International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 270-276 www.ijprajournal.com ISSN: 2249-7781

Development of In-Situ Formulation of Acyclovir for Treatment of Vaginal Herpes Infection

Arti Patidar[1], Prakash Kumar Soni[2]
Industrial Pharmacy Research Lab
Department of Pharmacy, S.G.S.I.T.S Indore (M.P.)

Date of Submission: 01-09-2021 Date of Acceptance: 12-09-2021

ABSTRACT
The present research work was aimed to develop In-situ gel formulation of acyclovir (ACV) for the treatment of vaginal herpes infection (HSV-2). The mixtures of Poloxamer 407 (20%) and Poloxamer 188 (10%) were used as polymers for gel formulations with Hydroxypropylmethylcellulose K100M (0.5%) and methyl cellulose (1%). Before development of formulation, drug characterization and preformulation studies were carried out like solubility, melting point determination, Differential Scanning Calorimetry (DSC), partition coefficient (Log P) determination, Preparation of calibration curve in different media and drug-excipient physical interaction study. After the preparation of In-situ gel formulation; pH, sol gel transition, drug content and in-vitro drug release study were determined. Morever ex-vivo vaginal membrane drug permeation study was performed on goat vaginal membrane. Cumulative % drug release in 12 hours was found to be 89.49%. and drug permeated in 12 hours found to be 56.80%.

KEY WORDS: Acyclovir, Poloxamer 407, Poloxamer 188, HPMC K100, Methyl cellulose and vaginal herpes infection (HSV-2).

I. INTRODUCTION

The vagina is a fibromuscular and tubular organ that extends from the uterine cervix to the vaginal vestibules and has a length of around 9 centimetres. Vaginal secretions are a combination of different fluids from a variety of sources, and the vagina is usually considered of as mucosal tissue without glands. Vaginal fluid has a pH range of 3.5 to 4.5. The presence of a dense network of blood vessels in the vaginal canal makes it an ideal route for drug delivery. The benefits of vaginal drug delivery are avoidance of enzymatic degradation, drug interactions, first-pass effect, easy to administer and high permeability for low molecular weight drugs. The physiology of the vaginal canal, phases of the menstrual cycle, age, pathological conditions, health conditions, and formulation factors all influence the rate and effect of drug absorption via this route. In the vaginal drug delivery system, various dosage formulations such as suppositories, gels, creams and ointments are used.[1, 2]

Type 1 (HSV-1) and type 2 (HSV-2) herpes simplex viruses are large, double-stranded DNA viruses in the family Herpesviridae. The main worldwide cause of sexually transmitted genital ulcers is HSV infection. Both of them are predominantly transmitted by communication of direct type: HSV-1 and HSV-2 also cause genital herpes and impact the health of individuals and the public. Immunocompetent individuals with genital HSV infection can have a great deal of psychosocial distress associated with frequent, painful, and recurring genital lesions.

Infection with HSV-2 is common worldwide and is almost exclusively sexually transmitted and causes genital herpes. The major source of genital herpes is HSV-2, which may also be caused by the type 1 herpes simplex virus (HSV-1). HSV-2 infection is permanent and not curable.[3, 4]

Acyclovir is one of the most widely used and effective antiviral drug prescribed for treatment of genital herpes and cold sores. Acyclovir is conventionally available in the form of gels, cream/ointment, injection, tablet dosage form and is to be administered topically, parenterally and orally, respectively. The topically applied creams/ointments are the most widely used dosage forms; however they suffer from drawbacks of leakage, messiness and tendency to escape from the body.

Thus, it was proposed to develop In-situ gel formulation of acyclovir which undergoes sol-to-gel transformation under the physiological conditions; i.e., temperature, pH and specific ions.[5]The formulation of In-situ gel of acyclovir for vaginal drug delivery was planned for the prolonged release of drug, improved patient

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 270-276 www.ijprajournal.com ISSN: 2249-7781

compliance and overcoming drawbacks of presently available conventional formulations.

Therefore, the present research work was aimed to develop in- situ gelling topical vaginal formulation of acyclovir for longer vaginal residence time, uniform spreading on vaginal mucosa, faster drug absorption, and reduce frequency of drug administration, resulting into better patient compliance and comfort.

II. MATERIALS AND METHOD

1.1 Materials

Acyclovir was obtained as a gift sample from ..... Poloxamers (P407, P188) were supplied from..... HPMC K100 and methyl cellulose was obtained as a gift sample from..... All other chemicals were of analytical grade.

1.2 Preparation of SVF (simulated vaginal fluid)

The simulated vaginal fluid was prepared using composition as shown in table 1

Table 1: Composition of SVF (simulated vaginal fluid)[7]

S.No.	Ingredients	Quantity
1.	Sodium Chloride	3.51gm
2.	Potassium Hydroxide	1.4gm
3.	Calcium Hydroxide	0.22gm
4.	Bovine Serum Albumin	0.018gm
5.	Lactic acid	2gm
6.	Acetic acid	1gm
7.	Glycerol	0.16gm
8.	Urea	0.4gm
9.	Glucose	5gm

1.3 Preparation of In-situ gel formulation

Vaginal In-situ gel formulations of acyclovir were prepared by cold method, a solution was prepared by mixing poloxamer 407 and poloxamer 188 in cold DM water at 4°C with continuous agitation. The solution was left at 4°C until a clear solution was obtained. Then other solution of HPMC K100 was prepared in DM water and methyl cellulose was also prepared in DM water then drug was added. This gel was left at room temperature for 24 hours. The compositions of In-situ gel formulation are given in table 2

Table 2: Formulation composition of acyclovir In-situ gel

S No.	Ingredients	Quantity (%w/v)
1.	Acyclovir	1
2.	Poloxamer 188	10
3.	Poloxamer 407	20
4.	Methyl cellulose	1
5.	HPMC K100	0.5
6.	Water q.s. to	100

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 270-276 www.ijprajournal.com ISSN: 2249-7781

III. EVALUATION OF ACYCLOVIR IN-SITU GEL FORMULATION

1.4 Determination of sol gel transition temperature

For this, the solution of formulation was placed in a test tube and dipped in water bath maintained at a temperature of 37±5°C for 2 min. The temperature of conversion of the solution to gel form was noted down by using thermometer. The gel was formed in the absence of flow of the formulation in the test tube.

1.5 Determination of pH

The pH of formulation was measured by calibrated pH meter (Cyberscan 510) at room temperature.

3.3 Determination of drug content

One gm of In-situ gel was taken in volumetric flask of 100 ml and dissolved completely in adequate quantity of water and then volume was made up to 100 ml with DM water. From above solution 1 ml was transferred to 10 ml volumetric flask and volume was made up to 10 ml with DM water. Drug concentration was determined by using UV spectrophotometer (Shimadzu 1700) at 252nm.

3.4 In-vitro drug release study

Dialysis method was employed for studying and comparing the in vitro drug release profile of developed formulation of acyclovir. In this method 1 ml of formulation was filled in separate dialysis membrane bags. The dialysis membrane bags were then placed in beakers containing simulated vaginal fluid (pH 4.2) maintained at 100 rpm and 37°C±0.5°C. Samples were withdrawn at different time intervals for duration of 12 hrs. The withdrawn samples were analyzed for their drug contain on UV spectrophotometer. A double beam UV spectrophotometer (Shimadzu®1700) was employed for this purpose. Percent cumulative drug release at different time interval was calculated. The experimental conditions of in vitro drug release study are shown in table 3

Table 3: Experimental conditions for in-vitro drug release study

Apparatus	Multi station magnetic stirrer
Membrane	Dialysis membrane (12-14kDa) HiMedia
Release medium	Simulated vaginal fluid
Volume of media	200 ml
Speed of rotation	100rpm
Sampling volume	5 ml
Sampling time interval	0.5, 1, 2, 3, 6, 9, 12 hrs.
Temperature	37°C± 0.5°C

- **Treatment of dialysis membrane**

Dialysis tubes were washed in running water for 3-4 hours, for complete removal of glycerol. Then the tubes were treated with 0.3 % (w/v) sodium sulfide solution at 80°C for 1 min. for complete removal of sulfur compounds. After that it was washed with hot water at 60°C for 2 min. followed by acidification with 0.2 % (w/v) sulfuric acid solution and again washed with hot water to remove traces of acid.

- **Procedure**

The treated dialysis membrane was cut into pieces of 5 cm each. One end of each tube was closed with dialysis closure clip (HiMedia). 1 ml of each formulation (equivalent to 10 mg acyclovir) was filled in separate dialysis membrane bags and

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 270-276 www.ijprajournal.com ISSN: 2249-7781

their other ends were also closed using clips. The filled dialysis membrane bags were dipped in 200 ml of simulated vaginal fluid taken in a beaker and maintained at 37°C± 0.5°C and 100 rpm using multi station magnetic stirrer. 5 ml of samples were withdrawn from each beaker at specific time intervals and replaced with equivalent amounts of fresh simulated vaginal fluid. The samples were analyzed at 252 nm for their drug content using double beam UV- visible spectrophotometer (Shimadzu 1700). Cumulative % drug release at different time intervals was calculated in table 5.

3.5Ex- vivo vaginal membrane drug permeation study

Ex-vivo vaginal membrane drug permeation study of developed acyclovir In-situ gel formulation was performed on freshly excised goat vagina procured from slaughter house. Modified franz diffusion apparatus was employed for this purpose. Experimental conditions for ex-vivo vaginal drug permeation study are reported in table 4

Table 4: Experimental condition for permeation study

Apparatus	Modified Franz diffusion apparatus
Receptor Media	Simulated Vaginal Fluid
Volume of media	13 ml (receptor compartment)
Sampling volume	3 ml
Sampling time interval	0.5, 1, 2,3,6, 9, 12 hrs.
Temperature[6]	37°C± 0.5°C

- **Procedure**

The procurement of goat's whole vagina was done from a slaughter house. The excised vaginal organ was then transported to the laboratory in simulated vaginal fluid (pH 4.2). The vaginal membrane was removed carefully followed by their washing with simulated vaginal fluid (pH 3.5-4.5). Once washed, the storage of the vaginal membrane was done in fresh simulated vaginal fluid.

The freshly excised vaginal membrane was mounted between the donor and receptor compartment and clamped in such a way that its upper surface faced the donor compartment. The receptor compartment was filled with 13 ml of simulated vaginal fluid maintained at 37°C± 0.5°C using magnetic stirrer. 2 ml of the developed acyclovir In-situ gel (equivalent to 20 mg acyclovir) was added in donor compartment. 3 ml of samples were withdrawn from receptor compartment and replaced with equal amounts of fresh media at specific time intervals. Aliquots of withdrawn samples were suitably diluted and analyzed for drug content on a double beam spectrophotometer (Shimadzu® 1700) at 252 nm. The study was carried out for a period of 12 hours.

3.6 Apparent vaginal membrane permeability coefficient (P_{app})

The apparent vaginal membrane permeability coefficient of the In-situ formulationwas determined according to the following equation.

$$\mathbf{Papp} = \frac{\mathbf{dQ}}{\mathbf{dt}} \cdot \frac{\mathbf{1}}{\mathbf{A.60.60.Co}}$$

$$\mathbf{Jss = Papp\ x\ Co}$$

Where, dQ/dt represents the slope of steady state of the plot's linear portion. The plot here refers to that of the drug's amount in the receptor chamber (Q) versus time (t). A is the exposed vaginal membrane surface area (3.14 cm^2), C_0 is the drug's initial concentration taken in the cylinder made up of glass and 3600 represents the hour's conversion to seconds.The calculated P_{app} and J_{ss} of the In-situ formulation are recorded in the table 6.13.

IV. RESULT AND DISCUSSION

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 270-276 www.ijprajournal.com ISSN: 2249-7781

1.6 Determination of sol gel transition temperature

The gelation temperature is the temperature at which the liquid phase makes its transition to a gel. Gelation temperature of the prepared In-situ gel formulation was found to be 37.4°C.

1.7 Determination of pH

The pH of the prepared In-situ gel found to be 7.2.

1.8 Determination of drug content

The drug content of In-situ gel formulation was found to be 1.01% (w/v) or 10.1 mg/ml.

1.9 In-vitro drug release study

Cumulative % drug release at different time intervals was calculated

Table 5: In-vitro drug release data of developed formulation

S.No.	Time (hrs.)	Cumulative % drug release
1.	0	0
2.	0.5	5.1
3.	1	16.6
4.	2	39.74
5.	3	54.3
6.	6	76.08
7.	9	81.43
8.	12	89.49

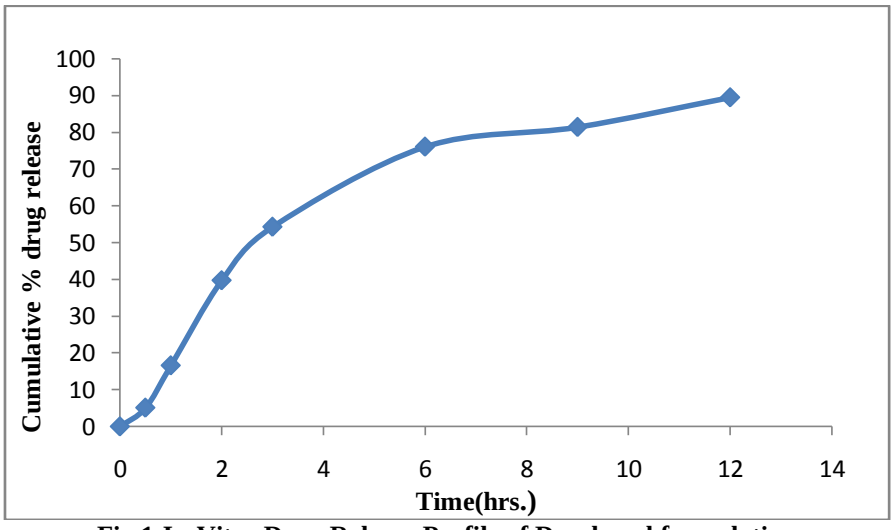

Fig.1:In-Vitro Drug Release Profile of Developed formulation

Cumulative percent drug release for developed acyclovir In-situ gel formulation in 12 hours was found to be 89.49%.

1.10 Ex- vivo vaginal membrane drug permeation study

The results of vaginal membrane drug permeation study (in terms of cumulative drug amount permeated) are reported in table 6

Table 6: Drug permeated data of developed formulation

S. No.	Time (hrs.)	Cumulative drug amount permeated (mg)	% Cumulative drug permeated

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 270-276 www.ijprajournal.com ISSN: 2249-7781

1.	0	0	0
2.	0.5	1.1	5.50
3.	1	2.06	10.30
4.	2	3.96	19.80
5.	3	5.87	29.35
6.	6	9.75	48.75
7.	9	10.45	52.25
8.	12	11.36	56.80

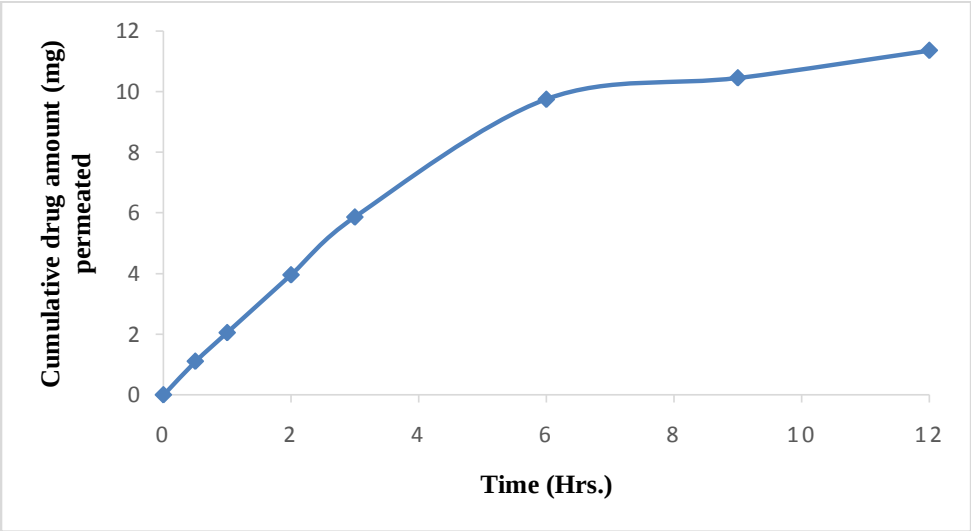

Fig.2:Drug permeation profile of developed formulation

Developed In-situ gel formulation of acyclovir showed drug permeated in 12 hours, which is found to be 11.36 mg (56.80%).

1.11 Apparent vaginal membrane permeability coefficient (P_{app})

The calculated P_{app} and J_{ss} of the In-situ formulation are recorded in the table 7

Table 7: Apparent vaginal membrane permeability coefficient (Papp) of developed In-situ gel formulation

S. No.	Samples for permeation study	P_{app} (cm/sec)	J_{ss} (µg.cm/sec)
1	Developed formulation	4.27×10^{-6}	0.000085

The developed In-situ gel formulation was found to effectively cross the vaginal membrane in 12 hours. The permeation from In-situ gel formulation was 56.80%.

Table 7 shows the vaginal membrane permeation parameters of developed In-situ gel formulation in 12 hours.

V. CONCLUSION

Vaginal infection is caused by an imbalance of yeast and bacteria in the vaginal region.The common types of vaginal infections include bacterial vaginosis, candida, chlamydia, gonorrhea, trichomoniasis and viral vaginitis like herpes simplex virus (HSV), or human papillomavirus (HPV).One of the most common sexually transmitted disease is genital herpes. Genital herpes is caused by herpes simplex virus.

Acyclovir is widely used in the treatment of genital herpes (HSV 2). There are many vaginal formulations like creams, ointments, and gels but these conventional dosage forms are associated with drawbacks of leakage, messiness, and tendency to escape from the body. Such problems can be addressed by drug delivery system, which can adhere to site and slowly delivers the drug for longer duration of time. Therefore, it was proposed to develop acyclovir In-situ gel formulation to

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 270-276 www.ijprajournal.com ISSN: 2249-7781

overcome the disadvantages of conventional dosage forms.

The characterization of acyclovir drug sample was done using UV-visible spectrophotometer and Differential scanning calorimetry (DSC). All the observations were similar to the value reported in the literature.

Calibration curves of acyclovir were prepared in demineralized water, simulated vaginal fluid (pH 4.2) and acetate buffer (pH 4.5) using UV-visible spectrophotometer (Shimadzu® 1700). The linearity of the calibration curves showed that the Beer Lambert's law was obeyed in the concentration range of 5-25 µg/ml at λ_{max} 252 nm.

Preformulation studies were carried out to determine solubility, partition coefficient and compatibility of drug with different excipients under various environmental conditions. Solubility data revealed that acyclovir is slightly soluble in water, simulated vaginal fluid and acetate buffer. The log P value of acyclovir was found to be -1.56 which was similar to the value reported in literature.

Various polymers were studied for the preparation of In-situ gel formulation. Out of various polymers poloxamer 407 and poloxamer 188 were selected to prepare In-situ gel on the basis of their properties and evaluation. Both poloxamers are temperature dependent so used in In-situ gel formulation.

The final In-situ gel formulation was prepared using HPMC, methylcellulose. Poloxamer 407 and poloxamer 188 were selected as a gelling agent in which 1% acyclovir is dissolved.

Developed In-situ gel formulation was evaluated for appearance, pH, temperature and drug content. In vitro drug release study of final formulation was performed in order to evaluate the drug release characteristics. Cumulative percent drug released for developed In-situ gel in 12 hours was found to be 89.49%. Vaginal drug permeation study of In-situ gel formulation was performed on goat vagina membrane by using Franz diffusion cell apparatus and cumulative amount drug permeation found to be 11.36 mg (56.80%).

On the basis of findings and observations of the present study, it can be concluded that the developed acyclovir In-situ gel formulation can be a better alternative to the presently available conventional dosage form by overcoming the drawbacks of present product and improving patient compliance.

REFERENCES

[1]. Hussain, A.; Ahsan, F., The vagina as a route for systemic drug delivery. J Control Release **2005,**103 (2), 301-13.

[2]. de Araujo Pereira, R. R.; Bruschi, M. L., Vaginal mucoadhesive drug delivery systems. Drug Dev Ind Pharm **2012,**38 (6), 643-52.

[3]. Gupta, R.; Warren, T.; Wald, A., Genital herpes. The Lancet **2007,**370 (9605), 2127-2137.

[4]. Sauerbrei, A., Herpes Genitalis: Diagnosis, Treatment and Prevention. Geburtshilfe Frauenheilkd **2016,**76 (12), 1310-1317.

[5]. Mohanty, D.; Bakshi, V.; Simharaju, N.; Haque, A.; Sahoo, C., A Review on in situ gel:A Novel Drug Delivery System. Int. J. Pharm. Sci. Rev. Res **2018**, 175-181.

[6]. Ramyadevi, D.; Rajan, K. S.; Vedhahari, B. N.; Ruckmani, K.; Subramanian, N., Heterogeneous polymer composite nanoparticles loaded in situ gel for controlled release intra-vaginal therapy of genital herpes. Colloids Surf B Biointerfaces **2016,**146, 260-70.

[7]. Nikhar, S.; Bansode, D.; Mahadik, K., Thermosensitive in-situ gel of tinidazole in treatment of bacterial vaginosis; Formulation and evaluation. Int. J. Ayurveda Res. **2002,**8 (1), 1-12.