4.0 Literature Review

4.0 Introduction

This chapter describes detailed literature that is reported on Tenofovir drug toxicity and drug safety data, analytical methods available for assay of Tenofovir nanoparticle formulation, Bioanalytical methods that are established to perform the pharmacokinetic activity of Tenofovir in humans and rats. Excipient safety and toxicity data of all the excipients that are used in developing the final nanoparticle formulation. Literature review currently supporting nanoparticle formulation in HIV and literature reported regarding poor CSF penetration of Tenofovir in biological fluids.

4.1 Drug safety, toxicity data

Data obtained from:

FDA Antiviral Drugs Advisory Committee meeting on NDA 21-356

VIREAD® is the brand name for tenofovir disoproxil fumarate (a prodrug of tenofovir) which is a fumaric acid salt of bis-isopropoxycarbonyloxymethyl ester derivative of tenofovir. In vivo tenofovir disoproxil fumarate is converted to tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5’-monophosphate. Tenofovir exhibits activity against HIV-1 reverse transcriptase.

Chemical Structure: Tenofovir Disoproxil Fumarate
Mechanism of Action: Tenofovir diphosphate inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate deoxyadenosine 5’-triphosphate and, after incorporation into DNA, by DNA chain termination.

Pharmacokinetics
The pharmacokinetics of tenofovir disoproxil fumarate have been evaluated in healthy volunteers and HIV-1 infected individuals. Tenofovir pharmacokinetics are similar between these populations.

Animal Toxicology
Tenofovir and tenofovir disoproxil fumarate administered in toxicology studies to rats, dogs and monkeys at exposures (based on AUCs) greater than or equal to 6 fold those observed in humans caused bone toxicity. In monkeys the bone toxicity was diagnosed as osteomalacia. Osteomalacia observed in monkeys appeared to be reversible upon dose reduction or discontinuation of Tenofovir.

Long-term oral carcinogenicity studies of tenofovir disoproxil fumarate in mice and rats were carried out at exposures up to approximately 16 times (mice) and 5 times (rats) those observed in humans at the therapeutic dose for HIV infection. At the high dose in female mice, liver adenomas were increased at exposures 16 times that in humans. In rats, the study was negative for carcinogenic findings at exposures up to 5 times that observed in humans at the therapeutic dose.

There were no effects on fertility, mating performance or early embryonic development when Tenofovir disoproxil fumarate was administered to male rats at a dose equivalent to 10 times the human dose based on body surface area comparisons for 28 days prior to mating and to female rats for 15 days prior to mating through day seven of gestation. Reproduction studies have been performed in rats and rabbits at doses up to 14 and 19 times the human dose based on body surface area comparisons and revealed no evidence of impaired fertility or harm to the fetus due to tenofovir. There are, however, no adequate and well-controlled studies in pregnant women.


4.2 Analytical method literature

Particle size, Zeta potential, PDI are important parameters for nanoparticle formulation along with drug entrapment efficiency. After this step we look into the invitro release of the dosage and then go ahead with its pharmacokinetic or pharmacodynamics parameters as per the objective of the study. Extensive literature review was carried out for standard analytical method which establishes the amount of drug entrapped in nanoparticle formulation. Few published methods like Alukda.D et al (2011), Rohan LC et al (2010), Meng, J et al (2011), Zhang.T a et al (2011) have used UV method to analyse the drug entrapment in nanoparticle formulation.

Appala raju et al (2008) reported simultaneous method for Tenofovir, Emtricitabine, Efavirenz tablet formulations and which involved high amount of organic concentration in mobile phase. Gradient run of the reported method sometimes results in HPLC system pump problems due to low viscosity of mobile phase by high concentration of organic solvent.

Kandgal.PB et al (2008) reported a method for tenofovir disproxil fumarate in tablet dosage form but the linearity range is less sensitive for lower concentrations of drug. Rohan LC et al (2010) have worked on Tenofovir gel formulation and reported HPLC method for evaluating in vitro release of drug, but no specific chromatography conditions were discussed.

Meng.J et al (2011) reported on Tenofovir chitosan nanoparticles where encapsulation efficiency was calculated by using UV spectrophotometer. Tao Zhang a et al (2011) have reported on pH responsive nanoparticles and drug entrapment efficiency was calculated by UV spectrophotometer with 2 µg mL\(^{-1}\) to 100 µg mL\(^{-1}\).

Alukda.D et al (2011) reported on solid lipid nanoparticle formulation of Tenofovir gel 1 mg where in vitro release is calculated by using UV spectrophotometer with a sensitivity of 2 µg mL\(^{-1}\) to 100 µg mL\(^{-1}\).
New stability indicating analytical method was developed for nanoparticle formulation analysis and validated it as per the guideline.

4.3 Bioanalytical method literature

Sentac et al. (2003) reported a bioanalytical method for tenofovir in human plasma by HPLC which included solid phase extraction by silica-bonded reversed-phase sorbent: Supelclean TM LC-18 SPE cartridges (500 mg, 3 ml, Sigma–Aldrich). The chromatography involved the use of mobile phase containing disodium hydrogen phosphate (Na$_2$HPO$_4$) buffer, tetrabutylammonium hydrogen sulfate and acetonitrile for different elution through a C$_{18}$ column with UV detection. The method proved to be accurate, precise and linear between 10 and 4000 ng/ml.

Jullien.V et al. (2003) reported spectroflorometric method of Tenofovir which has a lengthy sample preparation procedure. After precipitation of 200µl of plasma samples by methanol and evaporation of the supernatant, fluorescent derivatized compounds were obtained by a 40-min incubation at 80°C with chloroacetaldehyde 0.34% at pH 4.5. The assay was performed isocratically using 5 mM Sodium hydrogen phosphate buffer (NaHPO$_4$ pH 6), containing tetrabutylammonium (TBA) chloride 5 mM, and acetonitrile (85:15, v/v) as mobile phase, and a Cluzeau C$_8$ plus column maintained at 35°C. Detection was performed at excitation and emission wavelengths set at 236 and 425 nm, respectively.

Sparidansa.RW et al. (2003) reported a method for analysis of Tenofovir in plasma using derivatization of drug with chloroacetaldehyde solution. Tenofovir was isolated from a 200µl plasma sample using protein precipitation with trichloroacetic acid. The 6 fluorescent 1,N -etheno derivative is formed at 98°C in the buffered extract with chloroacetaldehyde. This derivative was analysed using gradient ion-pair liquid chromatography and fluorescence detection at 254 nm for excitation and 425 nm for emission. In the evaluated
concentration range (20–1000 ng/ml) The procedure was lengthy and time taking.

Bezy. V et al. (2005) reported a method which uses HPLC–ESI-MS/MS detection. Oasis® HLB Waters cartridges followed by optimised HPLC separation on an Atlantis® dC18 column with acetic acid–hydroxylamine buffer (ionic strength 5 mM, pH 7) acetonitrile elution gradient. Quantitation was performed by HPLC/UV at 260 nm. Linear calibration curves were obtained within a 30–10,000 ng/mL plasma concentration range. Solid phase extraction technique was used.

Rezk. NL et al. (2005) reported simultaneous estimation of Tenofovir, Emtricitabine in human plasma by HPLC using solid phase extraction. Using 200µL of plasma and BOND ELUT-C18 Varian columns. An Atlantis® dC-18 analytical column is used along with an 18 min linear gradient elution of phosphate buffer (pH 5.7) and methanol to provide sharp peaks for emtricitabine at 280 nm, tenofovir at 259 nm over the range of 10–10,000 ng/mL for both analytes.

Delahunty. T et al. (2006) reported estimation of Tenofovir by LC/MS/MS. Chromatographic separation was achieved with a Polar-RP Synergi, 2.0mm×150 mm, reverse phase analytical column. The mobile phase was 3% acetonitrile/1% acetic acid. The method was linear from 10 to 750 ng/ml with a minimum quantifiable limit of 10 ng/ml when 250µl aliquots were analysed.

El Barkil.M et al. (2007) reported determination of tenofovir in human plasma by single mass spectrometry detection. A solid phase extraction procedure (Bond-Elut® C18 Varian cartridges) provided high extraction efficiency (91% for tenofovir and 68.8% for the internal standard, 3-methylcytidine). An atlantis®-dC-18 analytical column was used with an isocratic mode elution of a mixture (pH 2.5) of ammonium acetate/methanol (98.5:1.5, v/v). Detection was performed at 260 nm and by using the ion at m/z 288. The signals from both detectors were validated over the range of 10–1000 ngmL−1 and were found to
be linear, accurate and precise. At the lowest limit of quantification, 10 ngmL−1 for UV and 5 ngmL−1 for MS

Gomes.NA et al. (2008) reported simultaneous estimation of Tenofovir, Emtricitabine in human plasma by LC-MS/MS. Chromolith Speed Rod RP18 column was used. The mass transition ion-pair has been followed as m/z 288.10→176.10 for TEN, m/z 248.20→130.20 for EMT and m/z 230.10→112.10 for Lamivudine (LAM). The method involves solid phase extraction from plasma, simple isocratic chromatographic conditions and mass spectrometric detection using an API 5000 instrument that enables detection at nanogram levels. Lamivudine was used as the internal standard. The proposed method has been validated with a linear range of 10–600 ng/ml for TEN and 25–2500 ng/ml for EMT.

Delahunty.T et al. (2009) reported simultaneous assay of tenofovir and emtricitabine in plasma using LC/MS/MS and isotopically labeled internal standards. mobile phase (3% acetonitrile/1% acetic acid.) stream flowing at 200µL/min. A Synergi Polar-RP, 2.0mm×150mm, reversed-phase analytical column was used to achieve the chromatographic separation. Detection of the analytes was achieved by ESI positive ionization tandem mass spectrometry. The precursor/product transitions (m/z) in the positive ion mode were 288/176 and 293/181 ions for TFV and Iso-TFV, respectively and the precursor/product transitions (m/z) were 248/130 and 251/133 ions for FTC and Iso-FTC, respectively. When the analyte/IS abundance ratios were plotted against the specified concentrations, the linearity of the concentration curves were in the range 10 ng/mL to 1500 ng/mL for both analytes (250µL plasma extracted), with a minimum quantifiable limit of 10 ng/mL for both analytes

The above reported methods are for determination of Tenofovir in plasma which involves expensive cartridges and mass spectrophotometer. Newer drug delivery systems like Tenofovir nanoparticle formulations are evolving and there is no standard sensitive analytical method was published till now. As a
result there was a need to develop a simple method with high sensitivity, stability.

In developing the analytical methods regulatory bodies like FDA and ICH guidelines were followed and validation parameters like accuracy, specificity, precision, robustness and ruggedness were taken into consideration. Standard textbooks like Snyder LR, Kirkland, Wells DA, Gary E. Shargel L were referred for further guidance during the method development and validation process.

4.4 Excipient safety and toxicity data

In preparation of Tenofovir nanoparticle formulations, biodegradable excipients like PLGA (Poly (lactic-co-glycolic acid 50:50) and non-biodegradable excipients like pH independent Eudragit RL and Eudragit RS were used as polymers. The safety and efficacy of this excipients are widely reported in literature.

Chemical structure: Poly (lactic-co-glycolic acid)

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\text{POLY} = \text{HO}[-\text{O}]-[-\text{O}]_{x}[-\text{O}]_{y}[-\text{H}]
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Astete, C. E. et al (2006) reported PLGA or poly(lactic-co-glycolic acid) is a copolymer which is used in number of Food and Drug Administration (FDA) approved therapeutic devices, owing to its biodegradability and biocompatibility. PLGA is synthesized by means of random ring-opening copolymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid. Common catalysts used in the preparation of this polymer include tin(II) 2-ethylhexanoate, tin(II) alkoxides, or aluminum isopropoxide. During polymerization, successive monomeric units (of glycolic or lactic acid) are linked together in PLGA by ester linkages, thus yielding a linear, aliphatic polyester as a product.
PLGAs are amorphous rather than crystalline and show a glass transition temperature in the range of 40-60 °C. Unlike the homopolymers of lactic acid (polylactide) and glycolic acid (polyglycolide) which show poor solubilities, PLGA can be dissolved by a wide range of common solvents, including chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate.

PLGA has been successful as a biodegradable polymer because it undergoes hydrolysis in the body to produce the original monomers, lactic acid and glycolic acid. These two monomers under normal physiological conditions, are by-products of various metabolic pathways in the body. Since the body effectively deals with the two monomers, there is minimal systemic toxicity associated with using PLGA for drug delivery or biomaterial applications. Also, the possibility to tailor the polymer degradation time by altering the ratio of the monomers used during synthesis has made PLGA a common choice in the production of a variety of biomedical devices such as: grafts, sutures, implants, prosthetic devices, micro and nanoparticles.

EUDRAGIT® RL 100 is a copolymer of ethyl acrylate, methyl methacrylate and a low content of methacrylic acid ester with quaternary ammonium groups. The ammonium groups are present as salts and make the polymers permeable. The physical properties of eudragit are it is colourless solid substance, clear to cloudy granules with a faint amine-like odour.

**Chemical structure:** Eudragit RL 100 and RS 100

Accessed from www.eudragit.evonik.com
Eudragit polymer is used in targeted drug release area like, pH independent Dissolution, Time controlled release and has properties like high permeability and pH independent swelling


Hans.ML, Lowman.AM (2002) reported the different methods of preparation of polymeric nanoparticles like solvent diffusion, solvent displacement, nano precipitation, solvent evaporation, multiple emulsion, salting out, ionic gelation, interfacial deposition, phase inversion nano capsulation, polymerization. Comparision of particle size diameter depending on the polymer, characterization like particle size, zeta potential is reviewed.

D. Kim, H (2005) reported on effect s of PLGA particles on blood coagulation and hemolysis In the present study, the poly(lactide-co-glycolide) acid(PLGA) nanoparticles were synthesized by using nanoprecipitation. The uptake of blood electrolytes from simulated blood fluid (SBF) and the stability (dispersion/aggregation) of nanoparticles in SBF was examined. Different loading amounts of PLGA and different contact time between PLGA nanoparticles and SBF were studied. The interaction of particles with the organic components of blood was also studied by using the measurement of red blood cell hemolysis and blood clotting with raw PLGA, surfactant modified PLGA, and PEGylated PLGA.

Semete.B et al (2010) reported extensively In vivo evaluation of the bio distribution and safety of PLGA nanoparticles as drug delivery systems with respect to toxic effects of PLGA nanoparticles along with industrial nanoparticles of similar size range such as zinc oxide, ferrous oxide and fumed silica. An in vitro cytotoxicity study was conducted to assess the cell viability following exposure to PLGA nanoparticles for a period of 7 days and there
localization in various vital organs studied. It was concluded that no toxic effects were observed with PLGA nanoparticles in comparison with industrial nanoparticles. The toxicity studies, cell viability, bio distribution across various vital organs like liver, spleen red pulp, kidney, hippocampus in the cerebrum, myocardium, terminal bronchiolus and lung, large intestines, ovarian follicles and corpus luteum, fallopian duct, glandular gastric mucosa, endometrium of uterine mucosa, thymus lymphoidal tissue and suggests the biodegradable and biocompatible safety of PLGA nanoparticles as drug delivery systems were studied.

Kumari.A et al (2010) reported extensively on Biodegradable polymeric nanoparticles based drug delivery systems for polymers like PLGA, poly lactic acid (PLA), poly caprolactone (PCL), chitosan, natural polymers like gelatin, Poly-alkyl-cyano-acrylates (PAC) for different drugs involving anti-cancer, diabetes, psychotic drugs, hormones, proteins etc and concluded the advantages of having nanoparticle drug delivery systems.

Zhang.T et al (2011) reported on pH-responsive nanoparticles releasing tenofovir intended for the prevention of HIV transmission. Established significant pH-responsive release of anti-HIV microbicides in the presence of human semen fluid simulant (SFS). After NPs preparation by emulsification diffusion. The vaginal pH hovers around 4.0 and seminal pH at 7.4, the seminal pH has a buffering capacity and nullify the vaginal acidic condition, so the effect of pH on release of Tenofovir nanoparticle drug delivery system is established. FDA approved biodegradable polymers are used in the study. Since the HIV virus can be present in human semen during the intercourse, it is promising to design a semen-triggered topical delivery system. On the other hand human vagina pH varies from 4 to 5, whereas human semen has a higher pH as reported by D.H. Owen, D.F. Katz, J. Androl. 26 (2005). Therefore, the local acidic pH will be altered during intercourse, which has been utilized in semen triggered delivery and pH-sensitive hydrogel.
Gupta.KM et al (2007) reported vaginal retention of such a delivery system is also important. The focus of the above study has to prepare a semen-triggered delivery system having a sustained release characteristic for vaginal delivery. So role of pH is important in Tenofovir formulation as evident from the above study.

Meng.J et al (2011) reported on tenofovir loaded chitosan nanoparticles to maximize microbicide mucoadhesion. They reported for the first time, a model anti-HIV tenofovir loaded chitosan NPs prepared by ionic gelation. The EE% of tenofovir, which was used as a model microbicide, could be improved significantly by using an ethanol solution as a solvent of chitosan but size of the particle was high. The in vitro release study, cytotoxicity assays, and mucoadhesive studies suggested that relatively large chitosan NPs have the potential to be a controlled release, safe, and bioadhesive microbicide delivery system.

4.5 Formulation literature

H. Fessi et al (1989) reported Nanocapsule formation by interfacial polymer deposition following solvent displacement is most widely referred article for nanoparticle formulation preparation. It reports preparation of nanocapsules by interfacial deposition of a preformed, well-defined, and biodegradable polymer following displacement of a semi-polar solvent miscible with water from a lipophilic solution. It was possible to prepare nanocapsules in the absence of any surfactant. But the poloxamer, a highly aqueous soluble surfactant, was needed for physical stability of the nanocapsule suspension.

Barichello.JM et al (1999) reported Encapsulation of Hydrophilic and Lipophilic Drugs in PLGA Nanoparticles by the Nanoprecipitation Method. More hydrophilic drugs, such as vancomycin and phenobarbital, were poorly encapsulated in PLGA nanoparticles. These drugs suffer from the problems of drug leakage to the external medium Insulin was preferentially surface bound on the PLGA nanoparticles.
Govender.T et al (1999) reported PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug investigated for drug incorporation efficiency enhancement with respect to influence of aqueous phase pH. By adjusting the pH and decreasing the ionization of drug it prevents drug leakage back into the aqueous phase as a result increasing the entrapment efficiency of the formulation. HEPES buffer is used for aqueous phase. They observed that there is an initial burst release followed by slow release in invitro study.

Peltonen.L et al (2004) reported Improved Entrapment Efficiency of Hydrophilic Drug Substance During Nanoprecipitation of Poly(l)lactide. In order to increase the loading of the hydrophilic drug in the hydrophobic nanoparticles. Lowering of the pH was the most efficient way to increase the drug loading; up to approximately 70% However, by lowering the pH of the outer media, the drug entrapment efficiency of water-soluble sodium cromoglycate was increased from 10% to 15% to as high as a level of approximately 70%. But this technique yield high particle size upto 800 nanometers.

Bilati.U et al (2005) reported development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles. The nanoparticles obtained varied in size from about 85–560 nm . The nanoparticle recovery step requires further improvements, as flocculation of particles is observed. Work was carried out on a wide range of solvents for PLA/PLGA with different polarities. To enable the entrapment of hydrophilic or lipophilic drugs. Alcohols as solvents can provide nanoparticles with different sizes. But the nanoparticle recovery still needs to be optimized in order to prevent nanoparticle coalescence,and to obtain a good final nanoparticle yield.

Bilati.U et al (2005) reported Nanoprecipitation Versus Emulsion-based Techniques for the Encapsulation of Proteins in to Biodegradable Nanoparticles and Process-related Stability Issues. Nanoprecipitation can sometimes be a good alternative to the classical and widely used double emulsion method especially in case of protein entrapment (lysozyme) leading to small and highly loaded
nanoparticles. solvents such as DMSO (instead of acetone or ethanol) and the use of nonsolvents such as methanol or ethanol (instead of water) certainly promote the formation of a narrow population of nanoparticles.

Ubricha.N et al (2005) reported Oral evaluation in rabbits of cyclosporin-loaded Eudragit RS or RL nanoparticles reported a good particle size and very high zeta potential which is directly correlated to its stability. Due to hydrophobicity of cyclosporin the drug entrapment is high on the Eudragit polymer. Pharmacokinetic studies were conducted on a small group of animals (n =3) and they have longer duration of AUC and elimination when compared to pure drug.

Damge.C et al (2007) reported Oral delivery of insulin associated to polymeric nanoparticles in diabetic rats reported the use of Eudragit RS as polymer for insulin nanoformulation. explained the attraction of the electropositive nanoparticles due to Eudragit® RS and the electronegative mucus layer which covered the intestinal epithelium. Due this attraction it increase the residence time of insulin nanoparticles next to the absorption surface of the gastrointestinal tract inside the intestine and create a drug gradient concentration towards blood.

Rosen.RK et al (2008) reported regarding Acceptability of Tenofovir Gel as a Vaginal Microbicide Among Women in a Phase I Trial: A Mixed-Methods Study Quantitative results indicate that tenofovir vaginal gel was acceptable to almost all users, while qualitative findings indicate that acceptability is complex, varies among different users, and is likely shaped by a variety of factors that manufacturers will need to consider to maximize use-effectiveness. Because of the differences in the qualitative and quantitative responses, the authors felt the need for future trials of candidate microbicides should include strategic collection of mixed-methods microbicide acceptability data.

discussed about the problems of encapsulation of hydrophilic drugs due to rapid partitioning of drugs in external aqueous phase.

Sharma.P et al (2010) reviewed Pure drug and polymer based nanotechnologies for the improved solubility, stability, bioavailability and targeting of anti-HIV drugs. However toxicity, adverse drug reactions, suboptimal bioavailability due to poor physicochemical properties, impaired biodistribution in HIV reservoirs are the common problems along with emergence of drug resistance, requirement of drug monitoring and lifelong adherence associated with antiretroviral treatment. Rational use of nanoscale drug delivery systems has the potential to tackle the above mentioned problems associated with antiretroviral therapy Nanotechnology based drug delivery systems hold promise to alleviate and improve the quality of life of the HIV infected patients

Wong.HL et al (2010) reported about the use of Nanotechnology applications for improved delivery of antiretroviral drugs to the brain and concluded that conventional HAART therapy will not be very effective in reducing the viral load and resulting in neurological problems. Suggestions of tailor made antiretroviral’s for brain delivery are done. Also discussed about various types of nano formulations for antiretroviral and emphasised on commercial availability of the nano formulation.

Koga.K et al (2010) reported Nano-sized water-in-oil-in-water emulsion enhances intestinal absorption of calcein, a high solubility and low permeability compound. As per BCS classification it falls under class III (High solubility and low permeability) and worked on various hydrophilic emulsifiers to check the leakage of calcein from formulations.

Das.S et al (2010) reported design of Eudragit RL 100 nanoparticles by nanoprecipitation method for ocular drug delivery. The use of Eudragit polymer for nanoparticle formulation. The positively charged nanoformulation was helpful in due to negatively charged mucosal fluid of the eye. Solvent
displacement technique was used. The nanosuspension was not subjected to lyophilisation step.

WHO/UNAIDS Meeting Report Next Steps with 1% Tenofovir Gel Johannesburg, South Africa 25-26 August 2010 discussed. The results of the CAPRISA 004 trial, released in July 2010, showed that 1% Tenofovir gel which reduced the risk of HIV infection in women by 39% compared with placebo, and by 54% in the women who reported more consistent gel use. These results were historic. After nearly two decades of research, this was the first clinical trial to show that a vaginal microbicide could provide a safe and effective way to prevent sexual transmission of HIV. The gel also provided a 51% protective effect against herpes simplex virus type 2 infections (HSV-2).

Yoo.JW et al (2011) reported pH-sensitive Eudragit nanoparticles for mucosal drug delivery reported. Drug delivery via vaginal epithelium has suffered from lack of stability due to acidic and enzymatic environments. The biocompatible pH-sensitive nanoparticles composed of Eudragit S-100 (ES) were developed to protect loaded compounds from being degraded under the rigorous vaginal conditions and achieve their therapeutically effective concentrations in the mucosal epithelium. quasi-emulsion solvent diffusion method. Loading efficiencies were found to be 26% and 71% for a hydrophilic and a hydrophobic compound, respectively. Both hydrophilic and hydrophobic model drugs remained stable in nanoparticles at acidic pH, whereas they are quickly released from nanoparticles upon exposure at physiological pH. The confocal study revealed that ES nanoparticles were taken up by vaginal cells, followed by pH-responsive drug release, with no cytotoxic activities. The pH-sensitive nanoparticles would be a promising carrier for the vaginal-specific delivery of various therapeutic drugs including microbicides and peptides/proteins.

Alukda.D et al (2011) reported on 1 mg Formulation of Tenofovir-Loaded Functionalized Solid Lipid Nanoparticles Intended for HIV Prevention. The SLNs were functionalized successfully using a layer-by-layer technique. The cytotoxicity assays showed a noncytotoxic effect on vaginal epithelial cells for
48 h. Although the EE% was relatively low, the SLNs appear to be a promising drug delivery template for microbicides.

4.6 Antiretroviral drug cerebrospinal fluid penetration

Anthony pillai.C et al (2006) reported about the distribution of the anti-HIV drug, Tenofovir (PMPA), into the brain, CSF and choroid plexuses. He reported that there is negligible transport of PMPA across the blood-brain barrier, but PMPA can cross the blood-CSF barrier. This reflects the differing physiological and functional characteristics of the blood-central nervous system interfaces. Self- and cross-inhibition studies did not suggest the involvement of a transport system in the central nervous system distribution of this drug. The ability of PMPA to accumulate in the choroid plexus tissue, but not in cerebral capillary endothelial cells due to hydrophilic nature of PMPA, indicate to the possibility of a transporter at the level of the choroid plexus. and in final conclusion he reported PMPA has been shown to cross the blood-CSF barrier and reach the CSF, but it cannot cross the BBB to reach deep brain sites.

Letendre.L et al (2008) reported poorer penetration of anti-retroviral drugs into the central nervous system appears to allow continued HIV replication in the brain this is indicated by higher CSF HIV viral loads. Inhibition of HIV replication in the central nervous system is probably critical in treating patients who have HIV associated neurocognitive disorders, anti-retroviral treatment strategies that account for brain penetration should be considered in consensus treatment guidelines.

Varatharajana.L et al (2009) reported regarding the transport of anti-HIV drugs across blood–CNS interfaces summarises the current knowledge and recommendations for future research in order to tackle HIV and prevent the formation of viral sanctuary sites, anti-retroviral drugs must be able to access the brain. But the normal function of the BBB and blood–CSF barrier is to shield the brain from harmful substances and provide a precisely regulated unique environment in the central nervous system and this hinders the
penetration of anti-HIV drugs into the brain, as a result viral replication in the brain leads to development of drug resistance and ultimately sub therapeutic concentrations of drugs that reaches the brain which leads to therapeutic failure.

Koopmans.PP et al (2009) reported in her review about the lack of drug penetration in brain and its serious effects with respect to viral loads and the advent of new antiretroviral drugs there reduced effect of cerebral disorders compared to older generation of drugs are discussed in detail in her article.

das Neves et al (2010) reported about the nanotechnology based system for treatment of HIV. Jose reported about new developments in nanocarrier systems for antiretroviral drugs are as a particularly interesting approach towards the improvement of HIV/AIDS treatment. This new technology may not be providing a way to cure HIV/AIDS, but nanotechnology based systems may improve drug therapy in infected patients as demonstrate by in vitro and animal in vivo studies. Various nanotechnology systems have been shown the ability to improve antiretroviral activity of several drugs, at the same time reducing their toxicity and potentially simplifying drug regimens. Nano formulations systems can provide higher and prolonged drug levels in known reservoir sites for HIV, this can result in better viral suppression and potentially longer time.

Ene L et al (2011) reported regarding the penetration of various anti-retroviral in central nervous system restricted entry into the brain, due to several factors like unique structure of the blood-brain barrier, and existence of efficient efflux mechanisms. This results in poor availability of drug in brain and the reservoir sites present in it will slowly get resistance to the current drugs and its dosage forms.