

# Mucoadhesive In Situ Vaginal Gels Loaded with Quercetin: A Review of Formulation Strategies and Therapeutic Outlook for Vaginal Infections

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## Abstract

Three of the most prevalent vaginal infections are bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis and affect a significant proportion of women of reproductive age, and can inexplicably return. The usual dosage forms, including creams, pessaries, and suppositories, easily leak, smear, or shift away from the mucous membranes shortly after being placed in the vagina, lowering the amount of time the drug may be available to act on the mucous membranes. It is really a brief stay home. In situ gel systems overcome this by being initially in a free flowing liquid form but then developing into a gel once they come into contact with the vaginal pH or temperature and subsequently, their gel becomes stiff and maintains its position for several hours. When incorporated into a mixture with mucoadhesive polymers (such as Carbopol 934, HPMC K4M or chitosan), the retention can be extended much further than the most basic formulation. Onions, capers and apples contain quercetin, a flavonoid that has been attracting increasing interest in the use of it as a phytopharmaceutical candidate. It demonstrates a broad-spectrum antimicrobial activity covering *Candida albicans*, *Escherichia coli* and biofilm producing microorganisms, along with beneficial anti-inflammatory and antioxidant properties. Despite its low topical availability, however, its use is limited for topical product applications for a long time. This present review follows the logics, chemistry of the polymers, polyphenols used to trigger the gelation of the in situ gels as well as the parameters of assessment for mucoadhesive in situ

gels showing quercetin therapeutic loading. Solubility-enhancement strategies (namely solvent blends, nanocarriers, and cyclodextrin complexation) and existing challenges for translation into clinical applications are considered. Vaginal gels containing phytopharmaceuticals appear to make sound sense and only further research is required to handle scaling up, stability, and regulation.

**Keywords:** Vaginal drug delivery, HPMC, Carbopol, In situ gel, Quercetin, Vaginal Candidiasis, and Phytopharmaceutical.

## I. Introduction

Worldwide, bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis are major contributors to gynaecology outpatient visits and encompasses a significant number of cases. It is estimated that almost 75% of women have at least one VVC episode in their lifetime and 8% of women worldwide have four or more episodes per year (recurrent) [1,2]. It isn't just a symptom. Noticing recurrent or untreated infections is associated with experiencing pelvic inflammatory disease, pregnancy complications such as miscarriage, stillbirth, premature labor, and quantifiable increase in risk of HIV acquisition [3]. Add to this the relentless impediment of antimicrobial resistance, such as the emergent strains of *Candida glabrata* that we are beginning to tag with the fluconazole resistance, and the metronidazole-tolerant strains of *Trichomonas*

vaginalis — and the case for safer and smarter therapy gets even stronger [4].

Traditional vaginal dosage forms have been used in the clinic for decades. But all creams, pessaries, suppositories and tablets face a common problem: low papillary residence time. The vagina has almost an automatic mechanism of self-cleaning, which is accomplished by fluid turnover and the aid of gravity is minimal. Most of an applied cream drains out or penetrates undergarments within an hour or two after application and may not attain therapeutic levels at the site of the infection before this happens [5,6]. A lack of messiness, leakage or multiple dosing is common complaints for patients, which collectively equates to poor adherence and increased relapse rates [7].

Why bother to perform in-situ gels, In theory the concept is simple. A solution that passes easily through an applicator at room temperature and that turns to a viscous, semi-solid network when it meets the body's pH (4.0–4.5), temperature (close to body temperature of 37 °C), or ionic milieu (vaginal fluid), does two useful things. It is uniformly distributed throughout the vaginal walls where creams may fail to reach and becomes undisturbed to remain in place within the vaginal folds. Combine with a mucoadhesive polymer that hydrogen-bonds with mucin, then the dosing time is hourly, at least once per day--some cases, once every 48 hours [8,9].

Quercetin is a molecule that has appeared in numerous pharmacology reviews that's derived from a plant. It is found in edible plants like onions, broccoli, capers, and many other edible plants, and has been studied for its anti-oxidant, anti-inflammatory, antiviral, anti-fungal and anti-bacterial properties [10,11]. The activity against

*Candida albicans* biofilms is documented and makes it relevant for application to the vagina: Biofilm formation is the action that triggers recurrent VVC [12]. In addition to the effects mentioned above, quercetin additionally interferes with bacterial efflux pumps and quorum sensing signals, which traditional antifungals and antibiotics are unable to do so [13]. Its big drawback, But aqueous solubility, just 1 µg/mL at 25 °C, does not lend itself to easily formulating a useful vaginal product.

But why phytopharmaceuticals, There is a part economic, and a part biological, to the argument. Multi-target photoactive azole antifungal resistance, as well as that against nitroimidazoles, are very well documented and the antimicrobials of another chemical scaffold are warranted, and plant-derived flavonoids do. It is likely to be more effective at multiple targets of bacterial or fungal disease, therefore, resisting the development of resistance as compared to mono-target synthetics. In addition, they tend to have higher safety margins (creating a buffer that is good for chronic or repeated dosing). Quercetin has been specifically designated as 'generally recognised as safe' (GRAS) in food use and has been reported to have reported lethal doses (LD50) of more than 5 g/kg for rodents, which gives formulator's relief.

This review gathers the literature on the in situ vaginal mucoadhesive gel systems in general, focussing on the quercetin loaded systems. We overview the pertinent anatomy and physiology, polymer selections, gelation triggers, and criteria for evaluation outlined in published work, and the open-ended issues confronting everyone in this space. This is not intended as an encyclopedia of published formulations, but an explanation of the design principles (and compromises) used today.

**Table 1.** Common vaginal infections and their causative organisms

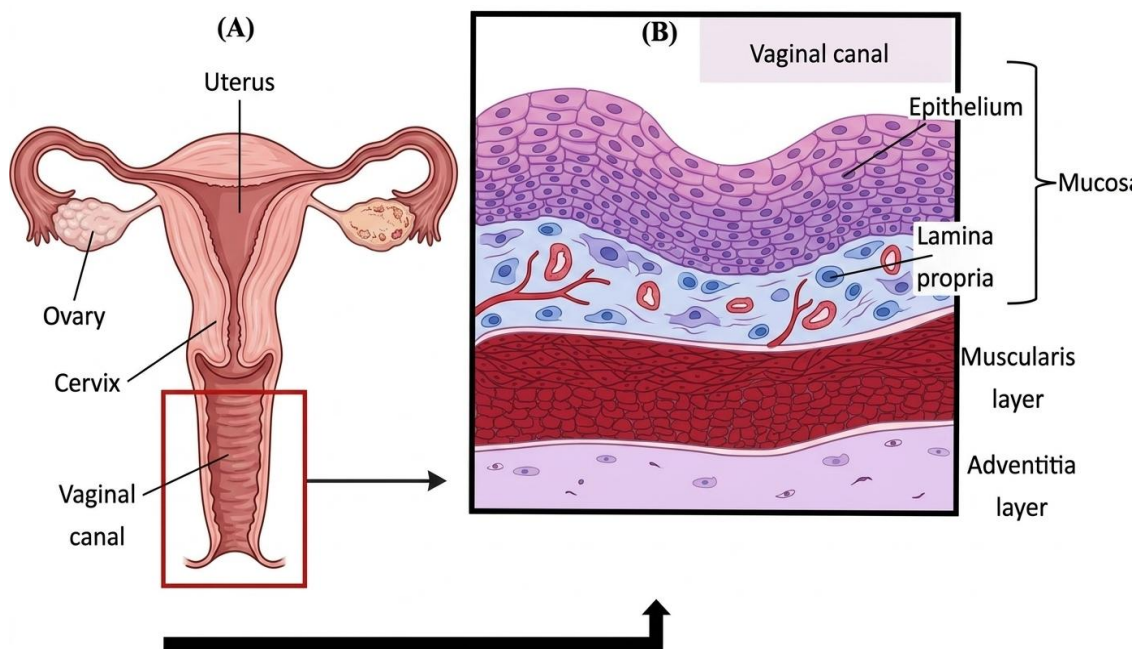
Infection	Causative organism	Typical symptoms	Standard therapy
Bacterial vaginosis	<i>Gardnerella vaginalis</i> , <i>Atopobium vaginae</i>	Thin grey discharge, fishy odour, raised vaginal pH	Metronidazole, clindamycin
Vulvovaginal candidiasis	<i>Candida albicans</i> (≈ 85–90%), <i>C. glabrata</i> , <i>C. krusei</i>	Pruritus, curd-like discharge, erythema	Fluconazole, clotrimazole, miconazole
Trichomoniasis	<i>Trichomonas vaginalis</i>	Frothy yellow-green discharge, dyspareunia	Metronidazole, tinidazole
Aerobic vaginitis	<i>Streptococcus agalactiae</i> , <i>E. coli</i> , <i>S. aureus</i>	Yellow discharge, inflammation, dyspareunia	Topical clindamycin, kanamycin

## II. The Vaginal Route as a Drug Delivery Site.

### 2.1 Anatomy and physiology

The mature female genitalia is a 7-10cm fibromuscular tube, covered by a non-keratinised stratified squamous epithelium overlying a well vascularised lamina propria. The epithelium has rugosities or folds which are transverse and this adds to the absorptive surface area remarkably. This structure has led to some peptides and lipophilic small molecules being transferred orally that can be better than vaginally [14]. This is because the epithelium's thickness in the follicular phase as a result of the action of estrogen and thins in post menopausal women, a thing to bear in mind when creating gels for older patients.

Histologically the vagina exhibits four layers of cells—the basal layer, then the parabasal layer, followed by the intermediate and final superficial layer—with flatter, more highly glycogenated cells on each layer moving outward. There are several pathways for drug permeation, such as: transcellular passive diffusion (dominant pathway for lipophilic drugs), paracellular diffusion through tight junctions and intercellular spaces (dominant pathway for small hydrophilic drugs), receptor-mediated uptake (relevant pathway when entering by a receptor-mediated process as in the case of biologics), or via a vesicular pathway. In the case of quercetin, log P is ~1.5, implying that transcellular diffusion would be expected to be the rate limiting step, typically the dissolution process from the gel matrix, but not necessarily from the membrane crossing.



**Figure 1.** Anatomy of the female reproductive tract showing the position of the vagina, with magnified inset of vaginal epithelial layers (basal, parabasal, intermediate, and superficial) and the overlying mucus film.

In normal women of reproductive age, vaginal fluid production approximately 0.5 - 0.75 mL per day and the bacterial flora consists mainly of Lactobacillus species, such as *L. crispatus*, *L. gasseri*, and *L. jensenii*. The presence of these bugs converts glycogen secreted by the epithelium being shed into lactic acid that helps to maintain a low vaginal pH, 3.8–4.5 [15]. This means that this acidic environment is the body's first barrier to germs invading and thureness is a sign of the pH (acidity) levels rising above 4.5, the no-bass level, and which

is a diagnostic marker for BV. Any medication applied to the vagina that disrupts the lactobacillary flora or changes the local pH of the vagina can be beneficial or harmful.

### 2.2 Factors that influence the absorption of vaginal drugs:

So, several physiological variables affect the percentage of a drug reaching any systemic or local target site after its administration. These factors include the state of the epithelium (which

changes throughout the menstrual cycle and with changes in hormonal levels), the quantity and consistency of vaginal fluid, sexual activity, douching behavior, and inflammation. These interact with factors such as molecular weight, lipophilicity, ionisation, dose volume, which are not always predictable in their effects in connection with drugs. In vitro permeating through rabbit vaginal mucosa does not necessarily predict in vivo when using a particular drug [16].

### 2.3 Why pick the vaginal route,

The self-evident sense of the route makes it immediately apparent that local treatment of the vaginal infection would be performed by a route that makes obvious sense: Drug at the site of infection, minimal systemic exposure, lower dose and fewer side effects [17]. Another advantage of the vaginal other than avoiding the first pass metabolism in the liver, which is beneficial for delivering certain peptide hormones or progestins systemically. There are some negative points, such as cultural acceptability issues, variability in hormone levels, leakage, and use disorders when menstruating. Good compliance is a common problem with clinical studies -- particularly with multi-day courses [18].

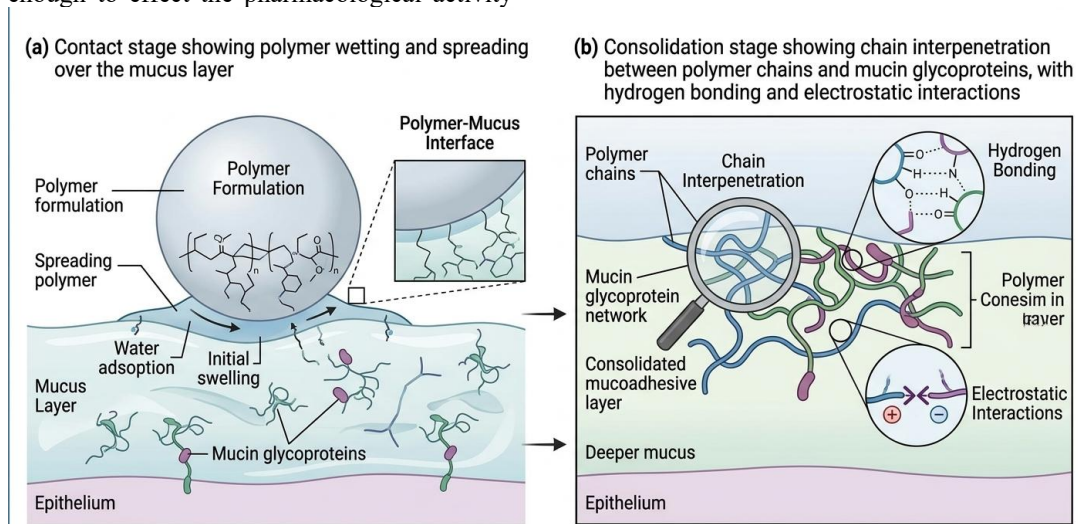
### III. Mcqueadhesive Drug Delivery Systems

#### The notion of mucoadhesion.

Mucoadhesion, on the most basic level, refers to the sticking to the mucus or mucus-coated epithelial surface of a synthetic or natural polymer long enough to effect the pharmacological activity

of the therapeutic agent. The mucus produced on the surface of the vaginal epitel is targeted mainly to mucin glycoproteins (glycoproteins with long chains bound to glycoside-forming glucose molecules also known as sialosides), a family of glycoproteins rich with sialic acid and sulphate structure and negative charge at physiological pH. Polymers with complementary functional groups (such as carboxylates and hydroxyls or amines) can interact with these residues via hydrogen bonding and/or electrostatic attraction and/or hydrophobic interactions or combinations of these.

Mucoadhesion can be said to occur in two general phases, from a process perspective. The first one is the contact step — when the polymer becomes in contact with the mucus surface, thereby positioning itself on it. The second stage is called the consolidation stage and consists of physicochemical interactions between polymer and mucus, passage of water from polymer to mucus or vice versa, and interpenetration of polymer chains and mucus. The bond formed is influenced by molecular weight of the polymer (higher, the more bonding, up to a maximum of approximately 200 kDa), polymer concentration, degree of hydration, contact time and application pressure during polymer application. With in situ vaginal gels, the stage isn't as important as it tends to be when making gels for the mouth, since it is already a gel that is already wet; the consolidation is more important as it determines the length of time the gel will remain in place.



**Figure 2.** Schematic of the two-stage mucoadhesion process: (a) contact stage showing polymer wetting and spreading over the mucus layer, and (b) consolidation stage showing chain interpenetration between polymer chains and mucin glycoproteins, with hydrogen bonding and electrostatic interactions.

**Table 2.** Advantages and limitations of the vaginal route for drug delivery

Advantages	Limitations
Avoids first-pass hepatic metabolism	Cultural and personal acceptability concerns in some populations
Local drug action with minimal systemic exposure	Drug leakage and messiness with conventional dosage forms
Large surface area through rugae increases absorption	Hormonal variations alter epithelial thickness and absorption
Self-administrable; no needles, no swallowing required	Not suitable during menstruation in many cases
Suitable for sustained local delivery of antimicrobials	Inter-patient variability in vaginal pH and flora

### 3.2 Theories of mucoadhesion

Over the years several theories have been suggested, none of which alone accounts for the entire phenomena observed in experiments. The wetting theory states that mucoadhesion can be considered as a surface energy issue; the polymer with low contact angle and high adhesion spreading on a mucus surface. The diffusion theory suggests that polymer chains and mucin chains intertwine over the interface (tangled mesh) whose strength depends on the chain mobility as well as the depth of penetration. The adsorption theory involves secondary forces, such as hydrogen bonding, van der Waals (vdW) forces between the two surfaces. The fracture and electronic theories explain what happens at the moment of detachment and the importance of charges transfer, respectively [21]. In practice, multiple mechanisms are functioning but the one that prevails depends on the length of time the polymer contacts the hydration, the polymer, and the hydration's state.

### 3.3 Mucoadhesive polymers used in vaginal delivery

There are two broad categories of polymers which are natural and synthetic. Chitosan is likely the most researched of all natural polymers. It mainly contains amine groups which turn into positively charged groups at the acidic pH of the vagina and attach to mucin with high positive charge. Chitosan shows intrinsic antibacterial activity, and has been demonstrated to be biocompatible with vagina tissue up to 2% w/v [22,23]. Other natural agents include sodium alginate, and xanthan, which may also be used as gelling agents in ion triggered systems.

An under-emphasized problem with the natural polymers is their batch variation. The mucoadhesive properties of chitosan derived from various shellfish and varying degree of deacetylation may differ significantly. The molecular weight of xanthan gum is dependent on the supplier and fermentation batch. This variability is acceptable for some preliminary formulation, and on commercial scale-up it can become an annoyance; when it happens, many companies will switch to cheaper-to-market the "synthetic" roots, and even the packing ratio may suffer due to the marketing price tag of the 'natural' label.

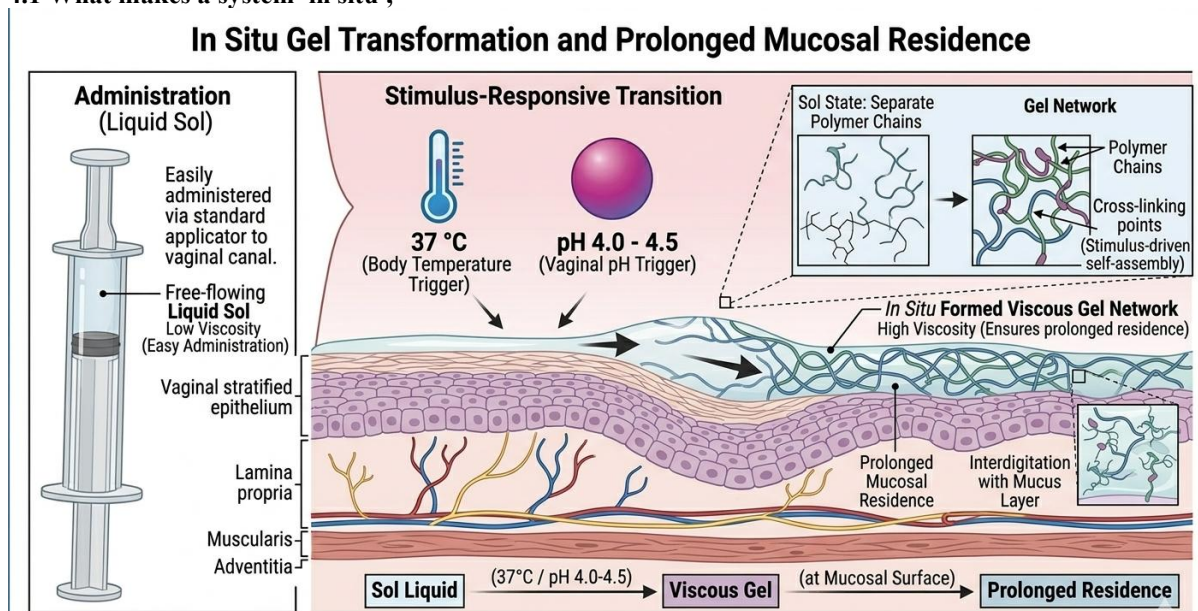
It is, however, synthetic polymers that dominate the published literature of vaginal in situ polymers. Carbopol 934 and 974P are anionic, poly(acrylic acid) derivatives that gel quickly when neutralised. They have carboxyl groups that are capable of hydrogen bonding with mucin sialic acid, and an appropriate swelling property at the pH of the vagina is beneficial. A related polymer, polycarbophil, has been well-known for some time and has been in use as a component of over-the-counter (nonprescription) vaginal lubricants, such as Replens. The addition of hydroxypropyl methylcellulose (HPMC) especially K4M and K15M also results in bulk viscosity and contributes to mucoadhesion by hydrogen bonding [24,25]. The majority of successful formulas thus far published have two polymers: a gelling material such as Pluronic F127 or Carbopol, which is responsible for switching from sol to gel; and a mucoadhesive such as HPMC or chitosan, which is responsible for retention.

**Table 3.** Common mucoadhesive polymers used in vaginal in situ gel formulations

Polymer	Type	Charge at vaginal pH	Primary role
Carbopol 934	Synthetic, polyacrylic acid	Anionic	Gelation (pH-triggered) and mucoadhesion
HPMC K4M	Semi-synthetic cellulose ether	Neutral	Viscosity modifier and mucoadhesion
Chitosan	Natural polysaccharide	Cationic	Mucoadhesion, mild antibacterial activity
Sodium alginate	Natural polysaccharide	Anionic	Ion-triggered gelation
Pluronic F127	Synthetic block copolymer	Neutral	Thermo-triggered gelation
Polycarbophil	Synthetic polyacrylic acid	Anionic	Long-residence mucoadhesion

#### IV. In Situ Gel Technology

##### 4.1 What makes a system 'in situ',



**Figure 3.** Conceptual illustration of in situ gel transformation. The free-flowing liquid (sol) transitions into a viscous gel network upon exposure to vaginal pH (4.0–4.5) or body temperature (37 °C), allowing easy administration through an applicator while ensuring prolonged residence at the mucosal surface.

In situ gels are liquids which remain liquid (clear and low viscosity) until they come into contact with the physiological environment. The trigger may be change in pH, rise in temperature, presence of certain ions or a combination of these. This is mainly a pragmatic benefit, describing the accurate dosage – namely there is less product formulation to squeeze into a syringe, a more uniform distribution

on the mucosa despite the gel dispersion and the patient's general comfort is enhanced due to the lack of thick paste to push around [26,27].

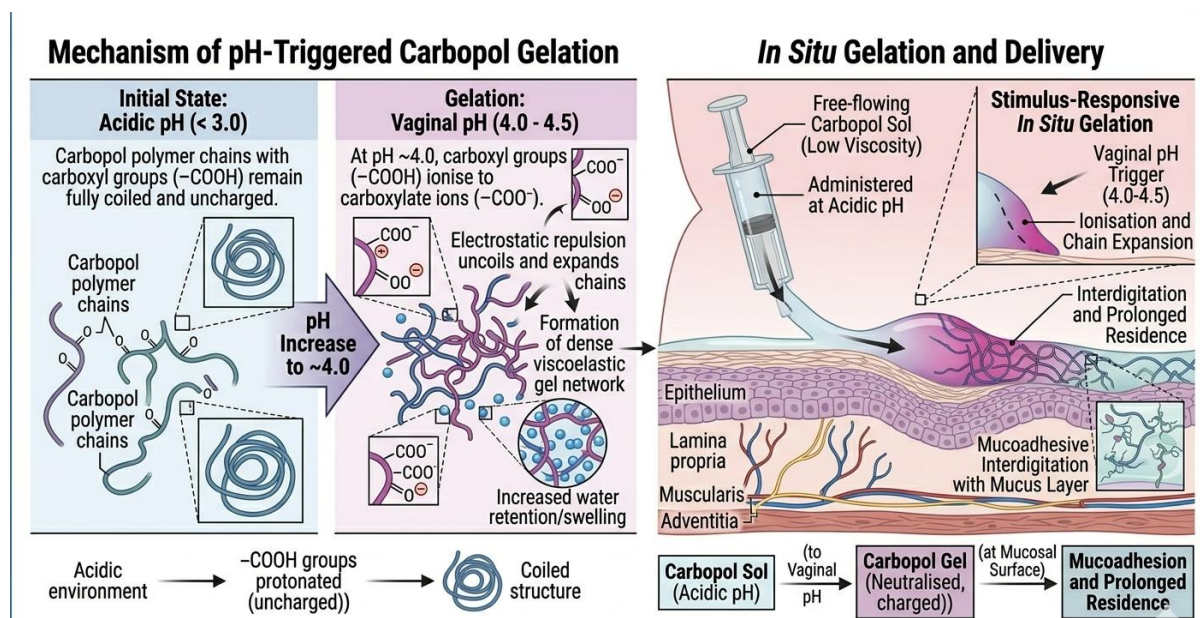
#### 4.2 Trigger systems

##### 4.2.1 pH-triggered systems

The typical one is based on a carbopol. When the pH drops below the critical value (around pH 3 for

Carbopol 934) the polymer chains remain coiled, and the solution becomes a runny liquid. Carboxyl groups become dissociated, the chains repel electrostatically, network expanded around and gels. If the formulation is for vaginal application it is

typically designed to become "gel chromatized" at the vaginal pH level of the vagina (approx 4.0-4.5). In most instances HPMC is not used as a gelling agent but instead is used to control the viscosity and to impart "mucoadhesion" [28].



**Figure 4.** Mechanism of pH-triggered gelation of Carbopol. At low pH, polymer chains remain coiled and uncharged. As the pH approaches the vaginal range (4.0–4.5), carboxyl groups ionise, chains repel each other electrostatically, the network swells, and a viscoelastic gel forms.

#### 4.2.2 Thermo-triggered systems

This is where the workhorse Pluronic F127 (poloxamer 407) comes in handy. It exhibits reverse thermal gelation (liquid at room temperature and gel at body temperature) above a certain concentration of ~18% w/w. The mechanism is heating of the compound causes a change in the central polypropylene oxide block from its disordered liquid state at lower temperature into its cubic crystalline lattice structure. Formulations composed of Pluronics in combination with any bioadhesive polymers such as HPMC or Carbopol are able to gel rapidly and maintain in place as a good gel [29,30].

#### 4.2.3 Ion-triggered systems

Less popular for vaginal use than the above two but applicable. However, gellan gum, sodium alginate and pectin can all form gels in the presence of divalent cations, primarily calcium ( $\text{Ca}^{2+}$ ). It's worth noting that there are calcium ions in the vagina, but

these are present at low levels and so are not often employed on their own and are instead used in addition to pH or temperature triggers [31].

#### 4.3 Polymers used in in situ gel construction

Other compositions published consist of a secondary mucoadhesive polymer and one or more solubility enhancing polymers, with the exception of the trigger polymer. A thermosensitive end is protected by Pluronic F127 and F68. Carbopol 934, 940 and 974P are pH-sensitive systems. Chitosan can not only be used as mucoadhesive but also as pH-sensitive due to its ability to dissolve when it has an acidic pH and precipitate when it has an alkaline pH. PEG 400, propylene glycol, ethanol and glycerol are frequently used as cosolvents to dissolve poorly water-soluble drug molecules, such as quercetin [32,33].

#### 4.4 Advantages and limitations

**Table 4.** Comparison of *in situ* gel trigger systems for vaginal delivery

Trigger	Representative polymer	Strengths	Limitations
pH	Carbopol 934, chitosan	Predictable gelation; well-documented	Sensitive to vaginal pH variation in disease states
Temperature	Pluronic F127, F68	Rapid gelation at body temp; large literature base	Weak gel strength alone; needs co-polymer
Ionic	Gellan gum, sodium alginate	Useful in lacrimal and oral systems	Ion concentration in vaginal fluid is modest
Combined (pH + thermo)	Pluronic + HPMC + Carbopol	Robust gelation across patient variability	More complex optimisation

Minus: disadvantages of use are "difficult administration" and "short duration of residence. Minuses: relatively complex formulation, sensitivity to minor changes in polymer concentrations, requirement of tight pH control during production at some part, occasional irritation by deviations from the formulation's pH or ionic strength during the production and loading of the drug by some hydrophobic active ingredient, limited loading capacity of drug by some hydrophobic drug [34].

## V. Quercetin as a Therapeutic Candidate.

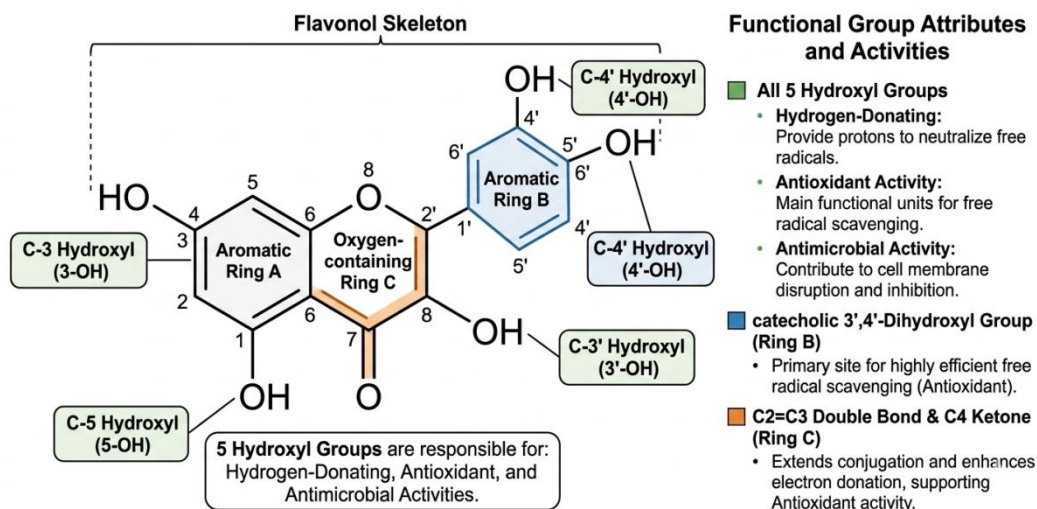
### 5.1 Origin and chemistry

Another of the more common flavonols in the human diet is quercetin (3,3',4',5,7-pentahydroxyflavone). The highest amounts, usually 200–300 mg/kg of dry weight, with high levels present as glucoside conjugates, occur in onion (particularly in red and yellow varieties) [35]. Other important sources include capers, lovAGE, dill, apples, broccoli, and some of the berries. The chemical structure of quercetin is shown as two

aromatic rings (A and B) linked with an oxygen-containing pyran structure (C). Biologically, these three rings bear five hydroxyl groups, which confer most of its biological activity especially its hydrogen donating, free radical scavenging activity [36].

The occurrence of quercetin in the free form is nearly imperceptible in dietary plants. It can be detected in the glycoside form: quercetin-3-O-glucoside (isoquercitrin), quercetin-3-O-rutinoside (rutin), quercetin-3-O-rhamnoside (quercitrin) and others. It makes water solubility a bit more substantial and alters the absorption profile because the sugar has to be cleaved (intestinal  $\beta$ -glucosidases or the cultured bacteria in the gut) first for the aglycone to be absorbed. For pharmaceutical formulation though, the aglycone is more commonly involved as that is the form in which pharmacological active component is known and the commercially available raw aglycone.

### Detailed Chemical Structure and Functional Attributes of Quercetin



**Figure 5.** Chemical structure of quercetin showing the flavonol skeleton with two aromatic rings (A and B) connected by an oxygen-containing pyran ring (C). Five hydroxyl groups (at positions 3, 5, 7, 3', and 4') are responsible for the molecule's hydrogen-donating, antioxidant, and antimicrobial activities.

### 5.2 Physicochemical properties

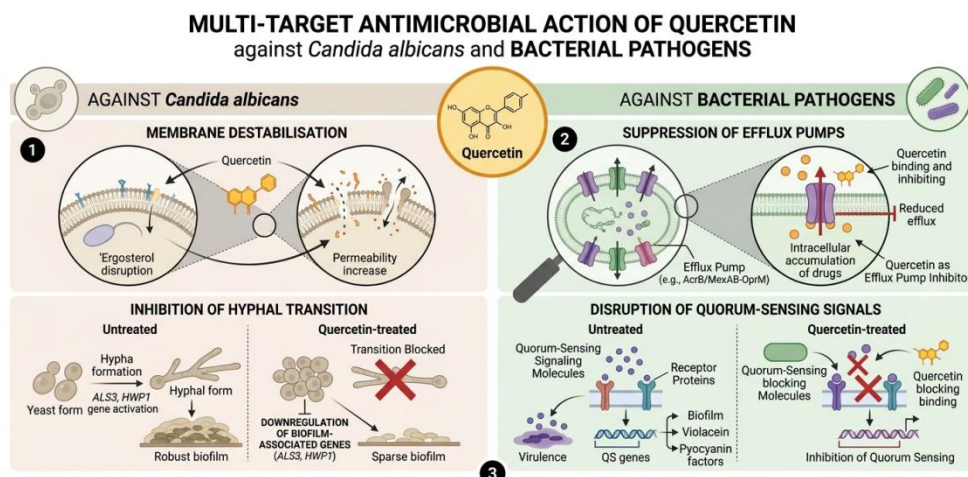
Quercetin is a yellow crystalline powder, which melts around 316 °C with decomposition. It is practically insoluble in water (1 µg/mL at 25 °C), slightly soluble in alcohol, and soluble in glacial acetic acid and in dimethyl sulfoxide. It has a log P value of approximately 1.5-1.8, which is considered as moderate lipophilicity. The ionisation behaviour is complex because it can ionise around six regions involving the hydroxyl function (Ka1 ~ 6.4, Ka2 ~ 8.0, Ka3 ~ 10.1, higher) and thus in the vaginal pH (4.0-4.5) it is almost completely un-ionised and lipophilic [37]. This is a paradox: a problem for aqueous systems of gels but it is a good thing for membrane permeation when dissolved.

### 5.3 Pharmacological activities relevant to Physiology and Biochemistry.

Quercetin himself has a huge pharmacological profile... it's almost outrageously extensive!... but a few of its activities are germane to the treatment of vaginal infections. It has been studied for its antifungal activity against *Candida* species and MIC values were identified to range from 16–256 µg/mL depending on the species and

method of testing [38, 39]. More important, quercetin (and nature) targets *Candida* biofilms (factors that mediate recurrent disease and render other antifungals ineffective). Mechanisms proposed comprise hyphal transition inhibition, down-regulation of genes involved in the synthesis of extracellular matrix, and down-regulation of genes involved in biofilm formation e.g. ALS3 and HWP1 [40].

The antibacterial activity too is very wide. Owing to its multiple modes of action, quercetin has antibacterial properties against *E. coli*, *S. aureus*, *P. aeruginosa* and *S. mutans* bacteria, with the five mechanisms listed above [41,42]. They have anti-inflammatory activity because they inhibit NF-κB signalling pathway, pro-inflammatory cytokines (such as IL-6, TNF-α, and IL-1β) and cyclooxygenase-2 (COX-2) and lipoxygenase enzymes [43]. Thus, the antioxidant effect (direct-radical scavenging, as well as the induction of the cellular antioxidant mechanisms (direct-antioxidative enzymes) through Nrf2- mechanism, provides an effective tissue-protective mechanism in inflamed mucosa.



**Figure 6.** Multi-target antimicrobial action of quercetin against *Candida albicans* and bacterial pathogens: membrane destabilisation, inhibition of hyphal transition, downregulation of biofilm-associated genes (*ALS3*, *HWP1*), suppression of efflux pumps, and disruption of quorum-sensing signals.

#### 5.4 Bioavailability problem

The oral bioavailability of quercetin is extremely low, as shown by some human studies (less than 2%). It is now clearly established that these are the reason: such poor solubility is a result of extensive first-pass metabolism (glucuronidation and sulphation); efflux by P-glycoprotein causes rapid elimination; and a plasma half-life of approximately 2 hours. Most of these issues are avoided in topical/ local delivery of the active agent, except for solubility being the rate limiting step. Microcrystalline cellulose would require some type of solubility enhancement to obtain inhibitory concentrations in the vaginal fluid, if it is to be used in a hydrogel matrix [44].

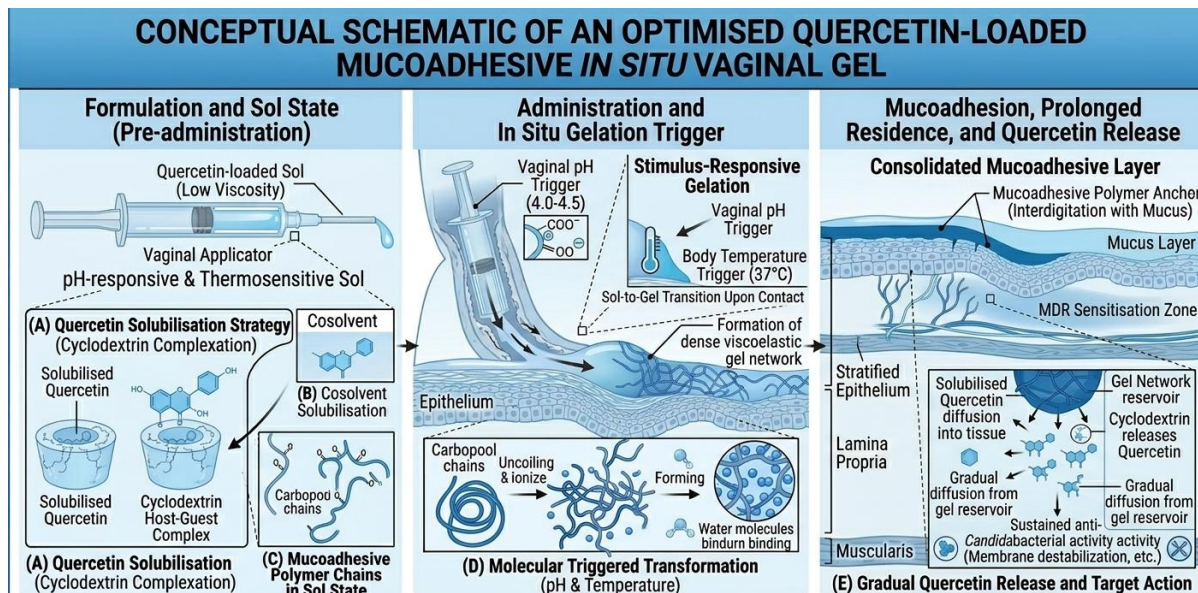
There are ways to enhance the delivery of quercetin. There are approaches which can improve the delivery of quercetin.

Numerous attempts have been made to solve the solubility issue. The most easy way and provides some improvement has been by using cosolvents (PEG 400, propylene glycol, ethanol, or DMSO). Some advanced methods are solid dispersions (with carriers PVP K30 or Solu-plus), cyclodextrin inclusion complexes (both with  $\beta$ -cyclodextrin and hydroxypropyl- $\beta$ -cyclodextrin are effective), liposomes, niosomes, nanoemulsions, polymeric nanoparticles, and self-emulsifying drug delivery systems [45, 46]. Cyclodextrin complexation is especially notable for use in a vaginal application since not only is the dissolution rate enhanced, but it also renders any potential irritation irreversible.

**Table 5.** Selected pharmacological activities of quercetin relevant to vaginal infection

Activity	Mechanism	Target organism / pathway
Antifungal	Inhibition of hyphal transition, biofilm matrix synthesis	<i>Candida albicans</i> , <i>C. glabrata</i>
Antibacterial	Membrane destabilisation, DNA gyrase inhibition	<i>E. coli</i> , <i>S. aureus</i> , <i>G. vaginalis</i>
Antibiofilm	Downregulation of <i>ALS3</i> and <i>HWP1</i> genes	<i>Candida</i> biofilms, polymicrobial biofilms
Anti-inflammatory	NF- $\kappa$ B inhibition, COX-2 / LOX suppression	TNF- $\alpha$ , IL-6, IL-1 $\beta$
Antioxidant	Direct radical scavenging, Nrf2 induction	ROS, oxidative tissue damage

### 5.5 Using Mucoadhesive In Situ Vaginal Gels (ISVGs) With Quercetin



**Figure 7.** Conceptual schematic of an optimised quercetin-loaded mucoadhesive in situ vaginal gel. The formulation administered as a sol via vaginal applicator transitions to a gel on contact with vaginal pH and temperature. Mucoadhesive polymers anchor the gel to vaginal mucosa, while quercetin (solubilised through cosolvent or cyclodextrin complexation) diffuses gradually into the surrounding tissue.

It seems simple enough on the surface level – a drug that can treat a wide variety of microbes combined with a delivery system that keeps the drug trapped at the site of infection for hours. In reality, it is a more complicated situation. However, due to its solubility and stability issues, Quercetin doesn't sit pretty in a Carbopol gel. The polymer matrix needs to play three roles: gel formation, mucoadhesion, and solubility maintenance, all of which should not be too fast or too slow, or too much and too little drug release, burst and subtherapeutic release, respectively [47]. It has been reported in a number of formulations that the right proportions of polymers can produce either a useable product, or one of little value.

#### 6.1 Role of Carbopol and HPMC

The combination of HPMC K4M and Carbopol 934 is most commonly used in in situ vaginal gels, containing PEG 400 or ethanol as cosolvents. Carbopol solutions of 0.3-0.5% weight per volume (w/v) are usually sufficient to achieve an adequate gel strength without excessive difficulty of administration. Typically the concentration of HPMC is in the range of 0.5% - 1.5% w/v; if below this, mucoadhesion is minimal, and if above this concentration, then the formulation is so viscous that it will not be syringeable into a vaginal

applicator. It is loaded up to 0.5% w/v (25 to 50 mg/day/an application) of quercetin far before the level that has been reported as toxic to the vagina mucosa [48,49].

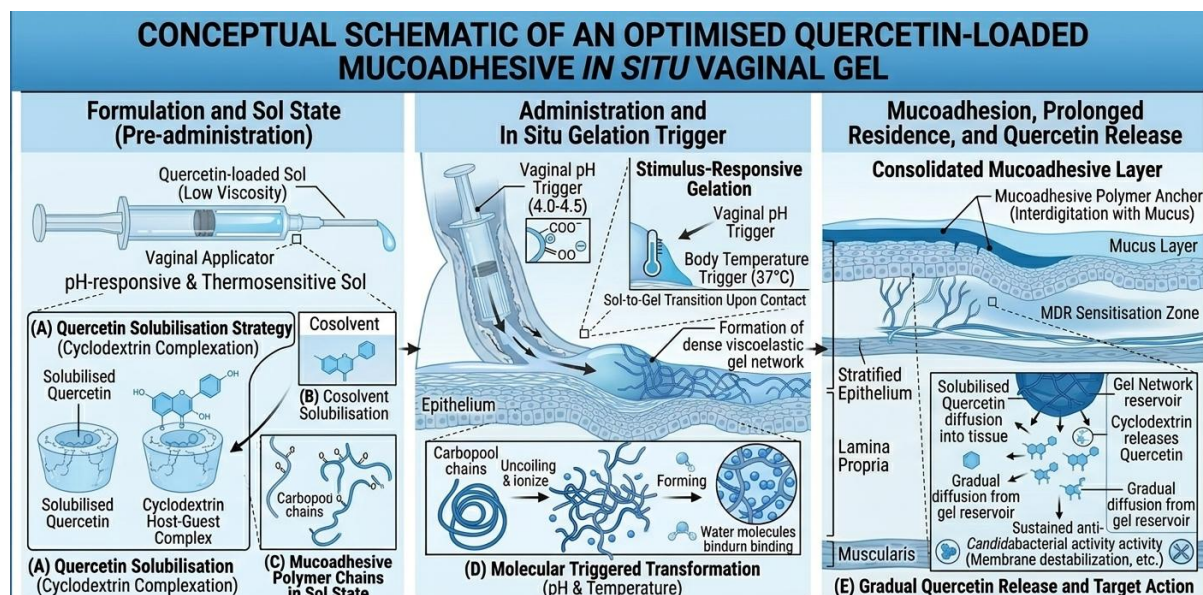
#### 6.2 Solubility enhancement strategies in the literature.6.5 Conclusions.

There are three common strategies to appear. First, solvent combinations consisting of PEG 400 (5–10%), propylene glycol (5–10%) and ethanol (5–15%). They are inexpensive, as well as legislative compliant, and provide boosts of solubility from 10-fold to 50-fold, generally adequate. Secondly, by complexation with hydroxypropyl-β-cyclodextrin (CD), the apparent solubility of quercetin is increased 200- to 500-fold and the drug can also be stabilized against oxidation at a quercetin-to-CD molar ratio of 1:1 or 1:2 [50]. Finally, Loading of the nanocarriers such as liposomes, niosomes, polymeric nanoparticles or solid lipid nanoparticles into the gel matrix. The third method provides the most promising in-vitro results but has an added manufacturing complexity, regulatory concerns and added costs.

#### 6.3 Drug release profile

The majority of in situ gels containing quercetin have experienced a biphasic drug release pattern, with small initial release (10–25% within 1 hour) and a prolonged release, which is maintained for 8 to 24 hours. The initial phase is considered to be due to drug presence at the gel surface or loosely bound to polymer and the sustained phase is

considered to be related to the drug diffusion in the swollen polymer network. It is noted that release kinetics fitted the Korsmeyer–Peppas with diffusion exponents ranging from 0.45 to 0.89, corresponding to that of non-Fickian (anomalous) transport, that is, release due to both diffusion and polymer relaxation [51].



**Figure 8.** Representative cumulative drug release profile of quercetin from an optimised mucoadhesive in situ vaginal gel in simulated vaginal fluid (pH 4.2, 37 °C), showing the characteristic biphasic pattern with initial burst ( $\leq 1$  h) followed by sustained release over 24 hours. The release behaviour fits the Korsmeyer–Peppas model with  $n$  in the range 0.45–0.89, indicating non-Fickian transport.

### 6.5 Mucoadhesion, retention in the vagina

The detachment forces of optimised quercetin gels are generally in the range of 4,000–10,000 dynes/cm<sup>2</sup>, while the retention time of these gels against simulated vaginal conditions is in the range of 6–10 hours. This is a good point higher than the one to two-hour-memory quoted for the market

creams. The in vivo presence-time relationship is confirmed in rats and rabbits, with gamma scintigraphy data obtained by in situ gel studies of non-quercetin demonstrating that 80% of the dose present at 4 hrs and approximately 40% were present at 8 hrs [52].

**Table 7.** Selected reported quercetin and flavonoid-loaded mucoadhesive vaginal/topical gels

Active ingredient	Polymer system	Trigger	Key outcome
Quercetin	Carbopol 934 + HPMC K4M + PEG 400	pH	Sustained release over 12 h; high mucoadhesion; reduced <i>C. albicans</i> CFU
Quercetin nanoparticles	Pluronic F127 + chitosan	Thermo + pH	Improved tissue penetration; sustained release > 24 h
Quercetin–HP-β-CD complex	Carbopol 974P + HPMC	pH	Solubility enhancement ~ 200-fold; biofilm disruption
Curcumin (comparator)	Pluronic F127 +	Thermo +	Demonstrated antifungal

flavonoid)	Carbopol	pH	activity in animal model
Resveratrol	HPMC + Carbopol	pH	Antioxidant activity preserved over storage

### 6.6 Antimicrobial performance

In an in situ gel form, quercetin has been found to result in 1- to 2-log reduction in *C. albicans* CFU on Sabouraud dextrose agar after 24 hours compared to conventional quercetin solution in similar comparative analyses with products [53]. These have been reported to be similar for *E. coli*

and *G. vaginalis* with a smaller data set. However, the biofilm-disrupting effect of quercetin seems to be maintained when applied via these gels, which is most clinically relevant, since biofilm associated recurrence is the primary reason that VVC is still returning [54].

**Table 6.** Summary of evaluation parameters and acceptable specifications for vaginal in situ gels

Parameter	Method	Acceptable range
Appearance	Visual inspection	Clear to slightly hazy yellow gel; no aggregates
pH	Calibrated pH meter	4.0 – 5.5
Sol viscosity	Brookfield, low shear	< 100 cP
Gel viscosity	Brookfield, post gelation	10,000 – 50,000 cP
Gelation time (37 °C)	Visual / tube tilt	< 60 s
Mucoadhesive strength	Texture analyser / balance	> 4,000 dynes/cm <sup>2</sup>
Drug content	HPLC / UV	95 – 105% of label claim
In vitro release (24 h)	Franz cell / dialysis	70 – 95% cumulative
Stability (6 months, 40 °C/75% RH)	ICH Q1A(R2)	Drug content within 10%

### Evaluation Parameters Related to Vaginal In Situ Gels 7.

Despite some variations on the protocols used, the published studies align themselves on about a standard set of tests. The following list presents some of the expectations which most pharmacopoeial and journal reviewers will have.

#### 7.1 Influence of visual and organoleptic assessment.

Ingenuity's color, transparency, and smell, and its uniformity. This gel is yellow to greenish-yellow when it has been completed by quercetin concentration. Cloudiness, phase separation or particle aggregation is a red flag and is typically a result of the lack of cosolvent or incompatibility of drug:polymers.

#### 7.2 pH determination

The normal vaginal pH is 3.8-4.5 and any product with a pH that is different from this may be a source

of irritation or alter the resident vaginal flora. In the literature, a calibrated digital pH-meter was used and its formulation pH range was set to be 4.2-5.0 prior to gelation and 5.0-5.5 following gelation in simulated vaginal fluid (SVF) [55].

#### 7.3 Rheological behaviour

The viscosity can be measured at various shear rates using the Brookfield-type viscometer (Cone and Plate or spindle) viscometers. The two most important are sol-state viscosity (where a low (preferably less than 100 cP) formulation flows through the applicator) and gel-state viscosity (where a high (preferably between 10,000 and 50,000 cP) formulation remains in place). The gelling time at 37°C should be less than 60 seconds, if you will have a longer time formulation may be leaking before it sets.

#### 7.4 Gel strength

It is quantitative and determined from the time it takes for a fixed weight (conventionally 50 g) to penetrate 5 cm and into the gel. Typically, the time range of 25 to 50 seconds is expected to represent good cohesion, and a short time, to mean the movement has occurred with sufficient shear to ease the cement's bond to the surface. Typically, in that range of 25 to 50 seconds, the cement has good cohesion, and because it is short, the bond to the surface has been broken by sufficient shear related to the movement.

### 7.5 Spreadability

Spreadability is measured by SLIP AND DRAG ON A GLASS SIDE plate and provides a quantitative figure. The higher, the more the surface of the vagina that will be covered when the same amount of force is applied, meaning there will be more even coverage than with a lower.

### 7.6 Mucoadhesive strength

Methods such as texture analyzer or modified balance methods. Excised vaginal mucosa (usually goat or sheep) is mounted, the gel is applied and then the force required to lift the gel is determined. Usually a values above 4,000 dynes/cm<sup>2</sup> are necessary for obtaining a useable retention time.

### 7.7 Drug Release studies

Samples are taken at several time points (0.5, 1, 2, 4, 6, 8, 12, 24 hours) and analysed using HPLC or UV spectroscopy at 37 °C in either a Franz diffusion cell or a dialysis membrane. Cumulative release is fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models for determination of a predominant release mechanism [57].

Down the road it is worth considering the composition of simulated vaginal fluids (SVF). In 1999, Owen and Katz released this very popular recipe for SVF composition (which was later adopted by most studies done since that time). The buffer mimics the electrolyte composition, pH and ionic strength of physiological vaginal fluids, and the amount of bovine serum albumin (BSA) (approx 0.018%) is sufficient to mimic the protein content. Tests for the dissolution of drugs that exhibit pH sensitive solubility (e.g., quercetin) are performed at multiple pH values and the last pH represents a value that represents BV-affected pH for a more representative picture of what the formulation will do in various patient subgroups.

### 7.8 Antimicrobial studies

Reference cultures of *Candida albicans* (ATCC 10231 or 90028) and *E. coli* (ATCC 25922) on cup plate or agar "well" diffusion. The zone of inhibition is measured after 24 and 48 hours. Time-kill kinetics provide more clinically relevant data and better use of newer publications has increased in years [58].

### 7.9 Stability studies

ICH Q1A(R2) guidelines define accelerated (40 °C / 75% RH) and long-term (25 °C / 60% RH) conditions. Formulations are usually stored for between three and six months with samples withdrawn at 0, 1, 3 and 6 months for assay (HPLC), pH, viscosity and mucoadhesion testing. Generally any drug content drop more than 10% over the test period is deemed as a formulation failure [59].

### 7.10 Optimization and Quality by Design

The newer approach to formulation development of an in situ gel for the vagina is to use the Quality by Design (QbD) approach instead of one factor at a time. The typical standard workflow follows a template of defining a Quality Target Product Profile (QTPP), Critical Quality Attributes (CQAs) (generally, gelation pH, gelation time, mucoadhesive strength, drug release and viscosity), and then mapping the interaction between formulation variables (Critical Material Attributes) and process variables. Typical methods used to model such relationships and to determine the design space of consistent formulation that satisfies specifications are statistical designs, including the Box–Behnken, central composite, and D-optimal designs.

For quercetin Carbopol/HPMC system, three formulation variables are generally major (Carbopol concentration (0.2–0.6% w/v); HPMC concentration (0.5–2.0% w/v); and cosolvent percentage (5–20% v/v of PEG 400 or its combination with propylene glycol). A Box–Behnken design with these as independent variables equally yields response surface plots, which show the interaction effect, for the responses: gelation time, mucoadhesion and 12 h cumulative release. In several published studies with the quercetin gel, higher drug loading was achieved with a higher concentration of Carbopol which also revealed the most non-linear trend between concentration vs. increase in mucoadhesion, whereas higher drug loading was also observed with increasing concentration of HPMC, with a linear trend. The rules take, verbatim, from these results are: Keep within the range of 0.3 – 0.45% Carbopol (carboxymethyl cellulose) use between 0.8 and

1.2% HPMC (hydroxypropylmethyl cellulose) and use 8–12% PEG 400 (polyethylene glycol) and the formulation will almost universally be within the target specifications.

## 8. Challenges in Future

### 8.1 Patient compliance

Even the most well planned vaginal gel won't work if patients do not use it. Multi day antifungal regimens consistently show 50%-70% adherence and in principal a long acting in situ gel could increase that due to reducing dosing frequency. What is catch, Vaginal route acceptability is culture (and partner) dependent. Counselling or patient education are equally important as formulation.

### 8.2 Vaginal irritation and biocompatibility are discussed.

#### Vaginal irritation and biocompatibility

In the literature information about slug mucosal irritation tests is available, together with reports of rabbits vaginal irritation studies, and all most published up to now report limited or mild irritation (Draize score from 0-1) of the mucous membranes of the slugs' mouth when using quercetin gels. Various studies are encountered in the literature for the use of quercetin gels in the slug mucosal irritation tests and the rabbits vaginal irritation tests, most of them showing no or only mild irritation (Draize score from 0-1) of the mucous membranes of both the slugs' mouth and the rabbit's vagina. Long-term (local) toxicity testing is however not yet well-conceived. Lactobacillary populations should also be of greater interest. Ironically, disruption of the flora of the soil may invite other infections [60].

### 8.3 Scale-up and manufacturing

Formulations, in a lab setting, do not directly and exactly scale to manufacturing. One problem is sterilisation: terminal autoclaving can cause alteration in the structure of the Carbopol or affect the quercetin content, and in the case of viscous polymeric solutions, filtration sterilisation is difficult. The typical solution is to use aseptic processing which is an added expense. Engineering issues that must be addressed before commercial scaleup to meet these requirements include polymer reproduced without change, batch-to-batch viscosity consistency and packaging to ensure 24-month drug stability.

### 8.4 Regulatory considerations

Most regulators classify vaginal in situ gels as topical drug products, with regards to in situ

formulations, the advice is split. For drug release testing, S.V.F. is needed and validated S.V.F. and Franz cell methodology is required, to achieve physiological conditions. The added interfaces to the phytopharmaceutical-based systems are reproducibility of bioactivity of the phytopharmaceuticals from batch to batch and the identification of marker compounds in the plant extract as well as standardization of the phytopharmaceutical extract.

### 8.5 Where the field is going

The three bright spots seem to be trends. Nanocarrier-loaded in situ gels formed by liposomes, niosomes and lipid nanoparticles in a gel matrix provide better solubility, controlled release and better penetration of tissues. Second, the use of dual-active drug combinations, that is, formulations consisting of a conventional antifungal agent (clotrimazole or fluconazole) used in sub-MICs associated with quercetin that, thanks to the synergy, limit the development of resistance. Third, the ability of smart polymers to sense inflammation-specific signals, such as the presented rise in the levels of cathepsin and release a drug only during the inflammatory process. All of these are still not clinically available but early results are promising.

Another, lesser-used direction would be the integration of probiotic strategies within the formulation of vaginal gels, which is likely to be equally as significant. The first step in preventing recurrence is to restore the lactobacillary population, a process to which several research groups are devoting their focus, with the addition of one effective pathogen-killing strain to form a combination product that does both. Keeping the bacteria viable during the manufacturing process, from packaging through storage is a challenge, but this usage makes sense enough from a clinical perspective. Relevance is not only doubled for quercetin, it doesn't seem to inhibit lactobacilli at therapeutic levels and it was reported by some studies to promote the growth of lactobacilli making co-formulation possible.

## 9. Conclusion

Therefore, mucoadhesive in situ vaginal gels can be used to address a very pertinent and long-standing problem: maintaining an active drug at the vaginal mucosa for extended periods of time without causing the patient "agony" in the process. Now the polymer chemistry is well understood, the means of evaluating is reasonably standardised and the clinical need, recurrent vaginal infections,

antimicrobial resistance, low compliance on conventional therapy, is not any different. With this extensive antimicrobial activity, biofilm-disrupting ability, anti-inflammatory and antioxidant activity, quercetin is an ideal fit for this delivery system. It is not a real solubility or stability problem, but can be solved by using the appropriate cosolvents or by complexation or nanocarrier approach. The field is in need of solid clinical validation data — side-by-side comparisons with marketed creams, more patients, a longer follow up period, and safety measures focused on microbiomes. From "proof of concept" to "good candidate," the next 10 years will determine if the platform can become a mainstay in routine operation.

As a research scientist, it's not been about finding new chemistry and biology, all of which have been known for decades; but it has been about creating new knowledge or creating old chemistry and biology with which to solve a clinical problem that people haven't solved properly yet. Whether or not patients would like to take a vaginal gel also depends on the gel's comfort, predictability and imperviousness to daily reminders. Those are formulation problems, and not biology problems. The right answers make for an archived paper, wrong answers make for a helping product.

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