CHAPTER 5: DEVELOPMENT OF AEROSOL FOAM OF EFAVIRENZ LOADED NANOPARTICLES FOR VAGINAL DELIVERY

5.1. Introduction

The biodistribution study for the nanoparticles was performed through intra peritoneal route of administration to estimate the pharmacokinetic parameters and quantify the level of drug distribution in various organs\(^1\)\(^2\). As the results showed higher distribution of drug in genital\(^3\), and the recent literature survey supported and discussed the requirement of ant viral drug treatment for higher population of women especially for AIDS, the formulated Efavirenz nanoparticles suspension was developed into foam for effective vaginal application in female patients.

In the near past conference (March 2014) held at Boston, vaginal drug delivery for sustained release of drugs was discussed and strongly recommended, which could help in preventing the HIV transmission from female to male\(^4\).

5.1.1. Mode of administration

Route of administration is the direction by which a drug or any other substance is brought into contact with the body\(^5\). This is classified either by location of drug application or the target site of action; various routes include oral, topical, rectal, vaginal, parenteral, inhalation etc\(^6\). Currently, the drug industry and the scientific community pose a huge interest to exploit various mucosal routes for drug delivery, mainly to bypass first pass metabolism, enzymatic or microbial degradation and for drugs which exhibits poor absorption through oral administration\(^6\). It is noticeable that human vagina is relatively an unexplored route of drug delivery even though it is a non-invasive route of drug
administration\textsuperscript{7,8}. Vaginal route is an alternative route for drugs given by oral administration especially for orally unstable drugs. It is vital site as identified with many merits compared to other routes for enhancing bioavailability and modified drug delivery. Vaginal mucosal delivery is considered to be one of the best routes for the delivery of drugs such as contraceptive, hormones, steroids, anti-fungal/anti-bacterial, anti-retroviral etc\textsuperscript{9}. Intra-vaginal controlled release drug delivery systems are effective in achieving desired release of the medicament, for both local and systematic action. The dosage forms like pessaries, vaginal tablets, vaginal inserts, cream, powders, douches, gel, and foam are inserted / placed into the vaginal cavity to produce site specific effect in women. So the vaginal route is an important path for the application of long term therapy, which helps in avoiding the frequent administration and maintains the constant blood plasma level, unlike other routes of administration\textsuperscript{11-17}

5.1.2. Physiological condition of the human vagina

Human vagina is slightly S shaped with fibro muscular tube which is 8.4 to 11.3 cm in length and 2.1 to 5.0 cm in diameter extending from the cervical part of uterus. It is positioned upward and behind at around 45° in between the bladder (front) and rectum and anus from behind\textsuperscript{18} (Figure 5.1).

The vaginal wall is made up of of three layers i.e. (1) epithelial layer (2) muscular coat and (3) tunica adventia. Numerous folds in the surface of vagina are displayed due to the presence of areolar tissue (outer covering), smooth muscle layer (middle) and an inner layer of stratified squamous epithelium forming ridges or rugae. These ridges are responsible for the vast surface area in the vaginal wall. This is also an important feature
of the vagina that helps in dispensing medicament and holding and providing support for the drug at the site\textsuperscript{18}.

Figure 5.1. Anatomy of human vagina

The presence of the smooth elastic fibres as well as loose connective tissues of the tunica in the vaginal region makes this organ much elastic in nature. There is a vast network of blood supply to this organ due to the abundance of arteries (blood) and lymphatic vessels (lymph) in the vaginal wall. Drugs administered through vaginal route bypasses hepatic first pass metabolism because the blood exiting vaginal cavity enters peripheral circulation through the venous plexus that vacates the blood initially into internal veins of iliac followed by haemorrhoidal veins. Even though the human vagina does not poses any secretory glands, the surface of the vagina remains moist due to the cervical secretions and transudation from blood. Hence these secretions in addition to the secretions by endometrium and the fallopian tubes constitute the vaginal fluid which moistens the mucosa continuously\textsuperscript{19}.
Thickness of the vaginal wall alters during menstrual cycle by approximately 200-300 mm. Major alterations are also observed in terms of the composition and quantity of vaginal fluid during the cycle. A woman of reproductive age produces vaginal fluid at the rate of 6 ml/day approximately, with 0.5-0.75 ml constantly present in the vagina\textsuperscript{19}.

There is a presence of wide range of enzymes in the vagina which includes \textit{lactobacillus acidophilus}, lysozymes, esterases, nuclease, etc., The vaginal microflora varies depending upon pH, hormonal levels, age, as well as administration of contraceptives, steroids and other anti-microbial medications\textsuperscript{20-25}. \textit{Lactobacilli acidophilus} are normally present in the vagina and secrete lactic acid which acts as buffer, and hence maintains an acidic environment (pH 3.8 to 4.2) in normal healthy women of reproductive age. This acidic environment helps inhibiting the growth of most of the microbes which may enter vagina. The vaginal pH changes with respect to age, menstrual cycle (different stages) and sexual arousal (Table 5.1)\textsuperscript{19}.

Table 5.1. Influence of age on the variation of pH, length and width of human vagina

<table>
<thead>
<tr>
<th>S.No</th>
<th>Changes of vagina</th>
<th>pH</th>
<th>Length of vagina (cm)</th>
<th>Width of vagina (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Before puberty</td>
<td>6</td>
<td>4.5-6</td>
<td>1-1.5</td>
</tr>
<tr>
<td>2</td>
<td>Reproductive age</td>
<td>4-5</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>Adult premenopause</td>
<td>4-5</td>
<td>7-8</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Post menopause</td>
<td>4-7</td>
<td>4.5-6</td>
<td>1-1.5</td>
</tr>
</tbody>
</table>
5.1.3. Vaginal drug delivery systems

It has been known for several decades that various pharmacologically active agents such as steroids show good absorption through vaginal mucosal layers. Feasibility of intravaginal absorption of drug was first proved experimentally by vaginal administration of progesterone through suppository formulation. The first controlled DDS intended for vaginal administration was formulated in 1970 in the form of vaginal ring for delivery of contraceptive drug\(^26\,27\) (medroxy progesterone acetate). The insertion and removal of a drug releasing vaginal device is the most practical advantage of this route. It also provides continuous release of drug at the target site thus ensuring patient compliance. Similar to other mucosal route, drug molecule administered through vagina is absorbed in three steps\(^8\):

(i) Through transcellular route (concentration dependent diffusion)
(ii) Paracellularly (through tight junctions) or
(iii) Vesicular or receptor mediated transport.

5.1.4. Benefits of intravaginal drug administration

An intravaginal controlled release DDS has been used\(^20\,21\) to achieve sustained and controlled delivery of either systemically active drugs such as labor-inducing agents and spermicidal agents, prostaglandins and steroids and also for the locally active drugs, due to the presence of vast network of capillaries in the vaginal mucosa\(^28\). Sustained release therapeutics through vaginal mucosa prevents the rapid hepato-gastro intestinal biotransformation, and increase the therapeutic activity of drugs\(^29\). Pre-systemic elimination associated with oral therapy can also be reduced. The perineum venous plexus that drains vaginal mucosal walls and the rectum flows into pudendal vein and
finally into the vena cava that circum navigates first-pass effect\textsuperscript{30}. Hence this route may be of greater advantages for drugs which are poorly bioavailable when taken orally, especially those which gets excessively inactivated by the liver. Moreover, this route can also be beneficial for drugs causing adverse gastrointestinal irritation.

The main advantages of this route of administration is that it possesses high surface area of 100 – 150 cm\textsuperscript{2} for average adult women, easy accessible for self administration with large solid dosage form without discomfort to the user. Vaginal route of administration provides effects on local therapy and systemic activity due to larger surface area, high level of blood flow, escape from first pass metabolism along with high permeability rate and self insertion mode of administration\textsuperscript{31-35}

5.1.5. Limitations of vaginal drug administration

1. Gender specificity.
2. Influence of sexual intercourse.
3. Personal hygiene.
4. Drugs sensitive to acidic environment of vagina.
5. Drugs causing local irritation.
6. Wetting under-garments due to leakage of drugs from vagina\textsuperscript{10}.

5.1.6. Application of vaginal DDS

1) This route displays potential advantages for vaginal immunization and also delivery of steroidal drugs and hormones.
2) Repeated administrations of vaginal contraceptive rings are proved to be safe and effective.
3) Treatment for HIV and other microbial infections in women through vaginal route is more effective and safe\textsuperscript{36-39}.
5.2. Polymeric nanoparticles for vaginal drug delivery

Many sexually transmitted diseases can be cured and prevented by administering the drugs through vaginal route\textsuperscript{40,41}. Most of the anti-microbial\textsuperscript{42-44}, anti-viral\textsuperscript{46-50}, steroids, etc., which are used in the HIV treatment and ART regime show low oral bioavailability and also causes drug resistance. This situation makes the researchers to find out alternative methods for prevention of disease and control modes and also it is considered as international priority.

Nano drug delivery has become more attractive recently even though it is not new, due to excessive research in materials and engineering through nanotechnology. Nanoparticles are either nanosphere or nanocapsule which lies in the range of 1 – 100 nm used widely for various applications in biology and medical field. Large numbers of researchers make use of it due to its increased unique function and properties. And also it is understood that material in nano structure which is smaller than most of the cells are more interactive with biological environment. With its wide range of applications such as cardio vascular, orthopedic, tissue engineering, devices in clinical, bioanalytical, diagnostics and therapeutics, cancer therapy, it is being used extensively for site specific drug delivery\textsuperscript{51}.

Nanoparticulate system for drug delivery can be divided into micelles, conjugates, composites, and polymeric nanoparticles. Colloidal system in nano size range developed using natural or synthetic polymeric materials that encapsulate or entrap the therapeutically active ingredients can be defined as medicinally active polymeric nanoparticles. This polymeric nano carriers act as a good vehicle to supply the API at the site of action\textsuperscript{51}.
5.3. Foam technology for vaginal delivery of drugs

Growing interest in dermatology makes different researches to come out with various solutions, with most valuable outcome of those studies as Foam technology\textsuperscript{52,53}. It is widely believed to be the future for dermatological drugs as considered by the pharmaceutical giant Foamix. Different types of particulate systems like polysaccharide nanoparticles, polymeric nanoparticles and lipid nanoparticles are used in the topical drug delivery. However they have some problems regarding drug release which can be overcome by using foam as a vehicle. Pressurized pharmaceutical foams are dynamic dispersal systems with active agents, solvents, surfactants, propellants that are sealed in the canister under pressure. By actuation of the value the outcome will be obtained in the form of fine droplets or foam. Aerosol foam formulation is a drug delivery system applied as spray, which deposits foam upon contact with surface of the biological system\textsuperscript{54-59}. This can be classified on the basis of their property such as aqueous/ non-aqueous, liquid/ solid, pharmaceutical and non-pharmaceutical. Its purpose in pharmaceutical field is in contraception, packaging, a vehicle to deliver drugs topically, dressings and purification of biological materials. Five most common methods that involved in the production of foams include:

1. Agitation of a solution using mechanical force
2. A stream of gas injected into the liquid
3. A stream of liquid injected into the liquid
4. Injection of gas and liquid simultaneously into the chamber
5. Impulsive reduction of pressure in the solution
Among these the method that is utilized for topical foam formulation is sudden reduction in pressure of the solution where ingredient gets combined with the propellant that is filled by pressure fill method. The efficient delivery of foam to the skin depends on propellants. Various propellants are available for the use, among those the most commonly used one is hydrofluoroalkanes and hydrofluorocarbons. Mechanism involved in the dispersion of particles via foam is by rapid evaporation of propellants after the actuation of valve. The excipient gets mix up with air which results in air bubbles having air/water interface. The surfactants stabilize the foam by quickly rearranging the interface to reduce surface tension. Further its stability is governed by Ostwald ripening and foam drainage. Stability can be assessed using optical microscopy, light scattering, and imaging by fluorescence or magnetic resonance. These evaluations are very important for developing efficient topical foams (Figure 5.2).

Figure 5.2. Illustration of mechanism of foam delivery for vaginal application
It helps in overcoming the limitations produced by other delivery systems like leakage, uneven distribution, irritation (high dose / insoluble matter), self clearing and low contact time. The foam is more advantageous over other conventional topical dosage form as it has specific benefit which includes:

- Can be easily applied over the targeted area with uniform spreading,
- Less sticky than creams and gels, High level of spreading , Improved local drug residence
- High contact time; Leaves no residue
- Application of accurate dose is possible (metered dose valve)
- Better patient compliance
- Minimizes rubbing process hence irritation and pain are reduced
- Contamination of product due to direct contact is reduce and
- Enhanced delivery and action of drug

Nature of foam can be categorized into four groups, they are: (i) drug specific- that are designed to deliver particular active agent, (ii) new methods for foam generation, (iii) topical foams, (iv) foams that enhance skin penetration of active agents. Foams show better role in case of insoluble drugs as they are dispersed in propellant and surfactant system which acts as a carrier and delivers it in an effective manner. As well as, its prolonged retention over the skin helps in better absorption.\(^{54}\)

Albert.Z.A and Lilian.F in their patented article ‘Pharmaceutical foam’ make clear about the topical delivery foam composition and other aspects related to such formulations
(Table 5.2). Furthermore they provide details about the quality of foam and their description that helps to understand the required foam quality in the formulations\textsuperscript{61}.

5.4. Rationale for Intra vaginal foam delivery of Efavirenz nanoparticles

Generally, vaginal route of administration is adopted to achieve both systemic and local effect. Some studies have confirmed that drug administration by intra-vaginal route showed higher bioavailability compared to oral route, due to its avoidance of first pass metabolism\textsuperscript{62}. Few systemically active drugs like hormones, contraceptives and many other drugs are administered for local action through this site\textsuperscript{63}. But in case of systemic delivery, vaginal route of administration is highly variable due to muscular and vascular thickness during the different stages of menstrual cycle and other factors like secretions, personal hygiene, nature of dosage form etc.\textsuperscript{64}

In this work, the developed nanoparticles formulation is intended for local action in HIV prophylaxis because, Efavirenz can be used for long term pre-exposure prophylaxis of HIV-I and has maximum stability at lower pH (around pH 4) and hence, recommended through the vaginal route\textsuperscript{65}

The aerosol foam formulations can also be suggested for both local and systemic action, where the sustained release Efavirenz nanoparticles could remain in the vaginal cavity and act as the microbicide in hetero sexual infections and act as long term prophylaxis, respectively.
Table 5.2. Description for foam quality

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Full, fine, stable (holds structure or only a very slow, small collapse over 30-60 sec)</td>
</tr>
<tr>
<td>1</td>
<td>Mostly fine with a couple of coarser bubbles on surface then stable, or fine then slightly coarser over time.</td>
</tr>
<tr>
<td>2</td>
<td>Slightly coarse initially but reasonably stable, or fine (possibly some slight dimples) with a couple of larger bubbles appearing on surface or a flat but fine and reasonably stable.</td>
</tr>
<tr>
<td>3</td>
<td>Slightly coarse bubbles then growing larger throughout or very coarse but stable, or fine (possibly with dimples) then many larger bubbles appearing on surface or fine then quick collapse</td>
</tr>
<tr>
<td>4</td>
<td>Coarse bubble quickly grows to larger throughout or fine but rough surface and quickly to large bubbles throughout or fine with many large bubbles immediately on surface</td>
</tr>
<tr>
<td>5</td>
<td>Out as large bubbles or immediate break to large bubbles</td>
</tr>
</tbody>
</table>
Also the vaginal route of antiviral drug administration is considered as the safe and effective route for the treatment of HIV infection and other sexually transmitted diseases. For proper delivery of foam into the vagina, vaginal applicators are used. The applicator contains a long tube for collecting the required dose of medicament from the container and a plunger to push the content into the vaginal cavity. The applicator could be fixed on the mouth of the aerosol canister and pressed down to fill in with the required quantity of foam. It is then inserted deep into the vagina and the plunger pushed to release the medicament into the vaginal cavity.

5.5. Materials and Methods

5.5.1. Development of Aerosol Foam containing the Nanoparticles:

All the formulations EFV 1, EFV 2 and EFV 3 of Efavirenz Eudragit E-100 nanosuspension were developed into aerosol foam by compression filling technique, in 250 ml canister. In order to get the product outcome in the form of foam discharge, hydrofluoro carbon (1,1,1,2-tetrafluoro ethane) and 0.1% w/v of sodium lauryl sulphate were used as propellant and foaming agent, respectively.

5.5.2. Evaluation of aerosol foam

5.5.2.1. Relative foam density

The pressurized container was shaken well and small amount of foam was dispensed out. A flat bottomed dish with approximate volume of 60mL was taken and uniformly filled with foam. After the foam had completely expanded it was leveled off by removing the excess foam with slide and then the dish was weighed (BL- 220H, Shimadzu). Similarly
mass of the same volume of water was determined by filling the dish with water and the weight was noted. Relative foam density was calculated by the formula\textsuperscript{61}

\[
\text{Relative Foam Density} = \frac{m}{e}
\]

Where, \(m\) = mass of the test sample of foam (g), \(e\) = mass of the same volume of water (g)

5.5.2.2. Visual assessment of foam

Foam structure and foam characteristics were depicted by visual examination, which includes bubble size, shape, clarity and collapse time. The formulation in the canister was sprayed and was visualized for the bubble size in order to identify its coarser or fine appearance; the foam structure, as well as the clarity of foam as it plays a role in aesthetic value. The foam was sprayed over the dish/hand and the time taken by the bubbles to disappear completely to leave the formulated drug over the surface was noted as the collapse time\textsuperscript{69}.

5.5.2.3. Determination of drug content per puff

Drug content in the delivered foam was determined by dispensing one puff of foam of approximately 20mL from the container into the beaker. Then 0.1N HCl was added to the sample and make up to 10mL and further analyzed under UV-Vis spectrophotometer at \(\lambda_{\text{max}}\) of 247nm, to estimate the concentration of the drug present in it\textsuperscript{68}. 
5.5.3. *Ex-vivo skin permeation study*

The *ex-vivo* permeation study was performed for the optimized formulation, to evaluate the amount of drug across the biological membrane. This study was performed using buccal mucous membrane of goat due to its easy availability and difficulty in procuring the vaginal mucous membrane of other mammals\textsuperscript{70-75}. Literature confirms that the buccal and vaginal mucous membranes are similar in various characteristics like moisture, pH, structure and composition\textsuperscript{76}. The layers of the buccal and vaginal membrane are made up of thin epithelial cells surrounded by muscular coat followed by loose connective tissue. Due to their similarity, many researchers have utilized as an alternative model to study drug permeability, bioadhesiveness, drug delivery and so on. The water permeability through buccal and vaginal membrane was reported to be similar and also drugs like vasopressin, 17β-estradiol and 4kD-dextran showed no statistically significant difference in diffusion and flux through human to buccal and vaginal membranes\textsuperscript{77-88}. Moreover, the buccal mucous membrane of animals like cow, goat, pig is similar human buccal membranes, wherein the thickness and composition do not vary significantly. The vaginal membrane of these animal models is also reported to be similar to human\textsuperscript{82-84}. Hence, with this literature background, goat buccal mucosa membrane was used as an alternative for vaginal model in the present study.

Excised goat skin was obtained from the slaughter house, the hairs and fatty tissues were removed and the mucosal membrane was separated. The membrane was placed in between the donor and receptor compartment of the Franz Diffusion Cell (Figure 5.3). About 1 ml (equivalent to 2mg) of the formulation was placed above the membrane
(donor cell) and simulated vaginal fluid media was filled in the receptor cell to touch the surface of the membrane. The entire set up was placed in magnetic stirrer maintained at 37°C and stirred at 100 rpm. At periodic time intervals, 5 ml of samples were collected from side tube of the receptor cell and replaced with the same volume of media to equilibrate the surface of the membrane. The collected samples were analyzed by UV-Visible spectrophotometer (SL150, Elico Instrumentation Ltd, Mumbai, India) to estimate the percentage of drug diffused at each time point\textsuperscript{89,90}.

Figure 5.3. Franz Diffusion Cell (in-house made)
5.6. Results and Discussion

5.6.1. Characteristics of the aerosol foam

The pharmaceutical vaginal foam was prepared by filling propellant and hydrofluoro carbon inside the pressurized container in such a way to deliver foam when the actuator was pressed. All the three formulations (EFV 2, EFV 3 and EFV 4) were selected on the basis of its physical stability and particle size distribution. Surfactant plays a vital role along with propellants, because the stability of the foam was totally dependent on these two. It helps in dispersion of the drug within air or liquid interface which consequently increases the drug release from the nanoparticles.

The results of the physico chemical evaluation of the aerosol foam formulations containing the polymeric nanoparticles of Efavirenz are shown in Table 5.3 and Figure 5.4 and Figure 5.5.

The relative foam density was found to be in the range of 0.06 to 0.07. The bubble size of the aerosol foam was smaller, as the time increases the bubble becomes bigger and finally ruptured out due to thinning of the film\textsuperscript{91}.

The shape and clarity of the appeared foam was spherical, and the formulations were clear, colourless and translucent in nature. The foam was found to be in the grading scale\textsuperscript{61} of 5 Hence it possess the characteristics such that fine foam becomes coarser bubble on standing, and also the foam appeared to be rich and creamy, which may get converted into creamy layer upon contact with the mucous layer on application\textsuperscript{92}. 
The bubble collapse time was found to be in the range of 1 – 5 minutes, wherein the formed bubbles were breaking quickly due to penetration of surfactant into the aqueous phase which decrease the bubble film thickness resulting in rupture of the bubble\textsuperscript{93,94}. The drug content per puff of the foam was found to be in the range of 34 – 38 µg per puff.

Table 5.3. Properties of Aerosol foam formulation containing Efavirenz Eudragit E-100 nanoparticles

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Relative foam density</th>
<th>Collapse time (min)</th>
<th>Bubble size</th>
<th>pH</th>
<th>Clarity</th>
<th>Drug content (µg/puff)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFV 2</td>
<td>0.071±0.002</td>
<td>1</td>
<td>Large</td>
<td>6.91±0.6</td>
<td>Translucent</td>
<td>35.23±1.5</td>
</tr>
<tr>
<td>EFV 3</td>
<td>0.0673±0.015</td>
<td>3</td>
<td>Medium</td>
<td>7.1±0.2</td>
<td>Translucent</td>
<td>34.21±2.0</td>
</tr>
<tr>
<td>EFV 4</td>
<td>0.0723±0.001</td>
<td>5</td>
<td>Large</td>
<td>7.3±0.2</td>
<td>Transparent</td>
<td>38.87±1.2</td>
</tr>
</tbody>
</table>
Figure 5.4. Foam characteristics of Aerosol foam formulation of Efaverinz Nanoparticles EFV 2 of Efavirenz nanoparticle.
5.6.2. *Ex-vivo* Skin Permeation study

The *ex-vivo* diffusion of Efaverinz from the polymeric nanoparticles was studied using excised goat skin membrane. Nanoparticle formulation containing 1:0.5 ratio of drug and polymer exhibited very slow permeation, due to their larger size and poor encapsulation in polymer. An increase in drug diffusion was observed with increase in the polymer ratio for EFV 2 and EFV 3 ratio formulations, wherein the later showed 100% drug diffusion within 14 h. A further increase in the concentration of polymer at EFV 4 levels reduced its diffusivity because of the yield of larger particles and precipitation of polymer\textsuperscript{95} (Figure 5.6).
The *in-vitro* drug release pattern of all the formulations was comparable to their corresponding *ex-vivo* profile. Yet statistically significant difference could be observed between the *in-vitro* and *ex-vivo* data of each formulation. The Efavirenz loaded polymeric nanoparticles formulation demonstrated 40-100 % release in dialysis method of study, and approximately 30-100% in Franz diffusion model at the end of 24 h. Since the excised skin membrane was thicker (due to mucosal and epithelial layers) than the uniform porous single layer dialysis membrane, the amount of drug diffused was considerably low in the *ex-vivo* study (Figure 5.7-5.10).

![Graph showing drug diffusion over time for different formulations](image)

**Figure 5.6.** Comparative *ex-vivo* skin permeation studies of different formulations of Efavirenz loaded polymeric nanoparticles in simulated vaginal fluid
Figure 5.7. Comparative *in-vitro* & *ex-vivo* drug release of Efavirenz loaded polymeric nanoparticles (EFV 1:1:0.5 ratio) in simulated vaginal fluid.

Figure 5.8. Comparative *in-vitro* & *ex-vivo* drug release of Efavirenz loaded polymeric nanoparticles (EFV 2:1:1 ratio) in simulated vaginal fluid.
Figure 5.9. Comparative *in-vitro* & *ex-vivo* drug release of Efavirenz loaded polymeric nanoparticles (EFV 3:1:2 ratio) in simulated vaginal fluid

Figure 5.10. Comparative *in-vitro* & *ex-vivo* drug release of Efavirenz loaded polymeric nanoparticles (EFV 4:1:3 ratio) in simulated vaginal fluid
5.6.3. Permeation data analysis

The effective surface area of the ex-vivo membrane exposed for permeation was calculated to be 2.54 cm$^2$. The cumulative amount of drug permeated per unit area vs. time plot (Figure 5.11.) was made to determine the flux, lag time and permeation coefficient$^{96}$ and the data are shown in Table 5.4)

Table 5.4. Ex-vivo permeability data analysis of different formulations of Efavirenz loaded polymeric nanoparticle

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Q$_{24}$</th>
<th>J$_{SS}$</th>
<th>K$_P$</th>
<th>T$_L$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFV 1</td>
<td>118.09 ± 6.5</td>
<td>6.15 ± 0.14</td>
<td>6.15 ± 0.14</td>
<td>1.3 ± 0.48</td>
</tr>
<tr>
<td>EFV 2</td>
<td>478.76 ± 13.8</td>
<td>22.76 ± 1.8</td>
<td>9.56 ± 0.75</td>
<td>0.84 ± 0.0005</td>
</tr>
<tr>
<td>EFV 3</td>
<td>954.76 ± 17.2</td>
<td>82.19 ± 4.7</td>
<td>34.53 ± 2</td>
<td>0.39 ± 0.021</td>
</tr>
<tr>
<td>EFV 4</td>
<td>168.7 ± 3.9</td>
<td>9.17 ± 0.23</td>
<td>9.17 ± 0.23</td>
<td>1.2 ± 0.026</td>
</tr>
</tbody>
</table>

The cumulative amount of drug permeated from the polymeric nanoparticles showed wide difference based on the polymer ratio in the formulation. The EFV1 (1:0.5 ratio) formulation exhibited least value of 118 µg/cm$^2$ and the EFV3 (1:2 ratio) trial showed 954 µg/cm$^2$ approximately at the end of 24 h. The formulation containing EFV 2 (1:1
ratio) of drug and polymer was found to be optimum with cumulative amount of drug release of 500 µg/cm². Accordingly the steady state flux, calculated the linear slope of the curve was 22.76 ± 1.8 µg/cm²/h in EFV 2 formulation, Also, the permeability coefficient value was found to be 9.56 ± 0.75 cm/h x 10⁻³ for this formulation. The lag time was found to decrease with increase in the polymer ratio from 1:0.5 to 1:2. But further increase in polymer level increased the lag time, due to decrease in the flux rate and permeability coefficient⁹⁷.

![Graph showing cumulative amount of drug permeated vs time for different ratios of drug to polymer](image)

Figure 5.11. *Ex-vivo* permeability data analysis of different formulations of Efavirenz loaded polymeric nanoparticle
5.7. Conclusion

The nanoparticles of Efavirenz-Eudragit E-100 were developed into aerosol spray by pressure filling process for its effective delivery through the vaginal route. Surfactant and propellent played significant role in the stability and delivery of required amount of nanoparticles via the actuator mechanism. The relative foam density of 0.06 – 0.07, smaller bubble size, formation of spherical clear colourless foam and bubble collapse time in the range of 1 – 5 min were found to be acceptable for its administration. Drug content per puff of the foam was around 34 – 38 µg. Ex-vivo diffusion of nanoparticles by Franz diffusion model through the excised goat skin membrane was better in EFV 2 and EFV 3 formulations, due to their size of particles around 100 nm and high polymer encapsulation. The permeation data analysis depicted that the formulations containing EFV 2 and EFV 3 drug and polymer exhibited higher cumulative amount of drug permeation (500 µg/cm² and 954 µg/cm², respectively) at the end of 24 h. Also, the steady state flux and permeability coefficient of the EFV 2 formulation was calculated to be 22.76 ± 1.8 µg/cm²/h and 9.56 ± 0.75 cm/h x 10⁻³, respectively. The lag time for the permeation process of EFV 2 formulation was 0.84 h. In conclusion, aerosol system containing polymeric nanoparticles of Efavirenz could provide effective dose delivery into vaginal cavity that act as suitable site for better drug release and permeation through the membrane to achieve required local and systemic action.
5.8. References


51. Anil Mahapatro, Dinesh KS. Biodegradable nanoparticles are excellent vehicle for site directed in-vivo delivery of drugs and vaccines. J. Nanobiotech. 2011, 9:55


74. Some ban, some restrict, a few don’t". The Indian Express. 2012-01-04. Retrieved 2015-06-06.


77. [http://www.interspeciesinfo.com/Interspecies/Mouth](http://www.interspeciesinfo.com/Interspecies/Mouth)


