**Killing Pathogens Where They Are: Inexpensive Formulation For Bacterial Vaginosis and Vulvo-Vagninal Candiasis Minimizing Systemic Exposure**

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**Background and aims.** Women, especially in Asia, suffer bacterial vaginosis (BV) and/or vulvovaginal candiasis (VC) silently due to socio-cultural factors affecting health-seeking behavior. There is an unmet need to address this problem with twin objectives of providing a formulation for discreet use, and ensuring unnecessary exposure of systemic circulation to antimicrobial agents.

**Methods.** With permission and under oversight of the Institutional Biosafety Committee, vide Permission Number CDRI-AM-24-155; *Gardnerella vaginalis* strain ATCC 14018 and *Candida albicans* strain ATCC 90028 were grown in petri dishes. The following composition of the gel was finalized: Clindamycin phosphate USP, 100 mg, voriconazole IP 500 mg, ethyl cellulose (EC) 45 cps, 1000 mg, acetone (processing solvent, lost to process, LR, 40 ml (31.428g), Carbopol940 1000 mg, purified water IP, qs. Phase-separation-coacervation under stirring for 30 minutes in a fume cupboard yielded microcapsules. G4elation was achieved by adding carbopol and water and mixing gently.

Target Quality Attributes (median particle size not less than 1μm, gel viscosity (*η*) between~7 mPa⋅s, and ~3 mPa⋅s were established using a Malvern Zetasizer and an Anton Parr MCR 72 rheometer respectively. HPLC analytical methods and LC-MS/MS bioanalytical methods were developed and validated according to relevant ICH guidelines. With permission and under oversight of the Institutional Animal Ethics Committee vide Permission Number IAEC/2024/139/Renew-0/Sr. No. 22 Dt. 18.11.2024, female rats (*N*=8) received intravaginal instillation of the formulated gel on Day 0. Blood samples were drawn at 0.5, 2, 4, 8, 12 and 24 hours in heparinized tubes and vaginal lavage was conducted at the same time. Samples were centrifuged immediately after collection and the supernatants stored at -80°C untill bioanalysis. After a washout and recovery period of two weeks, the same animals were randomly assigned to two groups of four. Both groups were instilled intra-vaginally with a suspension containing one million colony forming units (CFU) of each pathogen. One day later, and for five daily applications, the first group received intravaginal instillation of the formulated gel. The other group was treated similarly by the APIs incorporated into the same gel base, as comparator. CFU recovered by vaginal lavage on the seventh day after instillation were enumerated by plating and counting.

**Results.** Figure 1 (A, B) shows axenic cultures of *G. vaginalis* and *C. albicans*.



**Figure 1**: (**A**) *G. vaginalis.* (**B**) *C. albicans*. (**C**) PSD of optimized gel. (**D**) Effect of 10% deviation from process. The optimized process yielded a gel containing EC particles with a median size of 1 μm

The Herschel-Bulkley model: yeilded the curves shown in Figure 2. The value of yield stress(*τ*0) was 183.43 in respect of the formulated drugs, while this value was undefined in respect of with the gel prepared with unformulated drugs. The lack of fit of the latter indicates that the gel prepared with unformulated drugs may flow spontaneously, whereas the formulated gel would flow only under stress. The consistency index (*b*) in respect of formulated and unformulated gels were 2.51 and 102.95. The flow behaviour indices (*p*) for these non-Newtonian gels were 0.83 and 0.22 repectively. These values indicate that the formulated gel is superior in terms of consistency and flow (vaginal retention).



**Figure 3**: Flow curves of gels prepared with **A**: unprocessed and **B**: formulated drugs.

**Discussion.** Results of systemic pharmacokinetics and comparative efficacy are awaited and will be presented. It is anticipated that nano-pharmacy will not be deployed where it may not be necessary.

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