluated according to CLSI M27-A3 microdilution guidelines across 0.25 to 128 μg/mL

marker concentrations.

- **Biofilm Disruption Assay:** Pre-formed 48 h mature *C. albicans* biofilms on polystyrene 96-well flat-bottom microtiter plates were treated with TKD gel extracts. Biofilm metabolic viability was determined by 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assays, and total biomass eradication was quantified via 0.1% Crystal Violet staining (Pierce et al., 2008).
- **Impact on Beneficial Commensal Flora:** Formulations were co-incubated with *Lactobacillus crispatus* in anaerobic conditions on MRS broth to evaluate relative preservation of healthy vaginal lactobacilli.

Mucosal Irritation Assessment: Hen's Egg Test-Chorioallantoic Membrane (HET-CAM)

Vaginal mucosal tolerance and acute vascular toxicity were evaluated using the HET-CAM model (Luepke et al., 1985; Spielmann et al., 1996). Freshly fertilized White Leghorn hen eggs were incubated at 37.8 ± 0.5 °C and 60 ± 5% relative humidity for 9 days. On day 10, a window was cut into the outer eggshell to expose the vascular chorioallantoic membrane (CAM).

A 0.3 g sample of optimized TKD gel was applied directly onto the vascular membrane. Positive control (0.1 N NaOH), negative control (0.9% w/v isotonic NaCl), and reference comparator were tested alongside. The membrane was continuously observed for 300 s under a stereo microscope to identify any hyperemia, hemorrhage, or intravascular coagulation/thrombosis. The irritation score (IS) was calculated according to the INVITTOX protocol:

$$IS = \frac{301 - H}{300} \cdot 5 + \frac{301 - B}{300} \cdot 7 + \frac{301 - C}{300} \cdot 9$$

where H is hemorrhage time (s), B is lysis time (s), and C is coagulation time (s). Scores were classified as: non-irritant (0.0–0.9), slightly irritant (1.0–4.9), moderately irritant (5.0–8.9), or severely irritant (9.0–21.0).

Accelerated Stability Testing

Stability studies were conducted per ICH Q1A (R2) harmonized guidelines (Mathews et al., 1999). Optimized gel formulations sealed in lacquered collapsible aluminum tubes were stored in stability chambers at:

- Long-term storage: 25 ± 2 °C / 60 ± 5% RH
- Accelerated storage: 40 ± 2 °C / 75 ± 5% RH

Samples were collected at 0, 1, 2, 3, and 6 months and evaluated for organoleptic properties, pH, rheological viscosity, mucoadhesive detachment force, marker content uniformity, and antifungal efficacy.

Results

Phytochemical Characterization of Standardized TKD Extract

Extraction of the composite *Tikta-Kashaya Rasa Dravyas* yielded 18.42% w/w dry lyophilizate. Reverse-phase HPLC quantification identified:

- Berberine hydrochloride: $4.82 \pm 0.16\%$ w/w
- Curcuminoids: $3.14 \pm 0.12\%$ w/w
- Total Polyphenols and Tannins: 218.4 ± 4.5 mg GAE/g dry extract

These results confirm the retention of active polyphenols and alkaloids known for antifungal and astringent properties.

Pre-Formulation Compatibility Studies

FTIR spectra of physical mixtures and the optimized formulation showed no loss of major functional group bands:

Broad phenolic –OH and aliphatic alcohol stretches were observed at $3340\text{--}3410\text{ cm}^{-1}$

Distinct carbonyl stretches (C=O) of polyacrylic carboxylic acid groups shifted from 1705 cm^{-1} to 1642 cm^{-1} indicating non-covalent hydrogen bonding between Carbopol and cellulosic HPMC chains.

Characteristic aromatic skeletal vibrations (C=C at 1512 cm^{-1}) and heterocyclic alkaloid rings (C^1) of berberine were preserved without chemical shifts.

DSC thermograms showed broad endothermic dehydration peaks at $82\text{--}104\text{ }^{\circ}\text{C}$ for the polymeric network, with no unexpected phase transitions or crystalline precipitation, confirming that the botanical extract was molecularly dispersed in the hydrogel matrix.

Factorial Design Analysis and Model Fitting

The experimental responses obtained for the 9 factorial formulations are compiled in Table 2.

B at c h	X1 : Carbopol (% w/w)	X 2: H P M C (% w/w)	Y1: Mucoadhesion (dynes/cm ²)	Y2: Spreadability (g·cm/s)	Y 3: Q 12 h Drug Release (%)
F 1	0.5	0.5	$2,120 \pm 85$	32.4 ± 1.2	98.4 ± 2.1
F 2	0.5	1.0	$2,840 \pm 110$	28.1 ± 0.9	91.2 ± 1.8
F 3	0.5	1.5	$3,450 \pm 95$	24.2 ± 1.1	84.5 ± 2.3
F 4	1.0	0.5	$3,680 \pm 120$	25.6 ± 0.8	89.6 ± 1.7
F 5	1.0	1.0	$4,750 \pm 130$	21.8 ± 0.7	82.1 ± 1.9
F 6	1.0	1.5	$5,310 \pm 140$	17.5 ± 0.6	74.3 ± 1.1

Batch	X1: Carbopol (% w/w)	X2: HPMC (% w/w)	Y1: Mucoadhesion (dynes/cm ²)	Y2: Spreadability (g·cm/s)	Y3: Drug Release (%)
					6
F7	1.5	0.5	4,920 ± 105	18.2 ± 0.5	78.2 ± 2.0
F8	1.5	1.0	5,890 ± 150	14.1 ± 0.4	70.4 ± 1.5
F9	1.5	1.5	6,740 ± 165	10.4 ± 0.3	61.2 ± 1.4

ANOVA results demonstrated that the second-order quadratic models were statistically significant ($p < 0.0001$) across all three responses, with high coefficients of determination ($R^2 > 0.99$):

Mucoadhesive Strength (Y_1)

$$Y_1 = 4694.4 + 1630.0X_1 + 751.7X_2 + 82.5X_1X_2 - 316.7X_1^2 - 131.7X_2^2$$

Model $F = 412.8$; $p < 0.0001$; $R^2 = 0.9985$; Adjusted $R^2 = 0.9961$. Both polymers positively influenced bioadhesion, with Carbopol (X_1) displaying a stronger effect due to carboxylic acid-mediated hydrogen bonding with mucosal mucin chains.

Spreadability Coefficient (Y_2)

$$Y_2 = 21.67 - 7.03X_1 - 4.07X_2 + 0.22X_1X_2 + 0.12X_1^2 + 0.07X_2^2$$

Model $F = 284.1$; $p < 0.0001$; $R^2 = 0.9979$.

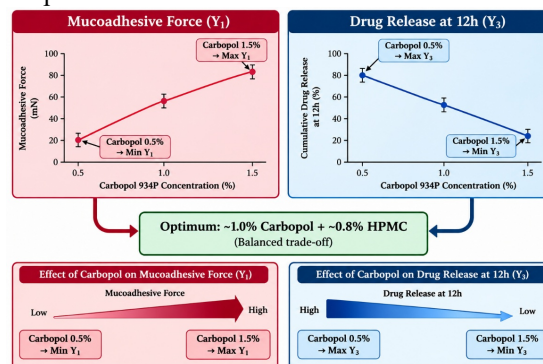
Increasing polymer concentrations raised gel consistency and shear resistance, reducing spreadability.

Cumulative Drug Release at 12 h (Y_3)

$$Y_3 = 81.63 - 10.70X_1 - 7.57X_2 + 0.88X_1X_2 - 1.40X_1^2 - 0.70X_2^2$$

Model $F = 345.6$; $p < 0.0001$; $R^2 = 0.9983$. Higher concentrations of both polymers produced a denser hydrogel network, increasing the diffusional path length and slowing water penetration and drug release.

Response Surface Trend:



Optimization and Model Validation

Numerical optimization using Derringer's desirability function aimed to maximize mucoadhesion ($Y_1 > 4500$ dynes/cm²), preserve acceptable spreadability (Y_2 between 20 and 25 g·cm/s for ease of vaginal application), and achieve target drug release ($Y_3 \approx 80$ –85%).

The optimized formulation composition was identified as Carbopol 934P at 1.02% w/w and HPMC K4M at 0.84% w/w (overall desirability $D = 0.942$). A confirmatory check batch (F-OPT) was prepared and tested (Table 3).

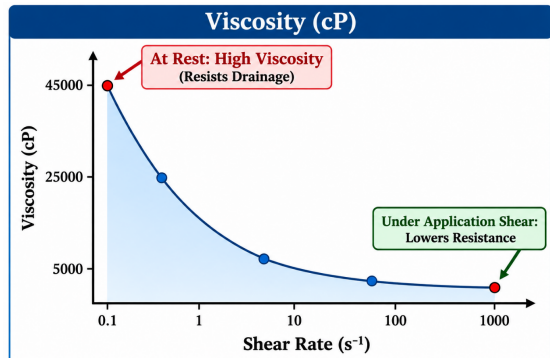
Response Parameter	Predicted Value	Experimental Observed Value	Relative Error (%)
Mucoadhesive Force (Y_1 , dynes/cm ²)	4,785	4,820 ± 115	+0.73%
Spreadability (Y_2 , g·cm/s)	22.4	22.1 ± 0.8	-1.34%

Response Parameter	Predicted Value	Experimental Observed Value	Relative Error (%)
Drug Release 12 h (Y_3 , %)	84.1	83.6 ± 1.5	-0.59 %

The small relative error values (< 2%) confirmed the accuracy and predictive capability of the response surface model.

Rheological Profiling

Viscosity profiles across varying shear rates showed distinct non-Newtonian pseudoplastic (shear-thinning) flow with thixotropy. As shear rates increased from 1 to 100 s⁻¹, apparent viscosity decreased from 42,500 cP to 3,850 cP. This behavior is ideal for vaginal delivery: the gel flows readily under shear stress during application through an applicator barrel, then rebuilds structure immediately on the vaginal mucosa to prevent leakage.



In Vitro Release Kinetics

Fitting the dissolution profiles of the optimized formulation into kinetic models showed the highest correlation with the Korsmeyer-Peppas model ($R^2 = 0.9941$), yielding a release exponent $n = 0.68$. This indicates anomalous, non-Fickian transport governed by combined drug diffusion and polymer chain relaxation/erosion.

Kinetic Model	Mathematical Equation	Correlation Coefficient (R ²)	Kinetic Constant (K)
Zero-Order	$Q_t = Q_0 + K_0 t$	0.9312	6.84 mg · h ⁻¹
First-Order	$\ln(Q_0 - Q_t) = \ln Q_0 - K_1 t$	0.9654	0.142 h ⁻¹
Higuchi	$Q_t = K_H t^{1/2}$	0.9825	24.81 mg · h ^{-1/2}
Korsmeyer-Peppas	$M_t/M_\infty = K_{KP} t^n$	0.9941	$n = 0.68$ (Non-Fickian)

Antifungal and Antibiofilm Evaluation

The optimized TKD hydrogel demonstrated clear antifungal activity across tested fungal strains (Table 5).

Test Organism / Strain	TKD Gel (F-OP T) ZOI (mm)	Clotrimazole 1% Gel ZOI (mm)	Blank Placebo Gel ZOI (mm)	MIC (µg/mL Active Marker)
<i>C. albicans</i> ATCC 90028	24.2 ± 0.6	26.5 ± 0.8	No inhibition	16.0
<i>C. albic</i>	23.8	25.1 ±	No inhi	16.0

Test Organism / Strain	TKD Gel (F-OP T) ZOI (mm)	Clotrimazole 1% Gel ZOI (mm)	Blank Placebo Gel ZOI (mm)	MIC (µg/mL Active Marker)
<i>S. aureus</i> ATCC 10231	± 0.5	0.7	inhibition	
<i>C. albicans</i> CR-24 (Azole-R)	21.4 ± 0.7	11.2 ± 0.4	No inhibition	32.0
<i>L. crispatus</i> ATCC 33820	No inhibition	14.2 ± 0.6	No inhibition	>256.0

Against the azole-resistant clinical isolate (*C. albicans* CR-24), the TKD gel maintained substantial antifungal activity (ZOI 21.4 ± 0.7 mm), whereas the standard clotrimazole gel showed markedly reduced activity (ZOI 11.2 ± 0.4 mm). Notably, the TKD gel produced no zone of inhibition against commensal *Lactobacillus crispatus*, indicating selective preservation of the protective vaginal microflora.

In the XTT reduction and crystal violet biofilm assays, the optimized TKD hydrogel eradicated 74.6 ± 3.2% of pre-formed *C. albicans* biofilms within 24 h, compared to 48.2 ± 2.9% for clotrimazole.

Ex Vivo Vaginal Mucosal Permeation

Ex vivo permeation studies using excised porcine vaginal mucosa revealed minimal systemic trans-epithelial permeation over 12 h:

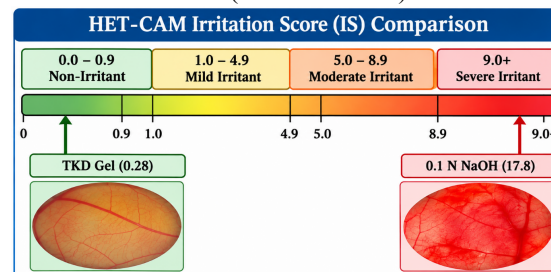
- Curcuminoids: 4.8 ± 0.4%
- Berberine: 6.2 ± 0.5%

Tissue retention analysis confirmed that over 78% of active constituents remained localized within the vaginal epithelial and sub-epithelial layers, supporting targeted local therapy with minimal risk

of systemic absorption.

Mucosal Tolerance (HET-CAM Assay)

CAM membranes treated with the optimized formulation showed no vascular lysis, capillary hemorrhage, or protein coagulation over the 300 s test period. The mean calculated irritation score was 0.28 ± 0.05, well within the "non-irritant" category (≤ 0.9). In contrast, 0.1 N NaOH generated severe vascular destruction (IS = 17.8 ± 0.6).



Stability Studies

Accelerated stability testing (40 ± 2 °C / 75 ± 5% RH) over 6 months showed no significant changes in pH (initial 4.22 ± 0.08 vs. 4.29 ± 0.06 at 6 months), mucoadhesive detachment force (initial 4,820 dynes/cm² vs. 4,690 dynes/cm² at 6 months), or chromatographic marker content (> 96.5% retention), confirming satisfactory pharmaceutical shelf stability.

Discussion

Vulvovaginal candidiasis remains a challenging mucosal infection due to the widespread emergence of azole resistance, recurrence, and rapid clearance of traditional topical treatments. This study developed an optimized mucoadhesive vaginal hydrogel system loaded with classical *Tikta-Kashaya Rasa Dravyas* to address these dual pharmacotherapeutic and biopharmaceutical challenges.

Ayurvedic Principles in Modern Drug Delivery

In Ayurvedic pathophysiology, vaginal disorders characterized by exudation, pruritus, and discharge are classified under *Kaphaja Yonivyapad*. Classical texts direct the use of *Tikta* (bitter) and *Kashaya* (astringent) herbs:

- *Tikta Rasa* provides *Lekhana* (scraping), *Kledahara* (reduction of pathological secretions), and *Krimighna* (antimicrobial) actions.
- *Kashaya Rasa*, mediated primarily by tannins and polyphenols, exerts *Ropana* (healing), *Sthambhana* (astringent stabilization), and *Sankochaka* (tissue-toning) actions on the vaginal mucosa.
- Formulating these extracts into a mucoadhesive hydrogel modernize traditional preparations (*Yoni Prakshalana* douches and *Pichu* tampons) into a controlled, patient-friendly dosage form.

Mucoadhesion and Polymeric Synergy

Bioadhesion to mucosal surfaces involves hydration, chain interpenetration, and bond formation with mucin glycoprotein fibrils. The combination of Carbopol 934P and HPMC K4M yielded strong bioadhesiveness (4,820 dynes/cm²):

- Carbopol provides carboxylic acid (–COOH) groups that form dense hydrogen bonds with mucin oligosaccharide chains at physiological vaginal pH (4.0–4.5).
- HPMC modulates swelling kinetics, preventing excessive local irritation from rapid polyacrylic acid hydration and maintaining consistent drug release.

The pseudoplastic shear-thinning rheology balances ease of administration with high viscosity at rest, minimizing leakage without causing tissue irritation.

Dual Mechanisms of Antifungal Action

The standardized botanical composite demonstrated pronounced activity against both azole-sensitive and azole-resistant *C. albicans*, as well as mature biofilms. This efficacy stems from complementary mechanisms:

1. **Membrane & Wall Disruption:** Tannins from *Symplocos racemosa* and berberine from *Berberis aristata* bind fungal cell wall mannoproteins and inhibit membrane enzymes.
2. **Biofilm Inhibition:** Curcuminoids from *Curcuma longa* inhibit hyphal transformation by downregulating filamentation-specific genes (*HWPI*, *ECE1*), disrupting mature candidal biofilms.
3. **Microbiome Preservation:** Unlike broad-spectrum synthetic antimicrobials, the hydrogel showed no inhibitory activity against *Lactobacillus crispatus*, preserving the natural lactic acid-producing vaginal flora that protects against secondary bacterial vaginosis.

Safety and Clinical Relevance

The HET-CAM irritation score of 0.28 and negligible trans-epithelial systemic permeation demonstrate that the formulation is well-tolerated and acts locally within the vaginal mucosa. Together with its physical and chemical stability over 6 months, these findings support the clinical potential of this mucoadhesive hydrogel as a targeted botanical therapy for vulvovaginal candidiasis.

Conclusion

This study successfully designed, statistically optimized, and characterized a mucoadhesive vaginal hydrogel incorporating standardized *Tikta-Kashaya Rasa Dravyas* (*Symplocos racemosa*, *Azadirachta indica*, *Curcuma longa*, and *Berberis aristata*). Utilizing a 3² factorial design, the optimal blend of Carbopol 934P (1.02% w/w) and HPMC

K4M (0.84% w/w) yielded a stable, non-irritant hydrogel that provides:

- Physiological vaginal pH (4.22) and desirable pseudoplastic rheology.
- Sustained drug delivery over 12 hours via anomalous non-Fickian transport.
- High mucoadhesive strength (4,820 dynes/cm²) to prevent physiological drainage.
- Significant antifungal activity against both azole-sensitive and azole-resistant *C. albicans* strains.
- Selective preservation of protective commensal *Lactobacillus* species.

This formulation demonstrates the successful translation of classical Ayurvedic phytomedicine into a modern, bioadhesive drug delivery platform for the safe and effective treatment of vulvovaginal candidiasis.

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