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Dolutegravir sodium loaded solid lipid nanoparticles: A vaginal drug delivery system for pre-exposure prophylaxis of HIV

Ruhi ANJUM [1 *], **PK LAKSHMI** [1]

[1] Department of Pharmaceutics, Faculty of Pharmacy, G Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad, India.

* Corresponding Author: E-mail: drlakshmisuresh@gmail.com (PK.L.); Tel. 09-00-004 44 52.

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ABSTRACT: The objective of this study was to formulate dolutegravir sodium an antiretroviral drug into topical (vaginal) semisolid solid lipid nanoparticle gel formulation using a rapid, economical one step process. Solid lipid nanoparticles formulations were prepared using solvent injection method combined with sonication, using different type of lipids (e.g. Phospholipon 80H, Phospholipon 90H and Soy lecithin) and surfactants (Poloxamer 407, Poloxamer 188, and Tween 80). The SLN gel was formed in one step process using poloxamer 407 & water forming aqueous phase and stearic acid & phospholipon 80H in ethanol forming lipid phase. The lipid phase was injected into aqueous phase forming a creamy gel at 70^0 C. Hence it does not require any gelling agent. It was optimized using a systematic approach of design of experiments. The formulation was evaluated for particle size, polydispersity index, zeta potential, flux, entrapment efficiency and results were found to be 455 nm, 0.411, -26.6 mV, 43.7±-0.12 µg/cm²/hr, 76.2% respectively. Drug release studies were conducted using two membranes via; dialysis membrane and goat vaginal tissue. The release pattern of the drug followed first order kinetics with Higuchi release mechanism. The release exponent 'n' of the Korsemeyer equation indicates the Fickian diffusional drug release. The *ex vivo* vaginal study of F2 formulation showed 64.89% of tissue deposition, which was 16 times more than the pure drug. This study concluded that the dolutegravir sodium, a sustained release solid lipid nanoparticle gel may have increased vaginal deposition and might show site targeted effect.

KEYWORDS: Dolutegravir sodium; solid lipid nanoparticles; solvent injection method; vaginal delivery; gel.

1. INTRODUCTION

Human Immuno deficiency virus (HIV) originated from simian immunodeficiency virus (SIV) which is a worldwide burden. HIV infects human immune system cells (CD4+T Cells (T-helper cells), dendritic cells and macrophages). CD4+ T lymphocytes are used by HIV as host to make copies and infect other cells of the body and thus immune system gets collapsed due to reduction in number of CD4+ cells which ultimately leads to AIDS [1]. Dolutegravir sodium (DTG.Na) is an integrase inhibitor, approved for the treatment of HIV [2]. It is more effective than other integrase strand transfer inhibitors (ISTIs) as these may gain resistance against the virus but DTG.Na plays an efficient role in being effective against such resistance [3]. DTG.Na belongs to BCS class II and is a second generation HIV integrase inhibitor used in the treatment of HIV and also used in the pre-exposure prophylaxis [PrEP]. It is prevention for people who do not have HIV but who are at substantial risk of getting it. Oral intake of DTG.Na may lead to adverse effects such as changes in immune system, hypersensitivity reactions and sometime organ dysfunction may occur. In order to avoid systemic side effects this can be formulated as site targeted drug delivery system i.e., targeting to vaginal tissue in the form of solid lipid nanoparticle (SLN) gel.

There are various nanotechnology based strategies used in the development of delivery system. Among which SLN holds a great potential in effective delivery of poorly soluble drugs by improving solubility and oral bioavailability. SLN was formulated in the 20th century as a different option for emulsions, liposomes and polymeric nanoparticles in order to regulate the drug release mechanism. SLNs contain a solid lipid, emulsifier, co-emulsifier and water.

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SLN's are prepared using different methods and different lipids, which are solid at room and body temperature. They exhibit significant benefits such as controlled release, better bioavailability, defence of chemically changeable drugs, and enhanced drug loading etc [4]. Nanoparticles have small size, large surface area, and high drug loading capacity and have great potential application for intravenous, ocular, oral, dermal, rectal, vaginal route of administration and are potential target for cytotoxic drug [5]. As SLN are biocompatible and biodegradable in nature hence these are used as a carrier for formulating wide variety of poorly water soluble drugs. This can be formulated as SLN gel owing to its low solubility where, poloxamer used in the formulation improve the solubility of the drug and lipid used will entrap the drug and prevent it from degradation.

2. RESULTS AND DISCUSSION

Solubility studies of drug in different lipids were studied as tabulated in Table 1. The excipients showing more solubilization potential for DTG.Na were selected for the preparation of SLN. Solubility of drug in two different type of buffers was studied, solubility in distilled water and citrate buffer was found to be 160 and 48.9 µg/ml respectively.

Table 1. Solubility studies of drug in different lipid.

Lipid and lipophilic surfactant	Melting point (°C)	Solubility (mg/gm)
Stearic acid	69.3	6
Phospholipon 80H	100	13.38
Phospholipon 90H	128	12.1
Poloxamer 188	52	25
Poloxamer 407	53-57	33

[a] All values represents mean ± SD (n=3)

All the prepared gels of selected DTG.Na SLN were spreadable, white, smooth and homogenous with semisolid consistency and show no syneresis and the pH was found to be between 4.0 - 4.6. The percentage drug content for all the prepared formulations was evaluated and the content of the drug ranged from 94.5% to 98.8%. The F2 formulation was found to have maximum drug content which indicates efficient loading. All the formulations were tested for spreadability parameter and was found to be good i.e., between 2.5 to 3.3 gm.cm/sec. Extrudability of all six formulations (F1 to F6) was exceptionally good. Viscosity of all the formulations was very good since the value was found in the range of (20000 - 21000 cps). Increase in viscosity might be due to increased solid lipid content and in the recent studies it was found that the formulation containing ethanol and poloxamer together may increase viscosity [6, 7, 8]. All the formulations were found to be spreadable, white, smooth with semisolid consistency and show no syneresis.

The Z average for F2 formulation was found to be 455.7 nm as shown in Figure 1. DTG.Na SLN exhibited lower particle size when prepared using Poloxamer 407. This result may be due to Poloxamer 407, as it has higher molecular weight and higher HLB value when compared to other used surfactants [9]. Surfactants or stabilizers are generally involved in the process to alter the surface properties and to impart stability to nanoparticles. By increasing the interfacial stability of solid lipid nanoparticles surfactant allows the formation of smaller droplet. The mean diameter of solid lipid nanoparticles may increase with the decrease in concentration of Poloxamer, the possible reason for the formation of larger size nanoparticles could be reduced interfacial stability, ensuring from an insufficient amount of surfactant leading to aggregation and coalescence of nanoparticles [10]. The nanosize range and a good uniformity in the SLN size may be attributed to the composition of the prepared lipid nanoparticles in which the optimum level of solid lipid (stearic acid) intercalates with that of surfactant [11].

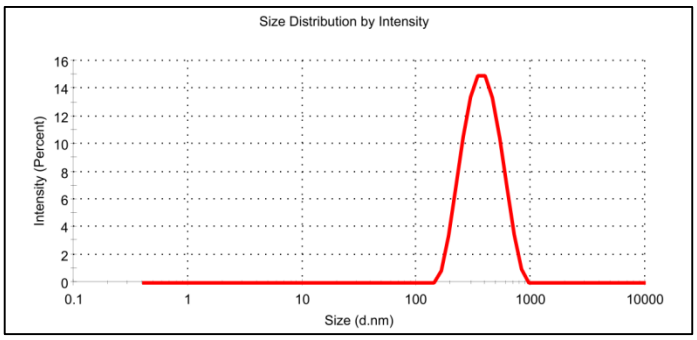

Figure 1. Particle size of optimized formulation.

The polydispersity index (PDI) is an indicator of particle size distribution. The PDI value of optimized formulation was found to be less than 0.5 which indicates a narrow size distribution and shows the correctness of the method of preparation. Zeta potential for F2 formulation was found to be -26.6 mV as shown in Figure 2. Zeta potential plays an important role in surface characterization which helps in determining the possible stability and surface charge of the nano particulate system. Usually, stability is increased by large negative (-) or positive (+) zeta potential value. It is compulsory for the formulation, as electrostatic repulsion between particles with like charges avoid aggregation of particles. In the present study, the ζ potential obtained for SLN formulation exhibited negative zeta potential value because of the presence of the stearic acid which is close to -30 mV ensuring physical stability. Similar results are depicted in earlier studies on voriconazole SLN [11]. Poloxamer being non-ionic surfactant was able to produce the stable SLN formulation. Although non-ionic surfactant might not ionize into charging group like ionic ones, but still demonstrated its zeta potential. The reason behind it might be due to molecular polarization. It was reported that poloxamer was one of the effective non-ionic surfactant added to avoid aggregation in the formulation. In addition to electrostatic stabilization poloxamer was also proved to provide steric stabilization [10].

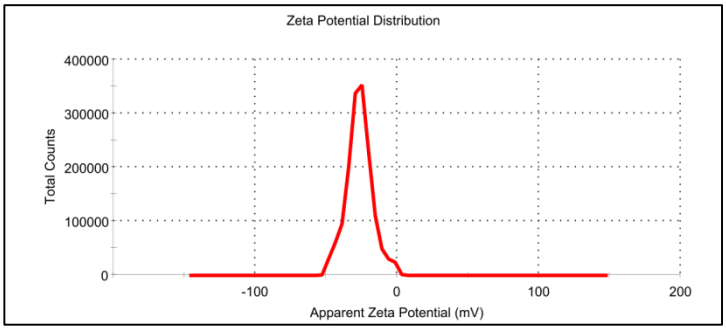

Figure 2. Zeta potential of optimized formulation.

SEM studies showed that the DTG.Na loaded solid lipid nanoparticles were almost spherical in shape as shown in Figure 3.

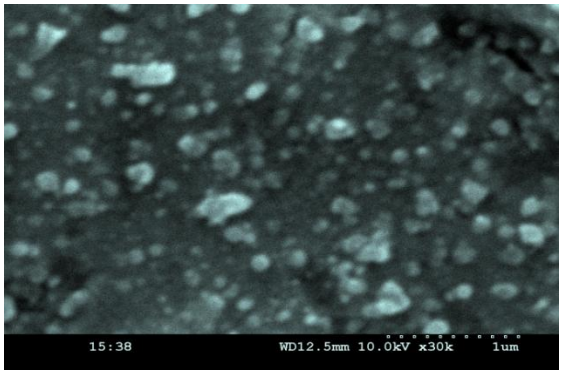

Figure 3. SEM image of optimized formulation.

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Determination of EE is an important parameter in case of solid lipid nanoparticles as it may affect the drug release and vaginal deposition. Lipids have shown positive influence on the corresponding percentage entrapment efficiency of DTG.Na SLN and it was found to be satisfactorily high for first six formulations. The results suggested that as the polymer concentration increases, the drug entrapment efficiency increases significantly due to its higher viscosity. But further increase in polymer concentration showed the decrease in entrapment efficiency which is basically due to decrease in drug loading, because:

Entrapment efficiency = actual drug loading: theoretical drug loading [Eq. 1]

Taguchi L^9 orthogonal array (3^4) design experimental trials were conducted using Minitab-18 which shows relation between independent and dependent factors (EE) as shown in Table 2. The ranks obtained for each factor will determine its effect on the response (entrapment efficiency). From Figure 4, it can be inferred that Factor A i.e., type of lipid has the greatest influence on the response. The other 3 Factors B, C and D i.e., type of surfactant, concentration of lipid and concentration of surfactant has equal influence on the response. Therefore, the order of independent variables on which entrapment efficiency depends was found to be: type of lipid > type of surfactant, concentration of lipid and concentration of surfactant as shown in Figure 4.

Table 2. Taguchi L^9 orthogonal array (3^4) design experimental trials.

Formulation code	Independent factors				Dependent factor
	Type of lipid	Type of surfactant	Concentration of lipid (%)	Concentration of surfactant (%)	Entrapment efficiency (%)
F1	1	1	3	1	70.3
F2	1	2	4	2	76.2
F3	1	3	5	3	73.5
F4	2	1	4	3	71.5
F5	2	2	5	1	75.4
F6	2	3	3	2	72.9
F7	3	1	5	2	30.4
F8	3	2	3	3	32.1
F9	3	3	4	1	31.0

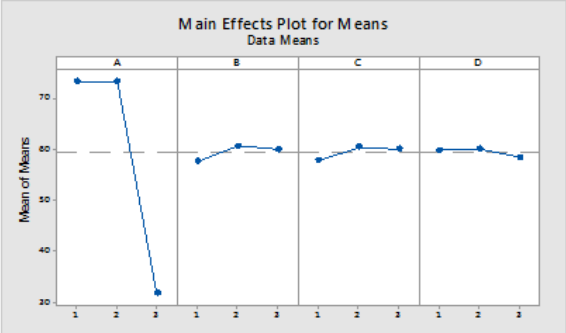

Figure 4. Main effect plot for mean.

The *in vitro* diffusion studies plays an important role to know the amount of the drug that has actually diffused across the dialysis membrane over a period of time and thus the release rate of each formulation was calculated and the efficiency of lipid and surfactant used in the formulation of DTG.Na SLN gel on drug release was known to ensure the release of drug from the SLN gel. The Taguchi formulations which have higher EE (above 50%) were further evaluated for drug release. This study was performed on Taguchi experimental batches using dialysis membrane. From the results, it was observed that the formulations containing Poloxamer 407 have shown greater drug release as shown in Figure 5. This may be due to the

higher HLB value and the amount of drug release was found in the order Poloxamer 407> Poloxamer 188> Tween 80 [12]. F1 and F4 have shown lowest drug release this may be due to lower HLB value of Tween 80. The result initially showed burst release followed by sustained release. The drug release pattern in SLN formulation showed biphasic release behavior consisting of initial burst release followed by sustained release. DTG.Na present on the surface of nanoparticles helps in initial burst release and larger diffusional distance gave sustained release [13].

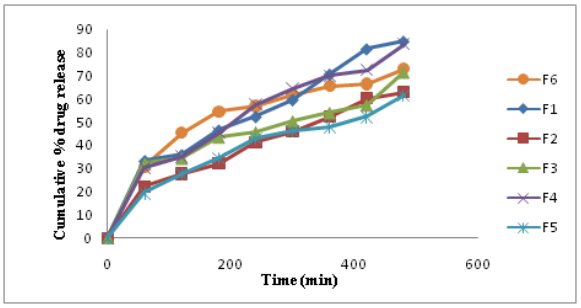

Figure 5. Cumulative percentage drug release of optimized formulation.

This study was performed for optimized Taguchi experimental batch using female goat vaginal tissue to know the amount of drug that has actually diffused across the tissue over the period of time, thus permeation parameters and kinetics of drug transport can be assessed. *Ex vivo* permeation studies of formulation F2 and pure drug were carried out. The drug release profile shown in Figure 6. It was observed that the formulation F2 showed the drug release of 32.56 % and pure drug showed the drug release of 18.66 %. The drug release from vaginal tissue when compared to the dialysis membrane was less, the reason being attributed that vagina is a biological membrane having three different layers, which might be the reason for the slow release of the drug entry into the vaginal tissues compare to dialysis membrane. Also, the drug has an ideal log p-value of 2.2 possessing ideal HLB, which may follow any one or more transport mechanism such as diffusion through cell along the concentration gradient (trans cellular or receptor-mediated transport) [14]. The *ex vivo* permeability parameters (flux and permeability coefficient) were calculated for the optimized formlation.

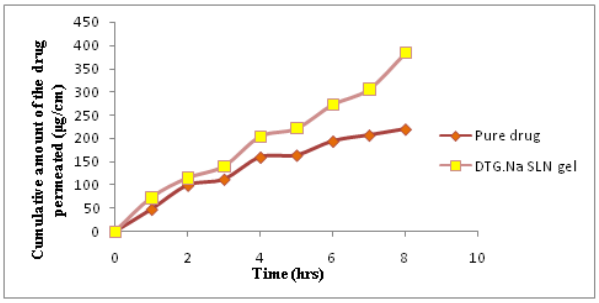

Figure 6. Cumulative drug release of optimized formulation and pure drug.

Vaginal tissue of female goat was used to perform the retention studies of DTG.Na SLN gel and plain drug. The percentage deposition profile of SLN loaded gel formulation was highest i.e., 64.89 % when compared to pure drug 4.02 % accumulation in the vaginal tissue. It was assumed that the remaining drug (77.38 %) was found superficially on the top layer of vaginal tissue. The solubility of DTG.Na was improved by formulating it into nanoparticulate system (SLNs) using different excipients. Where, poloxamer assist in improving solubility of DTG.Na and might also improve the affinity between lipid particles and vaginal membrane and the high lipid content in the formulation aids in the deposition of SLNs in the cavity for its action. From the results of *ex vivo* drug release kinetics for optimized formulation it was found that it follows first-order drug release kinetics and Higuchi drug release mechanism. From the value of release component "n" it can be concluded that the formulation has Fickian diffusion which refers to diffusion controlled rate release [15].

3. CONCLUSION

DTG.Na is successfully formulated as SLN loaded gel for vaginal delivery using different lipids and surfactants. The prepared formulation was evaluated for physicochemical parameters, entrapment efficiency, particle size, zeta potential, SEM, *in vitro* studies, *ex vivo* studies and vaginal tissue penetration studies. Among all the formulations prepared SLN prepared using Phospholipon 80H, Poloxamer 407 showed best results. This study concluded that the DTG.Na sustained release SLN gel may have increased bioavailability and might show site targeted effect.

4. MATERIALS AND METHODS

4.1. Material

Dolutegravir sodium was received from Mylan Pvt. Limited. Stearic acid was received from Finar, Phospholipon 80H and Phospholipon 90H was received from (Lipoid) Germany, Soy lecithin and Tween 80 was procured from SD fine chem. Limited, Poloxamer 407 and Poloxamer 188 was received from Yarrow, Mumbai.

4.2. Screening of components

4.2.1. Solubility of drug in different lipids and surfactants

The saturation solubility of DTG.Na in different lipids and surfactants was determined. For saturation solubility study of DTG.Na, a fixed amount of DTG.Na was taken in a test tube and to this solid lipid was added in increments of 0.1 g and the test tube was heated in a water bath with shaking at a temperature above the melting point of solid lipid. The amount of lipid in molten state required to solubilize the drug was noted. The complete dissolution was confirmed by formation of clear transparent solution. The same method was used for the saturation solubility study of DTG.Na in lipophilic surfactants [16].

4.2.2. Solubility studies of drug in different buffers

Solubility study of DTG.Na in different buffers was determined by equilibrating excess amount of drug in buffer solution of pH 4.2 and in purified water. Glass flask with a capacity of 50 ml was chosen. 10 ml of media was added to each flask and the amount of drug separately. The sufficient amount to saturate each media was added, which was characterized by deposition of substance not solubilized.

Incubator shaker was used to keep samples at 37^0 C during the test with agitation of 150 rpm for 72 hours (until it achieve the equilibrium condition). After this period, samples were immediately filtered (using a filter of pore size 0.45 µm) and diluted in a volumetric flask with the corresponding media. For quantification processes of anti-retroviral drugs, a UV-V spectrophotometer was used at the maximum absorbance wavelength for each media and the solubility values were calculated using calibration curves determined for the drug [17].

4.3. Preparation of SLN semisolid formulation

To formulate semisolid SLN formulations first the lipid phase was prepared by melting lipid (at 70^0 C) and lipophilic surfactant together. Then the drug was dissolved in ethanol which was added to the melted lipid phase. This solution was taken in the syringe and was injected rapidly into the aqueous phase containing surfactant (10 ml) while it was on magnetic stirrer at 400 rpm (which was heated to same temperature as that of lipid phase) and was sonicated using a probe sonicator to get nanoparticulate semi-solid formulation [16].

4.4. Experimental design

The Taguchi OA experimental design was used to study the effect of different lipids and surfactants at three levels. Four factors (independent variables) such as type of lipid, type of surfactant, concentration of lipid and concentration of surfactant were studied at all the three levels as shown in Table 3. Entrapment efficiency was taken as the response (dependent variable). An L9 orthogonal array was used for choosing the best and optimized formulation. The software used was Minitab-18 English [18].

Table 3. Taguchi L^9 orthogonal array (3^4) design of experiment.

Independent variables	Level 1	Level 2	Level 3
Type of lipid	Phospholipon 80H	Phospholipon 90H	Soy lecithin
Type of surfactant	Tween 80	Poloxamer 407	Poloxamer 188
Concentration of lipid (%)	3	4	5
Concentration of surfactant (%)	1	2	3

4.5. Evaluation of SLN gel

4.5.1. Visual appearance, pH and drug content

Visual appearance and clarity of prepared DTG.Na SLN gel was observed and checked for the presence of any particulate matter. The pH of the gel was determined using a pH meter (JENWAY 350, UK). One gram of each formulated gel was dispersed in 30 ml of distilled water and then the pH was measured and noted by bringing the electrode near the surface of the formulations and allowing it to equilibrate for 1 min [7].

One gram of selected DTG.Na SLN gel was taken in to a standard volumetric flask and mixed in a ratio of pH 4.2 citrate buffer : ethanol. The amount of drug per 1 g of gel was determined spectrophotometrically at 258 nm after filtration through the Millipore filter (0.45 µm) and drug content was calculate [19].

4.5.2. Spreadability and Extrudability

The spreadability of the gel formulations was determined by measuring the spreading diameter of 1 g of the gel by 20X20 cm glass plates after 1 min.

The mass of the upper plate was standardized at 10 g [19]. The spreadability of the formulation was calculated using the formula:

$$S = m * l/t \qquad \text{[Eq.2]}$$

Where,

S = Spreadability
m = weight tied to the upper slide
l = length of the glass slide
t =time

The formulation under study was filled in a clean, lacquered aluminum collapsible one-ounce tube with a tip of 5 mm opening, extrudability was then determined by measuring the amount of gel extruded through the tip when a constant load of 1 Kg was placed on the pan, then it was collected and weighed. The percentage of gel extruded was calculated, recorded and grades were allotted (+++Good; ++Fair; +Poor) [20].

4.5.3. Determination of viscosity

Viscosity of prepared gel was determined by VISCO lab 3000 viscometer which contains a piston style electromagnetic sensor and thermometer that provides continuous viscosity and temperature reading. The sample 1-2 ml was applied in the measurement chamber and the results were shown on the screen of VISCO lab 3000. The determination of viscosity for each formulation was done in triplicate and average of it was calculated [19].

4.5.4. Measurement of particle size, polydispersity index and zeta potential

The mean particle size, particle size distribution and polydispersity index of the prepared SLN formulations were measured using Photon Correlation Spectroscopy (Malvern Instruments, UK). The SLNs were diluted with distilled water (1:100) and the measurements were done at 25°C at an angle of detection of 90^0 . Zeta potential was studied by measuring the electrophoretic mobility using Malvern Zeta sizer Nano

ZS90 (Malvern Instruments, UK). The field strength applied was 20 Vcm^{-1}. Prior to the measurement, all samples were diluted in distilled water [21].

4.5.5. Surface morphology

The morphological characteristic of SLN was determined by scanning electron microscope (JEOL-JSM-6360 JAPAN). A drop of sample was placed on a slide and excess water was left to dry at room temperature. The specimen holder was used to attach the slide using a double coated adhesive tape and gold, coated under vacuum using a sputter coater (Model JFC-1100, Jeol, JAPAN) for 10 min and investigated at 20 Kv [22].

4.5.6. Entrapment efficiency

Entrapment efficiency of the drug was determined by quantifying the amount of free drug in the dispersion medium using below equation

$$\text{Entrapment efficiency (EE \%)} = \frac{\text{Mass of drug in submicron particles}}{\text{Mass of drug used in formulation}} * 100 \quad \text{[Eq. 3]}$$

10 mg of DTG.Na loaded SLN gel was dissolved in 5 ml of ethanol. 1 ml of the above solution was diluted to 10 ml with pH 4.2 citrate buffer and filtered using 0.45 µm filter membrane. The absorbance of the filtered solution was recorded by using UV spectrophotometer at 258 nm (UV-1601PC, Shimadzu, Japan) [23].

4.5.7. In-vitro drug release studies

Diffusion studies were performed using Franz diffusion cell. The cell was locally fabricated and the volume of receptor compartment was 25 ml. The dialysis membrane used for diffusion studies was positioned between donor and receptor compartment. DTG.Na SLN gel was uniformly applied on membrane and clamped together. The receptor compartment was filled with pH 4.2 citrate buffer and maintained by continuous stirring at 50 rpm with a magnetic bead and at 37^0 C. At each pre-determined time interval, 5 ml of sample was withdrawn and replaced with an equal volume of buffer. The samples were analyzed after appropriate dilution at λ_{max} of 258 nm using UV spectrophotometer [24].

4.5.8. Ex vivo permeation studies

In *ex vivo* studies, fresh vaginal tissue was removed from vaginal cavity of female goat obtained from the local slaughter house. The tissue was stored in saline water in cold condition. Diffusion studies were performed using Franz diffusion cell. It was placed in between the donor and the receptor compartment. The receptor compartment was filled with pH 4.2 citrate buffer and maintained by continuous stirring at 50 rpm with a magnetic bead and maintained at 37^0 C. At each predetermined time interval, 5 ml samples was withdrawn and replaced with an equal volume of buffer. The samples were analyzed after appropriate dilution at λ_{max} of 258 nm using spectrophotometer [25]. Various components of *ex vivo* permeation studies of DTG.Na gel were calculated.

4.5.9. Vaginal tissue retention studies

For determination of drug deposited in tissue, the cell was dismantled after a period of 8 h and tissue was carefully removed from the cell. The formulation applied on tissue surface was swabbed first with pH 4.2 citrate buffer and then with methanol. The procedure was repeated twice to ensure no traces of formulation are left onto tissue surface. The tissue was then cut into small pieces and drug present in tissue was extracted in pH 4.2 citrate buffer using bath sonicator and determined spectrophotometrically after suitable dilution and filtration [26].

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REFERENCES

[1] Maartens G, Celum C, Lewin SR. HIV infection: epidemiology, pathogenesis, treatment and prevention. Lancet. 2014; 384(9939): 258-271. [CrossRef]

[2] Serrao E, Odde S, Ramkumar K, Neamati N. Raltegravir, elvitegravir, and metoogravir: the birth of "me-too" HIV-1 integrase inhibitors. Retrovirology. 2009; 6: 25. [CrossRef]

[3] Kobayashi M, Yoshinaga T, Seki T, wakasa-Morimoto C, Brown KW, Ferris R, Foster SA, Hazen RJ, Miki S, Suyama-Kagitani A, Kawauchi-Miki S, Taishi T, Kawasuji T, Johns BA, Underwood MR, Garvey EP, Sato A, Fujiwara T. In Vitro antiretroviral properties of S/GSK1349572, a next-generation HIV integrase inhibitor. Antimicrob Agents Chemother. 2011; 55(2): 813-821. [CrossRef]

[4] Siafaka PI, Üstündağ Okur N, Karavas E, Bikiaris DN. Surface Modified Multifunctional and Stimuli Responsive Nanoparticles for Drug Targeting: Current Status and Uses. Int J Mol Sci. 2016; 17(9): E1440. [CrossRef]

[5] Das S, Chandhury A. Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. AAPS Pharmscitech. 2011; 12(1): 62-76. [CrossRef]

[6] Freitas C, Müller RH. Correlation between long-term stability of solid lipid nanoparticles (SLN) and crystallinity of the lipid phase. Eur J Pharm Biopharm. 1999; 47(2): 125-132. [CrossRef]

[7] Loo Ch, Basari M, Ismail R, Lau H, Tejo B, Kanthimathi M, Hassan H, Choo Y. Effect of compositions in nanostructured lipid carriers (NLC) on skin hydration and occlusion. Int J Nanomedicine. 2013; 8: 13-22. [CrossRef]

[8] Fakhari A, Corcoran M, Schwarz A. Thermogelling properties of purified poloxamer 407. Heliyon. 2017; 3(8): e00390. [CrossRef]

[9] El-Housiny, et al. Fluconazole-loaded solid lipid nanoparticles topical gel for treatment of pityriasis versicolor: formulation and clinical study. Drug Deliv. 2018; 25(1): 2015. [CrossRef]

[10] Khalil RM, El-Bary AA, Kassem MA, Ghorab MM, Ahmed MB. Solid lipid nanoparticles for topical delivery of meloxicam: development and in vitro characterization. 1st Annual International Interdisciplinary Conference, AIIC 2013; 779-798.

[11] Khare A, Singh I, Pawar P, Grover K. Design and Evaluation of Voriconazole Loaded Solid Lipid Nanoparticles for Ophthalmic Application. J Drug Deliv. 2016; 6590361. [CrossRef]

[12] Ekambaram P, Abdul HS. Formulation and evaluation of solid lipid nanoparticles of ramipril. J Young Pharm. 2011; 3(3): 216-220. [CrossRef]

[13] Kushwaha AK, Vuddanda PR, Karunanidhi P, Singh SK, Singh S. Development and evaluation of solid lipid nanoparticles of raloxifene hydrochloride for enhanced bioavailability. Biomed Res Int. 2013; 584549. [CrossRef]

[14] Mirza MA, Panda AK, Asif S, Verma D, Talegaonkar S, Manzoor N, Khan A, Ahmed FJ, Dudeja M, Iqbal Z. A vaginal drug delivery model. Drug Delivery. 2016; 23(8): 3123-3134. [CrossRef]

[15] Radha GV, et al. Design and evaluation of topical vaginal proniosomal formulations of tenofovir disoproxil fumarate for HIV prevention. Int Res J Pharm. 2019; 10(2): 94-102.

[16] Madan J, Khude P, Dua K. Development and evaluation of solid lipid nanoparticles of mometasone furoate for topical delivery. Int J Pharm Invest. 2014; 4(2): 60-64.

[17] Singh S, Dobhal AK, Jain A, Pandit JK, Chakraborty S. Formulation and evaluation of solid lipid nanoparticles of a water soluble drug: Zidovudine. Chem Pharm Bull. 2010; 58(5): 650-655. [CrossRef]

[18] Lakshmi PK, Sreekanth J, Sridharan A. Formulation development of fast releasing oral thin films of levocetrizine dihydrochloride with Eudragit® Epo and optimization through Taguchi orthogonal experimental design. Asian J Pharm. 2011; 5: 84-92.

[19] Lakshmi PK, Thangellapalli N, Chennuri A, Prasanthi D, Veeresh B. Wound healing activity of topical lawsone gel on rat model. Int J Pharm Sci Res. 2017; 8(7): 3162-3169.

[20] Rao K, Vijaybhaskar D, Pratima S. Formulation of topical oral gel for the treatment of oral sub mucous fibrosis (OSMF). Der Pharm Lett. 2011; 3(1): 103-112.

[21] Lu PJ, Fu WE, Huang SC, Lin CY, Ho ML, Chen YP, Cheng HF. Methodology for sample preparation and size measurement of commercial ZnO nanoparticles. J Food Drug Anal. 2018; 26(2): 628-636. [CrossRef]

[22] Üstündağ-Okur N, Yurdasiper A, Gündoğdu E, Gökçe EH. Modification of solid lipid nanoparticles loaded with nebivolol hydrochloride for improvement of oral bioavailability in treatment of hypertension: polyethylene glycol versus chitosan oligosaccharide lactate. J Microencapsul. 2016; 33(1): 30-42. [CrossRef]

[23] Soma D, et al. Solid lipid nanoparticles of irbesartan: preparation, characterization, optimization and pharmacokinetic studies. Braz J Pharm Sci. 2017; 53(1): e15012. [CrossRef]

[24] Deshkar SS, Patil AT. Development of mucoadhesive gel of fluconazole for vaginal candidiasis. Indo Am J Pharm Res. 2015; 5(11): 3599-3610.

[25] Berginc K, Skalko-Basnet N, Basnet P, Kristl A. Development and evaluation of an in vitro vaginal model for assessment of drug´s biopharmaceutical properties: curcumin. AAPS PharmSciTech. 2012; 13(4): 1045-1053. [CrossRef]

[26] Abdel-Mottaleb M, Mortada N, Elshamy AA, Awad GAS. Preparation And Evaluation Of Fluconazole Gels. Egypt J Biomed Sci. 2007; 23(1): 266-286. [CrossRef]