DESIGN, DEVELOPMENT AND EVALUATION OF ADVANCES NANOFORMULATION & NANOPARTICLE-DELIVERY FOR BRAIN FUNCTIONS AND BIOAVAILABILITY ACTIVITY FOR ANTI-VIRAL DRUG

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ABSTRACT- HIV, which causes AIDS, is one of the deadliest diseases and the sixth leading cause of death. The reported poor bioavailability of non-nucleoside reverse transcriptase inhibitors used to treat HIV infection is related to first pass metabolism, protein binding and enzyme metabolism. They are also less permeable to the blood-brain barrier. The central nervous system is considered the most important HIV reservoir. In the current study, efavirenz lipid nanoparticles were designed to provide improved drug permeability and protection through biocompatible lipid content and nano size. This creates a formulation with enhanced bioavailability and brain targeting ability. Solid lipid nanoparticles (SLNs) were prepared by high-pressure homogenization using a systematic design of experiments approach, and particle size, poly dispersity index (PDI), zeta potential, and entrapment efficiency were evaluated. . An average particle size of 108.5 nm and a PDI of 0.172 were obtained with a capture factor of 64.9%. The zeta potential is -21.2 mV and the drug is stable. Transmission electron microscopy and histopathological examination showed spherical and irregularly shaped lipid nanoparticles, respectively. Optimal SLNs were integrated into an in situ temperature-sensitive gel system. Various parameters of Efavirenz SLN gel were evaluated, such as gel temperature, pH, viscosity, light transmittance, muco-adhesive strength, diffusion, and in vitro and ex vivo dissolution studies. Drug release was found to be optimal for zero-order release kinetics (R2 = 0.3), suggesting that drug release is concentration-sensitive and diffusion-controlled. In vivo pharmacokinetic studies have shown that it is possible to successfully eradicate HIV in the brain and cure HIV patients after intranasal administration, and many of these problems are voluntarily overcome by the use of advanced anti-inflammatory drugs developed with nano-technology. These delivery systems carry antibodies in nanoparticles that can be made from synthetic or natural materials. However, due to health and environmental concerns, there is interest in developing antibiotics from natural products such as lipids, phospholipids, surfactants, proteins and polysaccharides. The composition, morphology, size and properties of nanoparticles can be used to improve antimicrobial activity, stability and efficacy. This

article provides an overview of the major classes of antiviral drugs, describes the current issues limiting their effectiveness, and suggests ways nanoparticles can be used to address these issues. We review recent research on nano particle-based antibody delivery and outline future directions.

Keyword: Nanoparticles, Nanoformulation, Anti-Viral Drugs,
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Introduction- Poorly soluble drugs are very common in pharmaceuticals and have low bioavailability. In recent years, the number of drug candidates in drug research has increased, and 70% of new drug candidates are drug candidates [1]. These drugs present significant challenges for researchers to determine bioavailability and improve drug delivery systems [2]. It is more difficult to cross the BBB (blood-brain barrier) to reach the brain [3, 4]. Infectious diseases are common and include typhoid fever, typhoid fever, measles, measles.

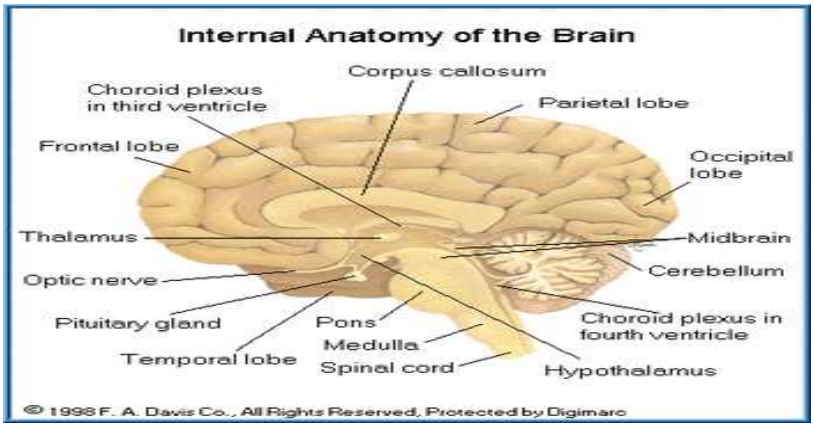
Current treatments using antiretroviral drugs for HIV infection are effective at lowering plasma levels, but not effective at eliminating the virus in other areas, such as the central nervous system, because they are inaccessible and cannot be stored in cellular and anatomical reservoirs where the virus can center. . . The central nervous system is the most important HIV repository [9]. With limited access to anti-AIDS drugs, the brain is thought to be a haven for the virus. This not only causes immunity, but mental function improvement, movement symptoms or mild neurocognitive impairment (MDR), HIV-associated dementia (HAD), HIV encephalitis (HIVE), and in many cases even death.

Aim of the research work- The aim of this study is to design and manufacture nanoparticles of the antiviral drug efavirenz, to increase their bioavailability in the HIV reservoir area by delivering them to the brain, to conduct research and to compare formulations of existing formulations.

BRAIN- The brain makes up the largest part of the central nervous system and is the main structure often referred to when talking about the brain. The brain, the main body of the central nervous system, is also protected from the environment by two main barriers: the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). The anatomy of the brain is shown in Figure 2.3. The brain is in the head, often close to the body's meanings such as vision, hearing, balance, taste and smell. The brain is the most complex organ in the vertebrate body. The cerebral cortex (the largest part) in a normal person is estimated to have 1.5-33 billion neurons [30], all connected by synapses to thousands of other neurons. These neurons communicate with each other through long protoplasmic fibers called axons that carry signaling pulses called action potentials to distant areas of the brain or body that target specific

beneficiaries. Microglia are a type of glial cell found in the brain and spinal cord [31]. Microglia make up 10-15% of all cells in the brain. As resident macrophages, they are the first and foremost of the immune system in the central nervous system (CNS) [32]. Microglia (and other glial cells, including astrocytes) are distributed over large non-overlapping areas throughout the central nervous system.

Figure 1: Anatomy of the brain



Blood –Brain Barrier- BBB (Blood-Brain Barrier) The BBB is an important barrier that prevents macromolecules, hydrophilic molecules, microorganisms, or nanoparticles from entering the brain [34]. The blood-cerebrospinal fluid barrier (BCSFB), composed of choroid plexus epithelial cells, also plays a role in nutrient and xenobiotic permeability. Access to the brain is limited and well-controlled, mainly due to three types of damage.

1. Physical Barrier- The BBB shows the largest surface area (approximately 20 m2), has weak endothelial cells with tight junctions preventing transport, lacks endothelial fenestrations, and reduces the rate of pinocytosis on the luminal side. The BCSFB problem occurs because there is a layer of polarized epithelial cells around the windowed capillaries held together by tight junction proteins.
2. Biological Barrier- Expression and function of various receptors, ion channels, and efflux/efflux transporters that regulate transport. In particular, ATP-binding cassette (ABC) membrane-associated transporters such as P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP), and breast cancer protein (BCRP, ABCG2) play an important role in restrictions. . Many Penetration drugs, including anti-cancer drugs and anti-HIV drugs.
3. Metabolic Barrier- Metabolic enzymes can inhibit transport. These transporters and enzymes may also cause drug-drug interactions that can lead to treatment failure and/or toxicity.

Methodology of Research-

TABLE 1 List of Materials used

Category	Name of Materials	Source / Supplier
Drugs	Efavirenz (EFV)	Sun Pharma Ltd. Sikkim, India
	Tenofovir Disoproxil Fumarate	Paradise Healthcare, Vadodara, India
Lipids	Glycerymonostearate	Ozone intermediate, Mumbai, India
	Compritol 888 ATO (Glyceryl behenate)	Gattefosse India Pvt. Ltd, Mumbai
	Tripalmitin (Glyceryl tripalmitate)	Sigma Aldrich
	Glyceryl palmitostearate	Chemdyes Corp., Rajkot
	Glyceryl distearate	Atur Enterprise
	Cetyl palmitate	National chemical, Vadodara
Surfactants	Poloxamer 188 (Pluronic F68)	Sigma Aldrich
	Poloxamer 407 (Pluronic F127)	Sigma Aldrich
	Poloxamer 245 (Pluronic P 85)	Chemdyes Corporation, Rajkot
	Polysorbate 20 (Tween 20)	S.D.Fine Chemicals. Ltd, Mumbai
	Polysorbate 60 (Tween 60)	S.D.Fine Chemicals. Ltd, Mumbai
	Polysorbate 80 (Tween 80)	Chemdyes Corporation, Rajkot
Gelling agents	Chitosan	Chemdyes Corporation, Rajkot
	Carbopol 934P	S.D.Fine Chemicals. Ltd, Mumbai
	Poloxamer 188 (Pluronic F68)	Sigma Aldrich
	Poloxamer 407 (Pluronic F127)	Sigma Aldrich
	Methanol	S.D. Fine Chemicals Ltd., Mumbai
	Acetonitrile	S.D. Fine Chemicals Ltd., Mumbai
	Isopropyl Alcohol	S.D. Fine Chemicals Ltd., Mumbai
	Potassium bromide	Merck Millipore
	Disodium hydrogen phosphate	Allied Chemical Corporation, Baroda
	Potassium dihydrogen phosphate	Allied Chemical Corporation, Baroda
	Sodium Chloride	Fischer scientific , Mumbai

	Sodium hydroxide	Fischer scientific , Mumbai
	Orthophosphoric acid	Fischer scientific , Mumbai
	110 (LA 395)	Himedia
	Freshly excised goat	Slaughter house
	-	PASM Hospital, Vadodara, Gujarat

Design of experiment- Its products are designed to meet the patient's needs and product needs. Drug development should include the definition of quality product (QTPP), identification and determination of critical features (CQAs), selection of appropriate manufacturing processes, determination of control strategies, identification of possible equipment and procedures affecting the CQA product. A method can facilitate continuous improvement and innovation in production and throughout the life of the product [16-18].In the current study, drug identification, selection of different products, social studies etc. Many preliminary studies such as particle size and maximum encapsulation of drug Efficacy in SLN [19, 20].

Preformulation studies - The main components of solid lipid nanoparticle systems include drugs, lipids and surfactants. After identifying the drug, various materials were selected as components of the proposed system. This selection was based on drug solubility and ability to form small particles. The selection was also based on the component's safety profile and approval status.

Drug-Excipients Compatibility Study- infrared spectra of pure chemicals stored at 25 ± 2 OC, 60% ± 5% relative humidity for 7 days, as well as physical mixtures of chemicals and selected materials, were recorded using an FT-IR spectrophotometer (Bruker Alpha-one, Bruker Optik). , Germany) in the range of 4000–400 cm-1 and compared significant changes

Figure 02 : Formulation of solid lipid nanoparticles by high pressurehomogenization

Muco-adhesive strength- Mucoadhesion was determined by a modified two-disc balance [37]. According to the literature review, although there are many in vitro and in vivo studies of the efficacy of mucoadhesive drug delivery systems, surprisingly, there is still no established protocol to measure

mucoadhesion or can be done according to its quality. Mucoadhesion Strength Method. In vitro testing with a two-disc balance is the best and easiest way to evaluate the mucoadhesive properties of formulations [43, 44]. In this way, as shown in Figure 3.3, one side of the scale is covered with wooden blocks and the other side with a container of water.More than 20 µl of test sample gel in contact with cellophane film (1 cm2) adhered to the horizontal end of the water box and on the perfect surface. Water was slowly added dropwise until the cellophane membrane separated from the gel.

Weight in grams of water required to separate the two surfaces was measured

$$\mathbf{F = w \times g}$$

Where F is the muco-adhesion force (dynes / cm^2),
w is the minimum weight required to break the bond (grams), g is the acceleration due to gravity (cm/s^2).

Drug Release Profile

***In-vitro* drug diffusion profile-** In vitro drug diffusion curves of SLN dispersions and EFV-loaded SLN gels were obtained using the dialysis bag/dialysis bladder method [7,24,48] as well as Franz diffusion cells [18,36,48]. For the Dialysis bag, the SLN dispersion and bulk suspension are packaged in a filter bag 110 (LA 395), Himedia, cut at 12000 Da) and extracted in 50 ml of methanolic phosphate buffered saline (pH 6.4) in a glass beaker, 40% v/v) [6, 7]. The beaker was placed on a magnetic stirrer and mixed with magnetic beads and covered with parafilm to prevent evaporation loss during the experiment [46]. Fractions are removed from the receptor chamber at 24-hour intervals and replaced with an equal volume of fresh diffusion medium.

***Ex-vivo* drug release profile-** In vitro release studies were performed in the nasal cavities of slaughtered goats using Franz diffusion cells [18, 39]. Carefully remove the proboscis and remove the tissue. Mount the severed nose in a Franz diffusion cell and fill the receptor chamber with methanolic phosphate buffer (pH 6.4, 40% v/v). Place the cells on a magnetic stirrer for gentle shaking and keep the temperature at 34 ± 0 °C. Place 5 EFV preparations (0.5 mL) on the donor site. Fractions are removed from the receiving chamber at 24-hour intervals and replaced with an equal volume of fresh diffusion medium. Aliquots were analyzed spectrophotometrically at 247 nm.

In-vivo studies- In vivo studies were performed in adult albino Wistar mice. Animal study protocols were approved by the Animal Ethics Committee (IAEC) and Animal Control and Care Committee (CPCSEA) [PIPH 04/15 CPCSEA921/PO/Ere/S/05/CPCSEA]. Animals were housed in polypropylene cages for mice. Rice bran was used as bedding. Laboratory rats were provided ad libitum with granulated food and purified drinking water. Rats were divided into two groups. Group I (a test group) consisted of 6 animals and the established SLN formulation (equivalent to 0.06 mg efavirenze) was

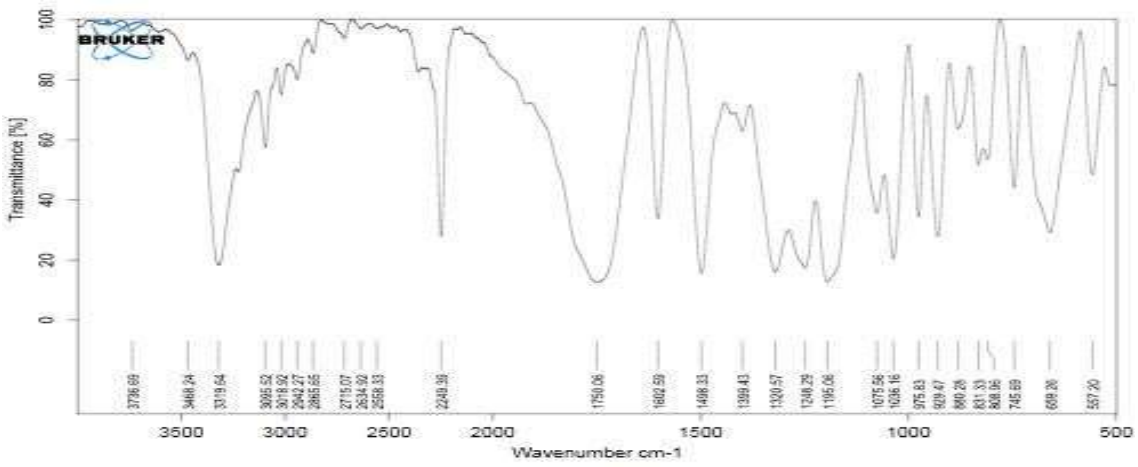
administered intranasally. The second group (standard) consists of 6 animals speaking commercial - EFAVIR - efavirenz capsules.(Powder equivalent to 25 mg efavirenz capsules, dispersed in 1 ml of water). This dose is calculated as the Human Equivalent Dose (HED) according to FDA guidelines. Plasma samples were collected from all animals and animals were sacrificed within 24 hours with an overdose of sodium pentobarbital. Brains were isolated, weighed, homogenized in PBS pH 6.4 at 5000 rpm using the Silent Crusher M homogenizer (Heidolph, Germany), centrifuged, and the supernatant collected for drug concentration determination [55].The amount of drug in plasma and brain homogenates is determined using HPLC, a developed and validated method for estimating efavirenz in plasma. Tenofovir disoproxil fumarate was used as an internal standard. Brain:plasma ratio, bioavailability and relative bioavailability were calculated using the formula brain:plasma = concentrate. Brain drug concentration/concentration Amount of drug in plasma fraction Bioavailability = Bioavailable dose/Used dose Relative bioavailability = Systemic drug availability/Systemic availability of an oral standard of the same drugs.

Results

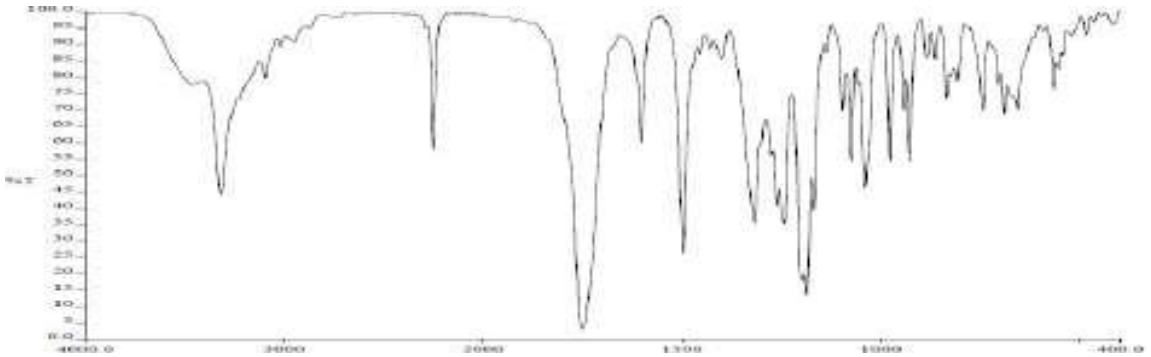
Identification of Drug- Before starting its construction, it is necessary to determine and ensure the purity of the purchased drug. Analytical tests and decisions to determine the appearance, solubility and melting point of chemical samples are summarized in Table 3.

TABLE 3: Identification tests for EFV with the inferences

Parameters	**Observations**	**Reported [1]**	**Inferences**
Appearance	White powder	White to almost white powder	Complies
Solubility	Practically insoluble in water (10 mg insoluble in >100 ml) Freely soluble in methanol (10 mg in < 1 ml)	Practically insoluble in water Freely soluble in methanol	Complies Complies
Melting point	139-142^{0}C	138 – 142 ^{0}C	Complies



(a)



(b)

Figure 2: IR spectra (a) Observed spectra of EFV (b) Reported spectra of EFV

TABLE 4: Major peaks observed and reported for EFV in IR spectra:

Observed (cm^{-1})	**Reported (cm^{-1})**	**Inferences [3]**
3319.64	3500-3100	N-H stretching
2249.39	2250-2100	C= C (Alkyne)
1750.06	1750-1730	C=O of ester
1602.59	1680-1630	C=O of amide
1498.33	1350-1000	C-N
1036.16	1300-1000	C-O

Summary- Infections with Humanimmune-deficiency virus (HIV) leadingto

Acquired immune-deficiency syndrome is one of the leading cause of death in the world [1, 2]. Current therapies for HIV infections with antiretroviral drugs is effective in reducing plasma viral levels, but are ineffective in eradicating the virus from sites like CNS which becomes a viral sanctuary site due to the inability of the drugs to reach to these sites. The CNS is the most important HIV reservoir site [3]. This not only results in virological resistance, but also is often associated with the development of various complications such as progressive deterioration in mental function, symptoms of motor abnormalities, mild neurocognitive disorder (MDR), HIV associated dementia (HAD), HIV encephalitis (HIVE) and even death in many cases. Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of choice and is recommended as a first line antiretroviral drug used in the high activity antiretroviral therapy (HAART) for the HIV infections [3-5]. EFV has low bioavailability (40-45%) [6] which is reported to be due to low water solubility of the drug, extensive first pass metabolism, metabolism by enzymes, high protein binding, effluxmechanisms, etc [7-9].

Thus, the main aim of the present investigations were to design and develop nanoparticles of EFV with the objectives of providing increased permeability and protection to drug with biocompatible lipid content, avoid first-pass metabolism and efflux-mechanisms, and select the route of administration to deliver EFV to brain/CNS in order to increase the bioavailability of EFV at the reservoir site of HIV. Suitable analytical methods were selected/developed and validated for determining the entrapment efficiency, *in-vitro*, *ex-vivo* drug release profiles and for estimation of EFV in brain and plasma respectively.

Conclusion- The present investigations, it may be concluded that solid lipid nanoparticles of a poorly soluble drug efavirenz were successfully developed and optimized using the systematic approach of design of experiments (DoE) by high pressure homogenization technique. Thermo sensitive *in-situ* gel was prepared with the optimized SLN dispersion. The intranasal administration of the formulation showed 150 times more brain targeting efficiency and 70 times better absorption potential of the drug from efavirenz loaded SLN formulation in comparison to the orally administered marketed formulation (capsule). Thus, it may be concluded that the developed formulation has better potential to target brain where the HIV viruses are reported to harbor even with low dose of efavirenz, rendering the treatment more cost-effective as well and acceptable to patients because of convenience of application of

in-situ gelling formulation. Hence, the developed formulation, after necessary investigations of clinical trials, has the promising potential for an attempt to completely eradicate HIV reservoir and cure AIDS.

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