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# Heterogeneous polymer composite nanoparticles loaded *in situ* gel for controlled release intra-vaginal therapy of genital herpes

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ABSTRACT

Herpes simplex virus causes serious and contagious genital infections in high percentage of female population world-wide. Acyclovir is a clinically successful antiviral molecule till date, in-spite of limitations as poor solubility, low half-life, reduced oral bioavailability and side effects at higher doses. In the present work, controlled release *in situ* gelling system loaded with polymeric nanoparticles of acyclovir containing a dose of drug equivalent to 105 mg/day has been developed. The formulation containing drug loaded polyvinyl pyrrolidone–Eudragit RSPO hybrid polymeric nanoparticles (Size ~99 ± 3 nm, Zeta ~+26.1 ± 1.5 mV) in 15% Pluronic F-127 gel exhibited improved permeability through vaginal membrane ($K_P$ = 2.20 ± 0.19 × $10^{-6}$ cm/s). The nanoparticles showed enhanced viability for vaginal epithelial cell lines up to concentration of 100–250 μg/mL. The formulation was evaluated for bioavailability and biodistribution through intra-vaginal administration in rat models. The nanoparticle *in situ* gel formulation maintained an average therapeutic drug level of 0.6 ± 0.2 μg/mL in plasma for 24 h. Significant improvement in mean residence time of the drug (12.52 ± 1.12 h) was observed with a two-fold increase in the relative bioavailability ($AUC_{0-24\,h}$ = 14.92 ± 2.44 μg h/mL) compared to that of the pure drug (7.18 ± 1.79 μg h/mL). The tissue distribution was 2–3 folds higher in animals treated with nanoparticles *in situ* gel compared to that of pure drug. Sustained release of drug *in vivo* was demonstrated, ensuring the suitability of the formulation for clinical therapy in female population.



## 1. Introduction

Herpes simplex viruses (HSV) categorized as HSV-1 and HSV-2, are highly infectious and are predominantly transmitted by oral and sexual contacts, respectively. These viruses outbreak in the mucus tissues of oral, nasal, rectal and genital regions and could spread further. According to a WHO report, the total number of patients infected with HSV-1 virus in 2012 was estimated as 3709 million, which was approximately 67% of the world wide population (all age and both sex) [1]. The global HSV-2 virus infections in 2003 and 2012 have been assessed to be 506 million and 517 million respectively, wherein the percentage of female population affected was comparatively higher than the male in most of the countries including Africa, Europe and South-East Asia [2]. In United States, approximately 22% of the pregnant women have been reported with HSV-2 infections, and about 85% of perinatal transmission occurs during the intrapartum period [3].

HSV genital infections are particularly common in adolescents and adults, yet the risk of mother-to-child transmission occurs in pregnant women. Hence, neonatal HSV infections persist as a substantial reason for morbidity and mortality in this vulnerable population [4]. During the course of pregnancy both types of HSV infections may be transmitted to newborns, which cause lesions in eye or skin, spread of symptoms throughout body, meningo encephalitis and abnormalities of embryo [5]. The diagnosis and management of the infections in pregnant women is essential for preventing the risk of neonatal HSV infections [6]. Based on a long term case study conducted with large population in Taiwan, herpes zoster has been reported as an early and clinically detectable manifestation of undiagnosed HIV infections [7].

Recently, immunotherapy based HSV vaccines have been identified and are being investigated in phase II and phase III clinical trials. The preliminary results have shown 15–50% decline in shedding rate after the vaccine therapy [8]. However, specific anti-viral therapies are required to treat the symptoms, reduce the spreading of infections and as a prophylactic for preventing the reoccurrence of the disease for the same patients.

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Amongst the various anti-viral drugs, acyclovir (ACV) is one of the oldest versatile molecules used in the treatment of viral infections caused by herpes simplex virus, varicella zoster virus and hepatitis virus. Irrespective of the introduction of many pro-drugs and anti-viral remedies, ACV is recommended and well tolerated for clinical therapy successfully due to its significant activity in reducing the viral capacity and preventing the reoccurrence of the infections [9]. Yet, the slight aqueous solubility (varying based on pH and temperature) and lower permeability coefficient of $1.19 \times 10^{-6}$ cm/s are certain shortcomings and hence, many researchers have focused on different approaches to overcome these limitations. Higher dosing has shown saturation limited oral absorption mechanism and some side effects in gastro intestinal tract (GIT), nephrotoxicity and neurotoxicity. Also, the poor oral bioavailability (10–20%) and very short half-life (2.5–3.3 h) necessitate the need for a controlled release dosage form and alternative route of administration, to improve maximum potential of the drug. [10–12] In view of these, we had reported the development and *in vitro* characterization of controlled release *in situ* gel system loaded with polymeric nanoparticles of acyclovir in our previous works [9,16]. Two formulations were identified as suitable for further evaluation based on *in vitro* release profiles. It is important to perform *ex vivo* permeability, cytotoxicity and *in vivo* biodistribution studies to ascertain the suitability of such formulations. Such an attempt has been made and the results are reported here. The novelty of this work is attributed to the improved bioavailability and biodistribution achieved using the *in situ* gel formulation containing 10 times lower dosage than that clinically used.

## 2. Materials and methods

### 2.1. Materials

Acyclovir (ACV) and Eudragit® RSPO (ERSPO) were received as gift samples from Matrix India Pvt. Ltd. and Glukem Pharma Pvt. Ltd. Hyderabad, India respectively. Polyvinyl pyrrolidone K30 (PVPK30) and ethyl cellulose (EC) were procured from SD Fine Chem Pvt. Ltd., Mumbai, India and Pluronic® F127 (PF127) was purchased from Sigma Aldrich, Mumbai, India. Other solvents and chemicals were analytical grade.

### 2.2. Controlled release system for once-daily therapy of acyclovir

The pharmacokinetic parameters of ACV are half-life of 2.5–3.3 h, volume of distribution of 32.4–61.8 L and elimination rate of 0.238 $h^{-1}$. The clinical trials with 200 mg oral dose of ACV every 4 h have resulted in mean steady state plasma concentration of 0.31–0.49 μg/mL [10]. Based on the available pharmacokinetics with one compartment model, the kinetics of controlled release of the drug for once daily therapy was designed, as reported in our earlier work [9]. Accordingly, the desired drug concentrations in plasma at 2, 4, 6, 8, 12 and 24 h are 0.47, 0.52, 0.63, 0.62, 0.66 and 0.44 μg/mL, respectively. The cumulative amounts of drug release at various time intervals were calculated using the desired drug concentration levels and are shown in Fig. 1. The probable minimum dose required to achieve the optimum therapeutic concentration has been calculated to be 105 mg/day [9,13]. This dose is almost 10 times lower when compared to conventional oral dose (200 mg tablets, 5 times/day) as recommended currently for the clinical therapy of genital herpes infections. Accordingly, a suitable controlled release system that could provide the estimated *in vivo* drug concentration was intended.

### 2.3. Development of ACV polymeric nanoparticles loaded in situ gelling system

Biodegradable and biocompatible polymers were selected for the development of drug loaded nanoparticles based on their outstanding contributions in drug delivery [14]. The polymeric nanoparticles loaded with ACV were formulated by nanoprecipitation method [15], with two different hybrid polymer blends: (i) PVP (20 mg/mL)-EC (10 mg/mL) blend and (ii) PVP (10 mg/mL)-ERSPO (5 mg/mL) blend. The respective nanoparticle formulations were labeled as AN-1 and AN-2. Even though, the entrapment efficiency ($80 \pm 2\%$) was similar in both the formulations, the size and surface charge were significantly different. Formulation AN-1 exhibited zeta potential and particle size of $-12.3 \pm 2.2$ mV and $403 \pm 6$ nm, respectively. The zeta potential of AN-2 was higher ($+26.1 \pm 1.5$ mV) than that of AN-1. In addition, the particle size of AN-2 ($99 \pm 3$ nm) was lower than that of AN-1, as reported in our earlier work [16].

The freeze-dried nanoparticles and pure drug were incorporated into Pluronic sol system prepared by cold method [17] and characterized for pH, gelation time, gelation temperature, viscosity of sol and gel and *in vitro* drug release. *In situ* gel (PEC) containing 18% w/v Pluronic F-127 loaded with AN-1 nanoparticles and the formulation (PERS) with 15% w/v gelling polymer containing AN-2 nanoparticles were found to satisfy the required *in vitro* drug release, as reported in our previous work [9].

### 2.4. Ex vivo vaginal membrane permeation study

Permeability of the nanoparticles loaded *in situ* gel formulation was evaluated across freshly excised vaginal membrane of goat using Franz diffusion cell with specific permeation area of 2.54 $cm^2$. Phosphate buffer pH 7.4 (15 mL) was used as the media to mimic the systemic physiological environment [18,19] and maintained at $37 \pm 2\,^{\circ}C$. Vaginal tissue layer was crimped between the two compartments (donor and receptor) of the diffusion apparatus, and required amount of the sample formulation was mounted above the membrane (donor part). The set up was positioned on magnetic stirrer, maintained at 500 rpm for effective mixing of the diffused drug in the receptor chamber and maintenance of sink condition. Aliquots of 5 mL of samples were collected at predetermined time points and replaced with fresh warm buffer. The amount of drug permeated through the membrane was analyzed using UV–vis spectrophotometer (Evolution 201, ThermoScientific, USA) at 252 nm ($\lambda_{max}$) [20,21].

### 2.5. Permeation data analysis

Quantity of drug diffused across the tissue membrane per unit cross section area in unit time is assessed as flux, and expressed as,

$$J_{ss} = \frac{Q}{A\,t} \quad (1)$$

where, $J_{ss}$ is steady-state flux (μg/$cm^2$/h), Q is the amount (μg) of drug crossing the membrane, A is the dynamic diffusion area ($cm^2$) and t is the time (h).

The cumulative quantity of drug passing per unit surface area of the tissue (μg/$cm^2$) is plotted against the respective time (h), where the slope of linear curve gives the value of steady state flux (Jss, μg/$cm^2$/h) and x-intercept value gives the lag time ($t_L$, h).

The permeability coefficient ($K_p$, cm/h) was estimated by using the Eq.,

$$K_p = \frac{J_{ss}}{C} \quad (2)$$