Original Article

Mucoadhesive *in-situ* Gel Formulation for Vaginal Delivery of Tenofovir Disoproxil Fumarate

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ABSTRACT

Introduction: Tenofovir disoproxil fumarate is an anti-retroviral medicine which belongs to microbicides class being formulated for a woman instigated technique of prevention of the human immunodeficiency virus infection. The objective of the present investigation is to prepare thermosensitive mucoadhesive *in-situ* vaginal gel of Tenofovir disoproxil fumarate that can present pre-exposure prophylaxis against human immunodeficiency virus in addition to providing excellent spreading as well as coating of the vagina, forming the therapy more effectual and bring about extended effect. **Materials and Methods:** The vaginal *in-situ* gel of Tenofovir disoproxil fumarate was prepared using thermosensitive polymer poloxamer 407 and mucoadhesive polymer carbopol 934 by a cold method. It was characterized for drug-excipient compatibility, viscosity, gelation study, gelling capacity, *in-vitro* drug release study, stability study and Hen's Egg Test-Chorioallantoic Membrane assay. **Results and Discussion:** Drug excipient compatibility study displayed that there is no interaction between drug and excipients. Formulation F2 was found as the most appropriate formulation on the basis of the evaluation parameters, as it displayed the preferred properties. The work of adhesion values was used as parameters for comparison of mucoadhesive performance and it was found as 0.324 ± 0.036 N. Hen's Egg Test-Chorioallantoic Membrane test showed that the formulation is nonirritant to the vaginal mucosa. Formulation F2 was subjected to accelerated stability studies at 40°C ± 2°C /75% RH ± 5% RH for 6 months. The results showed that it stayed steady for 6 months. **Conclusion:** It can be concluded that the development of a tenofovir *in-situ* vaginal gel which may offer effective and sustained protection against human immunodeficiency virus infection.

Key words: Antiretroviral drug, Thermosensitive, Mucoadhesive, *in-situ* gel, Non-irritant.

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INTRODUCTION

The Human Immunodeficiency Virus (HIV) is a retrovirus, which causes a decline in the immune system and ultimately can cause Acquired Immunodeficiency Syndrome (AIDS). It is stated in the UN AIDS 2013 report that HIV continues to be driven by gender inequalities and pernicious societal practices that lead to a higher susceptibility of the female population acquiring AIDS.[1] The antiretroviral therapeutic agents are preferred so as to overwhelm the HIV virus, slow down the development of HIV disease as well as avoid forward transmission of HIV.[2] Tenofovir, as an antiretroviral agent, is a nucleotide analog that inhibits HIV reverse transcriptase and shows potency *in-vitro* and *in-vivo* against HIV,[3] and so, tenofovir is one of the most common antiretroviral drug molecules used for HIV treatment.[4,5] Tenofovir disoproxil fumarate, a nucleotide analog HIV-1 reverse transcriptase inhibitor is 100 times more potent in its anti-viral activity compared to its prodrug Tenofovir.[6] Tenofovir disoproxil fumarate has enhanced permeability leading to a reduction in dose requirement and currently, it is administered orally in its prodrug form. Tenofovir disoproxil fumarate is effective against a range of HIV-1 subtypes, as well as CCR5-using and

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CXCR4-using HIV-1.[7] It also has a long intracellular half-life, low risk of resistance development,[8] and has been proposed for use in pre-clinical trials as a microbicide.[9] The vaginal drug delivery system was employed as a route for drug administration to achieve local plus systemic action. The benefits of the vaginal route are avoidance of the first-pass metabolism of a drug, reduction in hepatic as well as gastrointestinal side effects of the drug and overwhelming of pain, tissue damage and infection found after parenteral administration.[10-12] There are two major challenges in the vaginal drug delivery system. The first one is restricted contact time triggered by the physiological conditions imposed by the protective mechanisms of the vagina and the second challenge is poor patient compliance. Conventional vaginal formulations are available in market such as vaginal ointments and inserts inducing embarrassment to the patient.[13] Although it is known that patients can tolerate gels more easily as compared to vaginal ointments and inserts, but at a same time there is a difficulty in direct application of gels into the vaginal cavity.[13] Mucoadhesive polymers may localize in a specific region and extend residence time, thus improve the bioavailability of drugs.[14] Therefore, mucoadhesive dosage forms have become very important for the treatment of vaginal diseases. These types of dosage forms provide an interaction between the mucoadhesive polymer and vaginal mucosa and increase the residence time at the mucosal application site. Newly, the *in-situ* gelling systems have been found out as more opportune formulation for topical administration. The *in-situ* gels are stimuli-sensitive hydrogels that exist as a liquid before administration and immediately transition into gels following the contact with the vaginal mucosa. The *in-situ* gel offers a number of benefits for example, easy to administer into vaginal or other body cavities, excellent spreadability at certain temperatures, reduction in the frequency of administration, improvement in patient compliance and very comfortable as compared to the conventional formulations.[15] The most widely employed gels are poloxamers based gels as they are easily applied and also providing good spreading as well as coating of the vagina which makes the treatment more effective and undoubtedly result in extended effect.[16,17] Poloxamers were selected to develop *in-situ* gel which is a synthetic triblock copolymer of polyoxyethylene and polyoxypropylene that exhibits thermosensitive property in an aqueous medium.[18] Poloxamers have good compatibility but has very low mucoadhesive property. So, Carbopol 934 was included in the formula for improvement in the mucoadhesive as well as mechanical properties and for confirmation of longer retention time of developed *in-situ* gel formulation in the vaginal cavity. The objectives of the study were to design more effective treatment for HIV by preparing a new mucoadhesive *in-situ* vaginal gel of Tenofovir disoproxil fumarate that adhere to the vaginal mucosa and release the drug for longer period of time to enhance the therapeutic activity of the drug. Poloxamer 407 and Carbopol 934 were employed as polymers to provide *in-situ* and mucoadhesive property to the system. The resulting formulation was evaluated for gelation temperature, viscosity and pH measurement, mucoadhesive force measurement, *in-vitro* release study, irritation study and stability study.

MATERIALS AND METHODS

Tenofovir disoproxil fumarate was gently supplied as gift sample by Spectrum Pharma Research Solutions, Hyderabad, Poloxamer 407 was provided by BASF, Mumbai, India as gift sample and Carbopol 934 were received from Central Drug House (P). Ltd. India. All other chemicals, as well as reagents, were of analytical reagent grade, were purchased from S.D. Fine Chemicals Ltd., Mumbai.

Method of preparation of Tenofovir disoproxil fumarate vaginal *in-situ* gel

Thermosensitive and mucoadhesive poloxamer based vaginal *in situ* forming gels of Tenofovir disoproxil fumarate was prepared by the cold technique first described by Schmolka with slight modification.[19,20] Briefly, carbopol 934 was slowly added to a phosphate buffer pH 4.0 under continuous stirring at 4°C. Poloxamer 407 was then added gradually under continuous stirring until a clear solution was obtained. Agitation was performed in an ice bath at 4°C. Depending on the final viscosity of the preparation and the ratio carbopol 934 and poloxamer 407, poloxamer 407 could be dissolved first and carbopol 934 at last. The mixture was then placed at temperature 4°C for 48 hr to eliminate foam and air bubbles and the macroscopic homogeneity of each preparation was appreciated visually at the end of the storage period. Tenofovir disoproxil fumarate (1%) was initially dissolved in the mixture of methanol and polyethylene glycol 400 (3:5) and added to the cold poloxamer 407 solution containing various content of carbopol 934 (0.25-1.5%) with gentle mixing. Benzalkonium chloride (0.01%) used as a preservative (Table 1).

Table 1: Preparation of formulation batches F1 to F6.

Ingredients	Formulation code					
	F1	F2	F3	F4	F5	F6
Tenofovir disoproxil fumarate (% w/v)	1	1	1	1	1	1
Poloxamer 407 (% w/v)	18	18	18	18	18	18
Carbopol934 (% w/v)	0.25	0.50	0.75	1.0	1.2	1.5
Benzalkonium chloride (% w/v)	0.01	0.01	0.01	0.01	0.01	0.01
Water (ml) q.s.	10	10	10	10	10	10

Drug excipients compatibility study

A drug excipient compatibility study was performed for checking the compatibility between drug and polymers. The compatibility studies of Tenofovir disoproxil fumarate with other excipients were done by using Fourier Transform Infrared Spectroscopy (FT-IR). FT-IR spectra of pure drug and physical mixture of drug and other excipients were measured with the help of the FT-IR instrument using the Potassium Bromide (KBr) method. The samples to be tested were mixed with solid KBr. The mixture was then passed into a very thin pellet. The pellet was placed in the holder directly in the IR laser beam. Spectra were recorded using Shimadzu FT-IR 8400s loaded with IR solution version 1.2 software of the pure drug for any major interaction. Each spectrum was recorded in the frequency range of 3800-600 cm^{-1}.

Physicochemical characterization of Tenofovir disoproxil fumarate vaginal *in-situ* gel

The clarity of the vaginal *in-situ* gelling formulation after and before gelling was measured by visual examination. The pH of the vaginal *in-situ* gel formulation was recorded with a digital pH meter and allowing equilibrating for 1 min. The viscosity of all formulated batches of the *in-situ* gel was measured using Brookfield Digital Viscometer (DV-E model). The tests were performed in triplicate.

Gelation temperature

A 5 ml aliquot of the *in-situ* gelling solution was transferred to a test tube, submerged in a thermostat water bath. The temperature of the water bath was raised with an increment of 1°C and kept for 5 min to equilibrate at every new set of temperatures. After that, the sample was inspected for gelation upon slanting the test tube and it was believed to take place when the meniscus would no longer move. Each sample was measured in triplicate.[21,22]

Drug content

Drug content was determined by dissolving 10mL Tenofovir disoproxil fumarate vaginal *in situ* gel in phosphate buffer pH 4.0. After suitable dilution absorbance was recorded by using a UV/Vis double beam Spectrophotometer at 260 nm. The drug content was calculated with a slope of a standard curve.

Mucoadhesive force determination

The mucoadhesive force is a measure of a force necessary to separate the formulation from vaginal mucosal membrane. The apparatus urbanized for *in-vitro* mucoadhesive strength determination in a simulated vaginal environment is a modification of the earlier described mucoadhesion test assembly.[23,24] The method is based on the measurement of shear stress required to break the adhesive bond between a model membrane and the test formulation. The Tenofovir disoproxil fumarate vaginal *in-situ* gelling formulation is sandwiched between two model membranes fixed supports in the assemblies for a sufficient period of time. After the formation of the adhesive bond, the weight or force needed to separate the bond will be measured and calculated as mucoadhesive strength.

In-vitro drug release study

Franz diffusion cell was used for an *in-vitro* drug release study with water-jacketed receptor chamber (20 ml) and a donor chamber equipped with a thermostatically shaking water bath at 37 ± 1°C. The receptor chamber was containing phosphate buffer pH 4.0 solutions was constantly stirred by a magnetic stirrer. Both the chambers separated by cellulose membrane (Filter paper Whatman 41, 20-25µm, Whatman GmbH, Dassel, Germany) and each vaginal *in-situ* gelling formulation spread on the circular portion of the membrane. At predetermined time intervals, 1 mL of the solutions were removed from the acceptor phase at each sampling time for up to 8hrs. The aliquots were replaced with an equal volume of the freshly prepared release medium kept at the same temperature and amount of Tenofovir disoproxil fumarate release was measured by taking the absorbance at 260 nm against a blank with UV spectrophotometer at 260 nm (Shimadzu UV spectrophotometer, Japan). The result of *in-vitro* data was analyzed by statistical software to achieve the best fit kinetic model for *in-vitro* drug release from optimized formulation. The test was conducted in triplicate.[10]

Irritation test with Hen's Egg Test Chorioallantoic Membrane Test (HET-CAM)

For irritation study, modified HET-CAM test was performed as described by Velpandian and co-workers.[25]

In this test fertilized hen's eggs were obtained from a poultry farm. Three eggs were selected for each formulation weighing between 50-55 g. These (special pathogen-free) eggs were incubated at 37°C ± 0.5°C in a standard cell culture incubator up to day 3. On the 3rd day, 3 ml of egg albumin was removed by using sterile technique through a hole made by pointed pole and then the hole sealed by 70% alcohol sterilized paraffin with the help of the heated spatula. The eggs were kept in the equatorial position for the development of CAM. The eggs were candled on the 5th day of incubation and thereafter non-viable embryos were removed. On the 10th day, *in-situ* gelling formulation was instilled directly onto CAM surface with a pipette and observed within a specific time limit. The membrane is examined for vascular damage and the time required for injury to take place is recorded.

A 0.9 % Sodium Chloride (NaCl) solution was utilized as a control as it is stated to be experimentally non-irritant. The scores were recorded according to the scoring schemes mentioned below:

Score 0 indicates no visible hemorrhage (non-irritant); Score 1 indicates just visible membrane discoloration (mild irritant); Score 2 indicates structures are covered partially due to membrane discoloration and hemorrhage (moderately irritant); Score 3 indicates structures are covered totally due to membrane discoloration and hemorrhage (severe irritant).

Stability studies

Stability studies were carried out on optimized formulation according to ICH guideline for 6 months and after that checks all the physicochemical parameters of an optimized batch of formulated Tenofovir disoproxil fumarate vaginal *in-situ* gel.

RESULTS AND DISCUSSION

Drug excipients compatibility study

The FT-IR analysis of pure drug, drug and excipients physical mixture was done on Shimadzu FT-IR 8400s. The FT-IR spectra of pure Tenofovir disoproxil fumarate and physical mixture of Tenofovir disoproxil fumarate, Carbopol 934 and poloxamer 407 are depicted in Figure 1(a) and Figure 1(b) respectively. The FT-IR spectrum of Tenofovir disoproxil fumarate showed characteristics peaks at 1645 cm^{-1} (C=O stretching), 1325 cm^{-1} (O=C=O stretching), 2910 cm^{-1} (Aliphatic CH stretching), 3000 cm^{-1} (Aromatic CH stretching), 3383 cm^{-1} NH_2 stretching) and 3469 cm^{-1} (NH stretching) were identified, which was same in the physical mixture of Tenofovir disoproxil fumarate and excipients. Thus, it was confirmed that there is no interaction between drug and excipients.

Physicochemical characterization of tenofovir disoproxil fumarate vaginal *in-situ* gel

The physicochemical properties such as clarity, pH, viscosity, gelation temperature, drug content and mucoadhesive strength of the prepared formulation are depicted in Table 2.

The Tenofovir disoproxil fumarate vaginal *in-situ* gelling solution was transparent liquid at 4°C whereas transparent semisolid gel was formed at body temperature. The normal physiological pH of vaginal mucosa ranges from 3.5-4.5. The pH of all the vaginal *in-situ* gel formulation F1 to F6 found to be 4.0-5.0, within the range which vaginal mucosa can tolerate.

The rheological performance is a key element in the poloxamer formulation. The increase in polymer carbopol 934 concentration caused an increase in viscosity of formed vaginal *in-situ* gelling solution as well as vaginal *in-situ* gel. Results of viscosity profiles of formulations batches F1 to F6 at 37°C clearly indicate that the mucoadhesive polymer carbopol 934 had a viscosity-enhancing effect.

Gelation temperature is the temperature at which the liquid phase makes a transition to gel. An ideal vaginal *in-situ* gel should be a free-flowing liquid at room temperature for reproducible administration of formulation into the vaginal cavity where it undergoes an *in-situ* sol-gel phase transition to form a gel.[26] The temperature of a human vagina is 37.2°C,[27] so gelation temperature near to this temperature is a most suitable temperature. If gelation temperature is below the vaginal temperature, a gel might be formed before administration leading difficulties in manufacturing, handling and administering as well. And if gelation temperature is higher than 37.2°C, a formulation remains in a liquid state at vaginal temperature, resulting in drainage of formulation from the vaginal cavity at an early stage.

The drug content was found to be in acceptable range (91.65 - 98.58 %) for all the formulations, indicating the uniform distribution of the drug.

The mucoadhesive force is a vital and crucial characteristic for *in-situ* forming vaginal gels since it stops rapid drainage of formulation and therefore extends its retention time in vagina.[28] The mucoadhesive strength significantly increases as the concentration of mucoadhesive polymer increased in the range of 0.25-1.5%. The mucoadhesive force results indicate that the formulated Tenofovir disoproxil fumarate *in-situ*

vaginal gel with poloxamer 407 had acceptable adhesive property.

In-vitro drug release study

In-vitro drug release kinetics was carried out by the use of Franz diffusion cells in order to evaluate Tenofovir disoproxil fumarate vaginal *in-situ* gel release profile. The release profile of Tenofovir disoproxil fumarate from all the formulations reveals that as the level of mucoadhesive polymer carbopol 943 is increasing, the drug release is decreasing due to higher viscosity of the formulation (Figure 2). The retarding effect of mucoadhesive polymer carbopol 934 could be attributed to their ability to increase the overall product viscosity as well as their ability to distort or squeeze the extra micelle aqueous channels of poloxamer micelle through which the drug diffuses thereby, delaying the release process. The *in-vitro* drug release conditions may be very different from those likely to be encountered in the vagina. However, the results clearly show that the gels have the ability to retain the drug for a prolonged period of time about 8 hrs and that premature release will not occur.

The gels on visual inspection at periodic intervals during the *in-vitro* drug release experiments showed a gradual swelling after 4 hrs that resulted in an increase in the volume of most gels. No discernible relationship between the extent of swelling and gel composition could be established. Also, no apparent changes or disruptions in the integrity of the gels were noticed during the course of the experiment.

For measurement of release kinetics of Tenofovir disoproxil fumarate vaginal *in-situ* gel, the drug release data were analyzed by calculating coefficients of determination for evaluation of data for fitting the kinetic regression lines of zero order, first order, Higuchi, Korsmeyer-Peppas and it was observed that Korsmeyer-Peppas model showing the best correlation (R^2 = 0.9860).

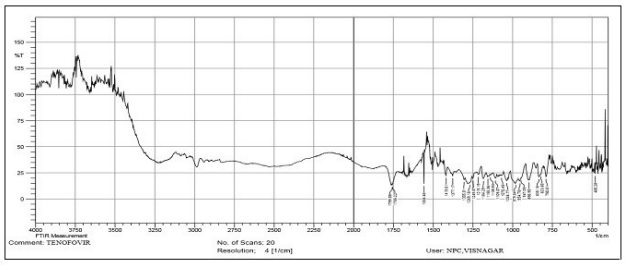

Figure 1: (a) FTIR spectrum of Tenofovir disoproxil fumarate.

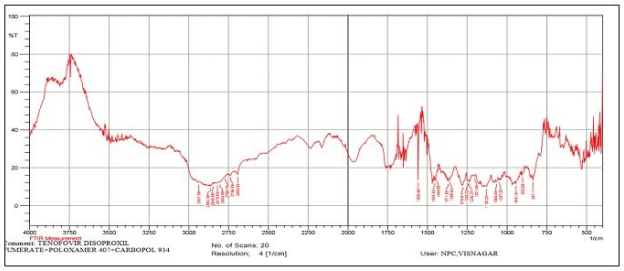

Figure 1: (b) FTIR spectrum of physical mixture of Carbopol934, Poloxamer 407 and Tenofovir disoproxil fumarate.

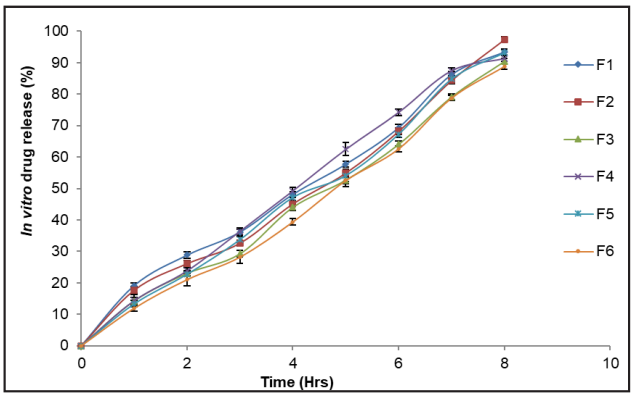

Figure 2: *In-vitro* drug release of formulation batches F1 to F6.

Table 2: Physicochemical characteristics of formulation batches F1 to F6.

Formulation batch	pH*	Viscosity of *in-situ* gelling solution* (cps)	Viscosity of *in-situ* gel* (cps)	Gelation temperature* (ºC)	Drug content* (%)	Mucoadhesive strength* (N)
F1	4.0 ± 0.16	160 ± 12.25	1911± 0.89	38 ± 1.25	95.23 ± 0.77	0.286 ± 0.012
F2	4.2 ± 0.12	240 ± 8.16	2116 ± 0.62	37 ± 1.63	98.58 ± 0.89	0.324 ± 0.036
F3	5.0 ± 0.16	360 ± 16.33	2310 ± 0.33	34 ±1.25	96.25 ± 0.62	0.368 ± 0.005
F4	4.0 ± 0.16	440 ± 12.25	2580 ± 0.60	30 ±1.63	91.65 ± 0.79	0.395 ± 0.013
F5	4.0 ± 0.21	520 ± 8.16	3320 ± 0.22	28 ± 1.25	92.23 ± 0.33	0.402 ± 0.005
F6	4.5 ± 0.21	640 ± 16.33	3532 ± 0.89	27 ± 2.02	94.98 ± 0.62	0.436 ± 0.019

*Data are expresses as Mean ± S.D. (n=3)

Formulation batch F2 selected as optimized formulation exhibits gelation temperature near to body temperature, acceptable pH for vaginal delivery, desirable mucoadhesive property to retain the drug in a vagina for a longer time, better drug content and high drug release in sustain manner up to 8 hr.

Irritation test with Hen's Egg Test - Chorioallantoic Membrane Test (HET-CAM)

Irritation of optimized formulation was tested by Hen's egg CAM test which is quick, sensitive and inexpensive.[29] In addition, irritation testing of formulation with incubated eggs is a borderline case between *in-vivo* and *in-vitro* system and they do not conflict with the ethical as well as legal obligations. Irritation potential of optimized formulation was tested by using HET-CAM and results were compared with normal saline as a control that was non-irritant (Score 0) while optimized Tenofovir disoproxil fumarate vaginal *in-situ* gel was non-irritant up to 1hr (Score 0) but mean score was found to be 0.33 after 5hrs which indicates that formulation is non-irritant to mild-irritant (Figure 3 and Table 3).

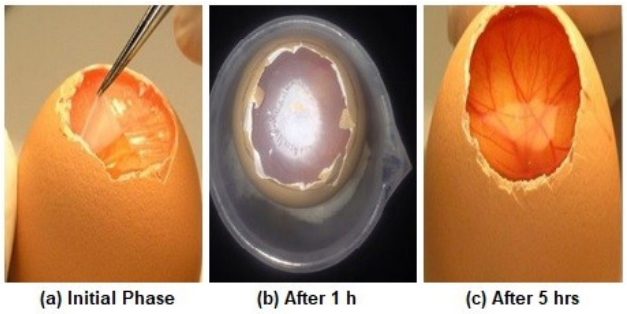

Figure 3: Hen's egg CAM test for irritation study.

Table 3: Score for HET-CAM test (Time in hr.).

Sample	0	1	2	3	4	5
Normal saline solution as a control						
Egg 1	0	0	0	0	0	0
Egg 2	0	0	0	0	0	0
Egg 3	0	0	0	0	0	0
Mean	0	0	0	0	0	0
Optimized formulation of Tenofovir disoproxil fumarate vaginal *in-situ* gel						
Egg 1	0	0	0	0	0	0
Egg 2	0	0	0	0	0	0
Egg 3	0	0	0	0	1	1
Mean	0	0	0	0	0.33	0.33

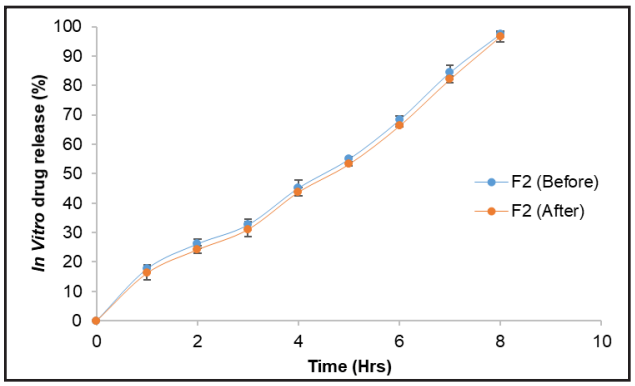

Figure 4: Release data after stability study of optimized batch (F2).

Stability studies

A stability study was carried out on the optimized formulation of tenofovir disoproxil fumarate as per ICH guidelines for 6 months. There are no major changes are found in physicochemical characteristics as well as on release profile in optimized formulation. Results of stability studies are presented in Table 4 and Figure 4. Hence, it is indicating that during stability study the optimized formulations remain stable as per ICH guidelines. So, we can conclude that after the 6 months stability as per ICH guidelines there are no physicochemical changes are observed. Hence, our optimized formulation is stable.

CONCLUSION

The present investigation deals with the formulation of mucoadhesive Tenofovir disoproxil fumarate vaginal *in-situ* gel using carbopol 934 as a mucoadhesive polymer. This *in-situ* vaginal gel was made with the

Table 4: Physicochemical evaluation of formulation after stability studies.

Formulation batch	pH*	Viscosity of *in-situ* gel* (cps)	Gelation temperature* (ºC)	Drug content* (%)	Mucoadhesive strength* (N)
F2 (Before)	4.2 ± 0.12	2116 ± 0.62	37 ± 1.63	98.58 ± 0.89	0.324 ± 0.006
F2 (After)	4.0 ± 0.08	2090 ± 0.32	36 ± 0.33	98.25 ± 0.12	0.318 ± 0.013

*Data are expressed as Mean ± S.D. (n=3)

intention to provide a topical prophylactic dose of Tenofovir disoproxil fumarate in the vagina against HIV. The Tenofovir disoproxil fumarate vaginal *in-situ* gel was found to have varying degrees of mucoadhesion, retention and prolonged release based on their formula. Formulation batch F2 showed the desired mucoadhesion, retention in the vagina as well as the prolonged release of the drug up to 8 hrs. Hence, it was selected for release kinetics, where it followed the Korsmeyer-Peppas model. Formulation batch F2 was stable at the end of the accelerated stability study. Collectively, these data suggest the possibility of using the *in-situ* vaginal gel as a delivery system for HIV microbicides for the prevention of HIV transmission.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

HIV: Human Immunodeficiency Virus; **HET-CAM:** Hen's Egg Test-Chorioallantoic Membrane; **AIDS:** Acquired Immunodeficiency Syndrome; **FT-IR:** Fourier Transform Infrared Spectroscopy; **UV:** Ultra-Violet; **KBr:** Potassium Bromide; **NaCl:** Sodium Chloride; **hrs.:** Hours; **Min:** Minutes; **q.s.:** Quantity Sufficient; **nm:** Nanometer; **ml:** Milliliter; **µg:** Microgram; **g:** Gram; **cps:** Centipoise; **N:** Newton

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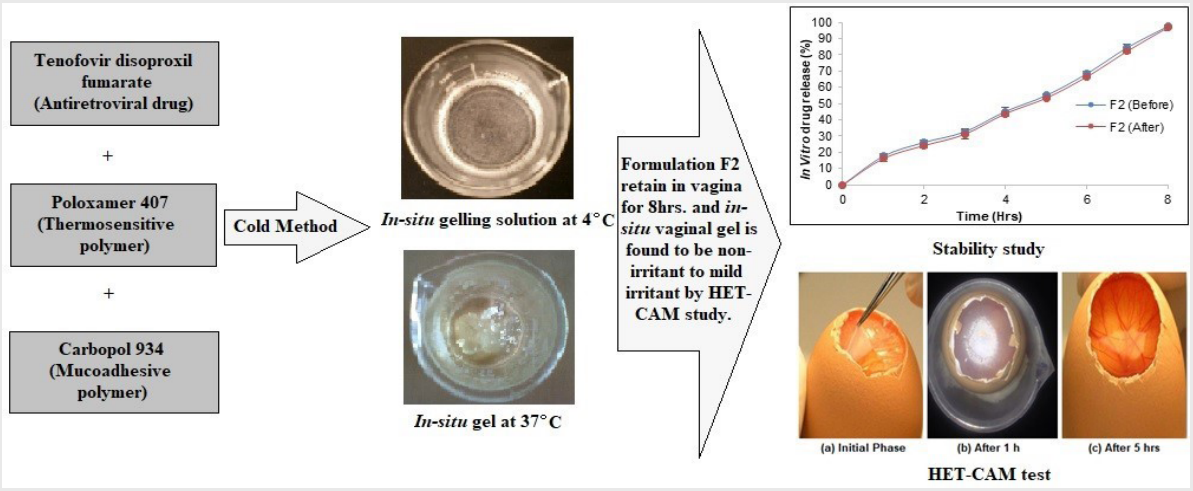
PICTORIAL ABSTRACT



SUMMARY

Tenofovir disoproxil fumarate is selected as a drug because it is one of the most common antiretroviral drug molecules used for HIV treatment. The objectives of the present investigation was to fabricate thermosensitive mucoadhesive *in-situ* vaginal gel of Tenofovir disoproxil fumarate to provide topical prophylactic dose of drug in vagina for HIV infection and also bring about extended retention of dosage from in the vaginal cavity. The vaginal *in-situ* gel of Tenofovir disoproxil fumarate was formulated using thermosensitive polymer poloxamer 407 and mucoadhesive polymer carbopol 934 by cold method. Prepared *in-situ* gel were characterized for drug-excipient compatibility, physicochemical characteristics like viscosity, pH and appearance, gelation temperature, *in-vitro* drug release study, mucoadhesive study, stability study and irritation study by HET-CAM Test. Drug excipient compatibility study revealed that there is no interaction between drug and excipients by means of FT-IR study. On the basis of evaluation parameters, Formulation F2 was selected as most appropriate formulation because it displayed desired mucoadhesion, retention in the vagina as well as the prolonged release of drug up to 8 hr. exploit it in therapy of HIV. It was also exhibited good mucoadhesive force (0.324 ± 0.036N) to retain formulation in vaginal cavity for longer period of time. Irritation study results revealed that the formulation is non-irritant to the vaginal mucosa. Stability study results showed that it was stayed steady for 6 months. Finally, these data suggesting the possibility of using the *in-situ* vaginal gel as a delivery system for sustained protection against HIV infection.

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