Journal of Cardiovascular Disease Research

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

DEVELOPMENT OF EFAVIRENZ LOADED PRONIOSOME FORMULATION FOR ENHANCED ORAL BIOAVAILABILITY: AN IN-VITRO AND IN-VIVO STUDY

Swarupa Arvapalli*[1], A. Anka Rao[2]

College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Andhra Pradesh, India.

1. Research Scholar, College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Andhra Pradesh.522502.

2. Associate Professor, College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Andhra Pradesh.522502.

***Corresponding author:**

Department of Pharmaceutics, KL College of Pharmacy, KL University, Vaddeshwaram,Andhrapradesh, India.

E-mail address: rupa03arvapalli@gmail.com

ABSTRACT

In the present investigation, proniosome of Efavirenz were formulated, optimized and evaluated for effective oral delivery in order to overcome the bioavailability issues of orally administered Efavirenz. The proniosome were prepared using a blend of Span-60, cholesterol and maltodextrin and were evaluated for in-vitro parameters, ex-vivo permeation and in-vivo performance. Results indicated that the vesicles were spherical in shape, their size ranged from 284.00 nm to 941.40 nm and they had high zeta potential. The entrapment efficiency for spans was higher compared to tweens. DSC and IR studies confirmed the absence of chemical interactions between the Efavirenz and proniosome components. In-vitro release study showed that formulations with spans exhibit controlled release profile and followed the Higuchi model. No significant change in vesicle size and entrapment efficiency was observed when the proniosomes were stored at 4 ± 1 °C and 25 ± 2 °C for three months. Proniosomes with span 60 showed no signs of erythema or edema and has highest flux across the rat skin (169.851 ± 2.13 μg cm−2 h−1). The relative bioavailability was 92% after transdermal administration of proniosomes and the tmax was increased to 8 h. So we conclude that the developed proniosome formulation would be a promising alternative to improve the bioavailability problems of Efavirenz.

KEY WORDS: Efavirenz, *In-vivo* study, ex-vivo permeation, proniosomes, *In-vitro* study.

INTRODUCTION

The problem of bioavailability of the administered drug is one of the major issues when it comes to oral delivery, which arises due to lack of dose proportionality, intra and inter-subject variability, unpredictable absorption, and weak dissolution[1]. Even if the oral route of drug administration is preferred mostly, this problem is existent for most existent and newly discovered drugs. To address the problem of low solubility of class-II drugs(according to B.C.S classification) proniosomal method is used in this research which can enhance the rate of dissolution[2-4]. Microencapsulation, spray drying, surface area enhancement through nanonization / micronization, surfactant inclusion ,solid dispersion, drug derivatization, and complexation are various methods for increasing the rate of dissolution of insoluble drugs[5]. Apart from this, storage and sterilization of such drugs is difficult in the original form. Since proniosomes are powder formulations ,dry in nature and contain carrier particles which are coated with surfactant and water soluble, they can easily dissolve in agitated aqueous media to form niosomal dispersion(niosomes formed are more uniform in size compared to standard niosomes) [6-8]. They are also easy to store and sterilize because of its free flowing powdery formulation which adds to the convenience of measurement, transfer , storage and distribution[9]. EFV traditionally is administered in high doses due to its low bioavailability(40-45%) which comes because of its high plasma-protein binding (more than 99%)[10]. Since the drug non-nucleoside reverse-transcriptase inhibitor and is highly lipophilic,[11] it's generally used in first-line paediatric therapeutic-cocktail (dosage range: 200-600/ day)[12]. However, its lipophilic nature((log P = 5.4)) and low water solubility (8.3 μg/ml) limits absorption when administered through the oral route. Thus, there is a need to enhance bioavailability and dissolution characteristics of EFV. While EFV has been previously formulated as nanoparticles and solid dispersible particles for addressing these issues, there were no studies of formulation of EFV using maltodextrin based proniosomal powders which can be administered orally. Thus, this paper encompasses a systematic study of formulation of proniosomal powders with EFV(Maltodextrin based). Further in the study, rat intestine has been experimented (*ex- vivo*) to assess the permeability of EFV from its proniosomal formulation.

MATERIALS AND METHODS

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

Materials

Efavirenz was a gift sample from Aurobindo Pharma, Hyderabad, India. span20, span40, span60, span80, and cholesterol were purchased from E.Merck, Mumbai, India. Pearlitol SD 200 (spray dried mannitol) was procured from Capricorn Pharma India Pvt Ltd. Dicetyl phosphate (DCP) was obtained from Sigma Aldrich, Bangalore, India. All chemicals and solvents used in the study were of HPLC grade.

Method of preparation of proniosomes

Proniosomes were prepared by the film deposition on carriers method[12]. The compositions of different proniosome formulations are represented in Table 1. In this method, accurately weighed amounts of surfactant mixture (250 µM) comprising of surfactant and cholesterol at various molar ratios, DCP (5%) and drug (10mg) were dissolved in 10mL of solvent mixture containing chloroform and methanol (1:1). The resultant mixture

***In-vitro* dissolution study**

In- vitro dissolution study of proniosomal powders and control (pure drug powder) was performed using USP type II (paddle) apparatus (Electrolab, TD L8, Mumbai, India) in Phosphate buffer (pH 7.4). The volume of dissolution medium used was 900mL and maintained at a temperature of 37±0.5°C with paddle speed set at 50rpm throughout the experiment. An aliquot of 2mL was collected at predetermined time intervals up to 4h and replaced with fresh dissolution medium. The samples were filtered by passing through 0.45 µm membrane filter (Millipore, USA) and analyzed by HPLC. Dissolution efficiency (DE) was used to characterize the drug release profiles and it is defined as the area under the dissolution curve up to a certain time "t" (measured by trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. The relative dissolution rate (RDR) was calculated by dividing the DE of proniosome formulations with control

Scanning electron microscopy

The surface morphology of the Efavirenz (pure drug), Pearlitol SD200 (carrier), and optimized proniosome powder (PN3) were investigated by using scanning electron microscope (SEM) (S-4100, Hitachi, Japan). The sample was fixed on a brass stub using double sided adhesive tape and it was made electrically conductive by thin coating of gold. SEM images were recorded at accelerating voltage of 15 keV.

Differential scanning calorimetry

The physical nature of the drug in optimized proniosome formulation (PN3) was evaluated by performing differential scanning calorimetry (DSC) analysis. The DSC thermograms of the samples were obtained by a differential scanning calorimeter (DSC 6; Perkin Elmer, Waltham, MA, USA). Each sample was kept in an aluminum pan and then crimped with an aluminum cover. All the samples were scanned at 10°C/min from 40 to 280°C under a nitrogen purge at 30mL/min.

Fourier transform infrared spectroscopy

The infrared spectra of Efavirenz, Pearlitol SD200 and optimized proniosome powder formulation (PN3) were obtained using Fourier transform infrared (FT-IR) spec trophotometer (Thermo Scientific, Barrington, IL, USA) by the conventional KBr pellet method. The scanning range was 4000–400 cm−1 at a resolution of 4cm−1.

Powder X-ray diffractometry

The powder X-ray diffractometry (PXRD) patterns of pure drug, Pearlitol SD200 and optimized proniosome powder formulation (PN3) were obtained using X-ray diffractometer (X' Pert PRO PANalytical, West borough, MA, USA). CuKα radiation, nickel filtered graphite monochromator with 45 kV voltage, and 40 mA current with X'celerator detector was used for measuring. The samples were run at 1° (2θ) min−1 from 3° to 45° (2θ).

Ex vivo permeation studies

All studies were conducted with prior consent of Institutional Animal Ethical Committee, St. Peter's Institute of Pharmaceutical Sciences. Male Wistar rats weighing between 200–250 gm used in the study were supplied by the Jeeva Life Sciences, Hyderabad (CPCSEA/IAEC/JLS/16/07/21/15). The animals were kept in separate cages with free access to water and food until the rats were sacrificed. After the rats were sacrificed with inhalation of excess ether, the abdomen was opened and a segment of the ileum was removed. The ileum was flushed with Krebs Ringer solution to remove the mucus and adhered intestinal contents. One end of the intestine segment was tied and niosome dispersion (proniosome powder hydrated with phosphate buffer pH 7.4) equivalent to 5mg of drug was introduced in to the lumen and tightly closed. The tissue was placed in an organ bath with continuous aeration and maintained at a temperature of 37°C. The receptor compartment consists of 50mL of phosphate buffer (pH 7.4). At predetermined time intervals, an aliquot of 1mL was collected and replaced with equal volume of medium. The samples collected were added with an equal volume of methanol, centrifuged and the supernatant was quantified for Efavirenz using HPLC. Control (pure drug suspension) containing the same amount of drug was included in the study for comparison

Pharmacokinetic study

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

The study was conducted with the prior approval of Institutional Animal Ethical Committee, at Jeeva Life sciences Hyderabad(CPCSEA/IAEC/JLS/16/07/21/15) Male albino Wistar rats (200–250 g) were selected for the study and had free access to food and water. Before dosing, the animals were kept for overnight fasting. The rats were divided into two groups containing six in each. Niosomes were prepared by adding distilled water to Efavirenz proniosomal powder and shaking the mixture manually for 5min. Control group was given with an oral suspension of Efavirenz (2.0mg/mL in 0.5% w/v of CMC-Na) and the other group received the optimized proniosomal formulation (PN3) at a dose of 10mg/kg body weight. The treated animals were kept in separate cages and maintained under laboratory conditions throughout the study. At predetermined time intervals, blood samples (500 µL) were collected from retro orbital plexus into heparinized micro-centrifuge tubes. The plasma was separated by centrifugation at 10,000 rpm for 10min in a micro-centrifuge (Remi equipments, India) and stored at −20°C until analysis.

Pharmacokinetic parameters

The peak concentration (Cmax) and its time (Tmax) were obtained directly from the plasma concentration vs. time profile. By using trapezoidal rule method area under curve (AUC0–t) was calculated. The AUC0–∞ was determined by dividing the plasma concentration at last time point with elimination rate constant (K). The relative bioavailability (F) was estimated by dividing the AUC0–∞ of proniosome formulation with control oral suspension

Results & Discussion

Preparation and characterization of proniosomes Efavirenz loaded proniosomes were prepared by film deposition on carriers methods by changing the hydrophilic–lipophilic balance using different spans as shown in Table 1. The stability problems associated with the aqueous niosome dispersions has been solved by the proniosome approach. The vesicular stability of the niosomes after hydration with biological fluids is also vital for achieving maximum therapeutic benefit from the proniosomes. Keeping this in mind different strategies have been employed to improve the stability of the vesicles. Cholesterol is added as a structural lipid to improve the membrane stability and entrapment efficiency of vesicular systems20. Cholesterol also alters the fluidity of chains in the vesicular bilayers and reduces the permeability by increasing the degree of orientational order. For the formation of physically stable niosomes a

In vitro dissolution study

The dissolution profile of Efavirenz from proniosomes and pure drug were shown in the Figure 2. The dissolution of Efavirenz from proniosomes was found to be three times higher than the pure drug (control). The percent drug release and percent DE was significantly higher for all proniosome formulations compared with control ($p < 0.05$) (Table 4). The RDR >1 indicates dissolution enhancement and in our case we could notice >1 for proniosome formulations (Table 4). The proniosome formulation (PN3) promoted higher dissolution of Efavirenz compared with other formulations. The higher dissolution of Efavirenz from proniosome powder formulation may be due to the altered physical state of the drug entrapped in bilayers of niosome. Also the presence of charged lipid dicetylphosphate in the niosomal bilayer may increased the permeability of the bilayer and reduced the size of the vesicles which results in maximum surface area exposed to the dissolution medium which leads to higher drug release from proniosomes

Scanning electron microscopy

Figure 3 explains the surface morphology of Efavirenz, Pearlitol SD200, and Efavirenz proniosomes which were examined by SEM. Figure 3b shows the porous surface of the pearlitol particle, which enables it to use as effective carriers and provides more surface area for surfactant loading. Further the SEM images reveal the absence of native crystalline structures of Efavirenz in the proniosome powder (Figure 3c). **Differential scanning calorimetry studies**

The thermotropic behavior and the physical nature of the drug in proniosomes were known from the DSC thermograms of Efavirenz, Pearlitol SD200, and proniosome formulation (PN3). It can be depicted from Figure 4 that the drug acquire crystalline nature as it demonstrate sharp intense peak at 147–152°C corresponding to its melting point. The Pearlitol SD200 also exhibit peak at 178–180°C. On the other hand, the absence of noticeable peak over the range of 147–152°C in proniosome powder is an indicative of the alteration of the native crystalline form of the drug to amorphous or molecular state which was confirmed by PXRD analysis

Ex vivo permeation studies

Ex vivo intestinal permeation study was carried out to show the ability of proniosomal formulations for improved absorption. The majority of the drugs get absorbed in vivo from the small intestine so it is more suitable to use the rat intestine tissue28. Thus, we have used ileum segment of the intestine to assess the ability of proniosomal formulations for improving the permeation of Efavirenz across the intestine. The Efavirenz proniosomal formulation showed an improved transport across the rat intestine when compared with the control (pure drug suspension). There was about 2.2 times increase in the intestinal permeation of niosomal Efavirenz compared with control. The flux was increased for proniosome formulation and the enhancement ratio above 1 reveals the improved transport and we could notice an ER >1 for proniosome formulation compared with control. The improvement in the intestinal permeation for proniosome formulation might be due to

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

Pharmacokinetic studies

The mean plasma Efavirenz concentration versus time profile after oral administration of proniosome formulation **(MPA4)** and control (Efavirenz suspension) **EVF-S** was shown in Figure 8 and the pharmacokinetic parameters were represented in Table 6. The plasma concentration of Efavirenz(MPA4) from pronosomal systems was higher when compared with the control. Tmax of Efavirenz proniosome was 6 after oral administration and its corresponding Cmax and T_{max} was 4.56µg/mLand 6 hr where as the Cmax and Tmax of Efavirenz suspension after oral administration was 0.8µg/mL and 1h, respectively. The relative bioavailability of proniosomal formulation to that of control was found to be 2.3.

suspension

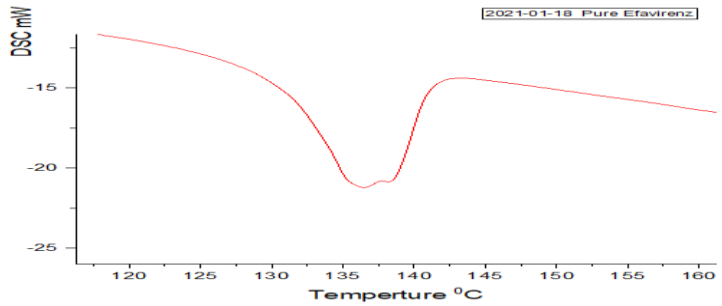
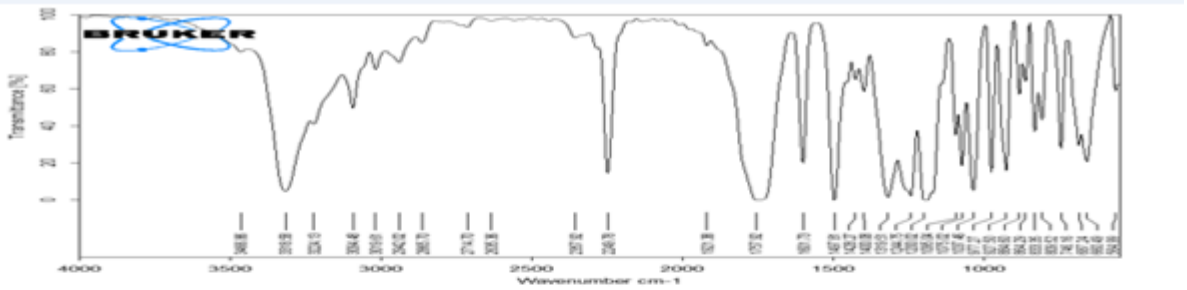
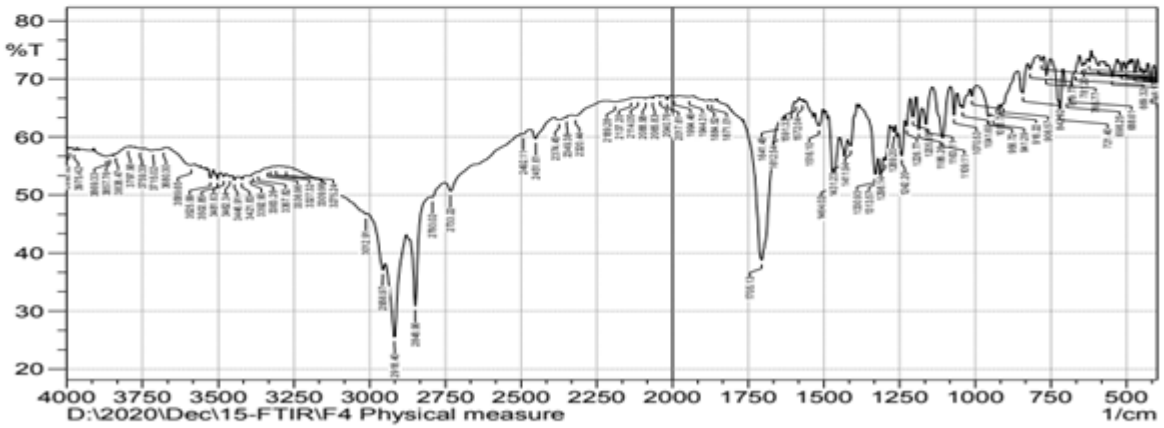


Figure :1 DSC of Pure Efavirenz



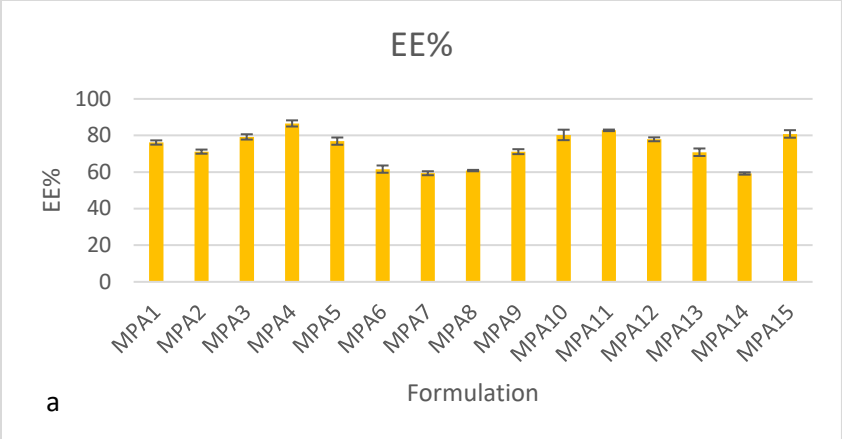
A



B

Figure:2 A) FTIR of Pure Efavirenz B)FTIR of Physical mixture of Optimized Formulation

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021



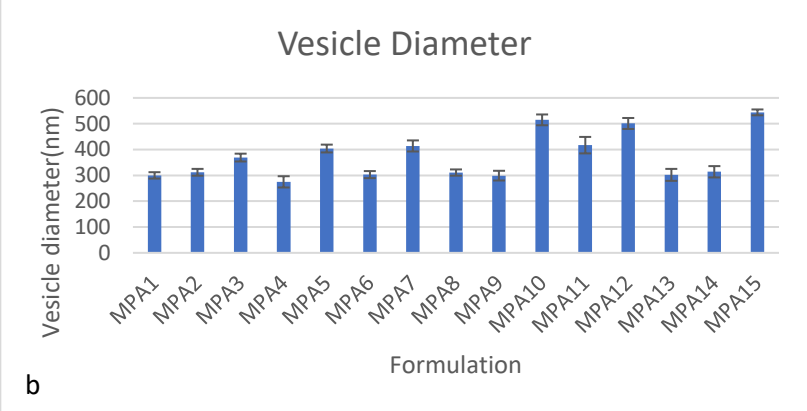


Figure:3 a) Entrapment Efficiency b)Zeta Potential

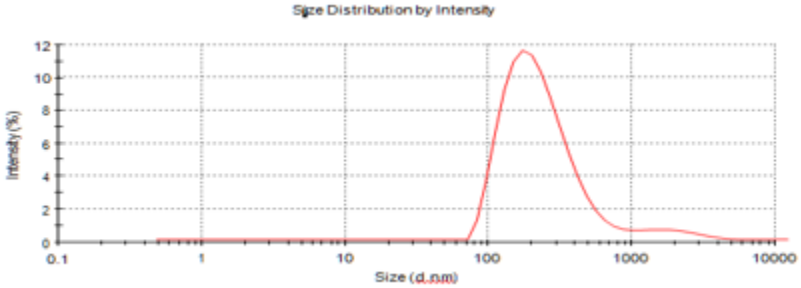


Figure:4 Particle size distribution for Optimized formulation

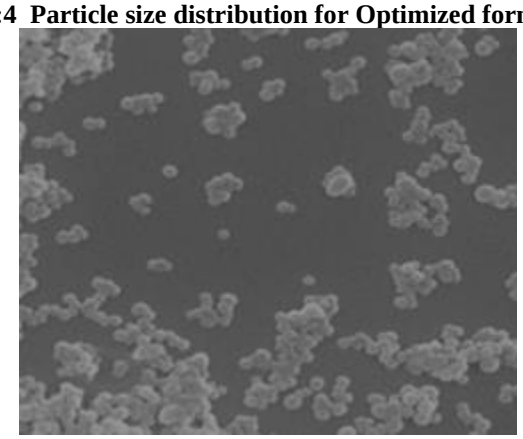


Figure:5 SEM image for Optimized formulation

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

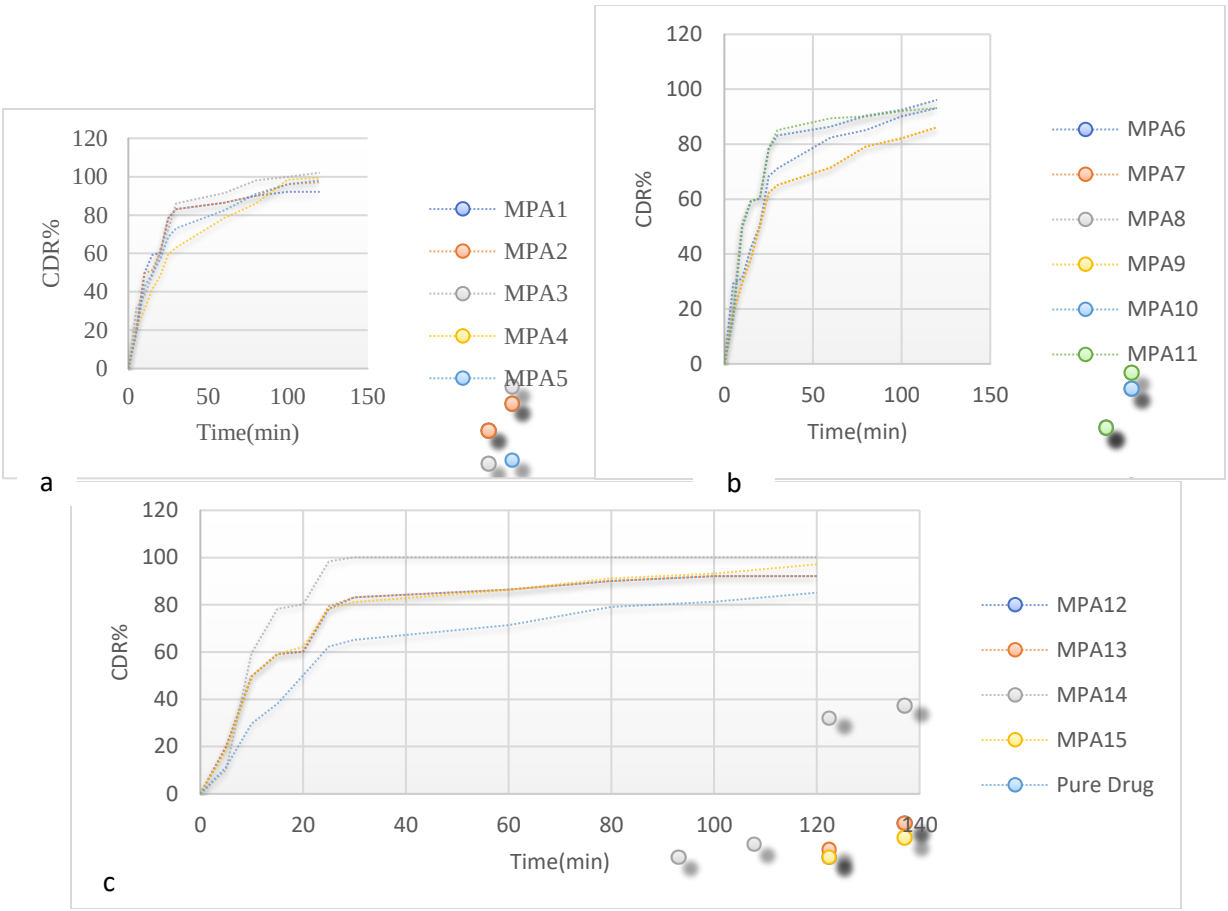


Figure:6 a)Drug Release% of MPA1 to MPA5 b) Drug Release% of MPA6 to MPA 11 c)Drug Release% of MPA12toMPA15 and Pure drug

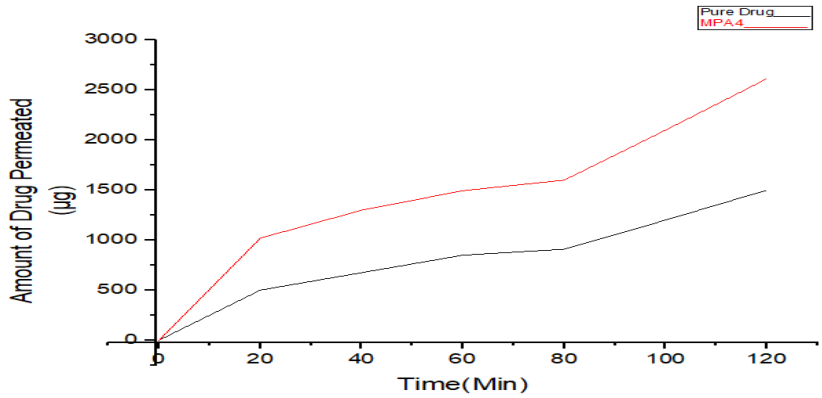


Figure:7 Comparison graph for Ex-vivo permeation of MPA4 with pure drug in rat intestine

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

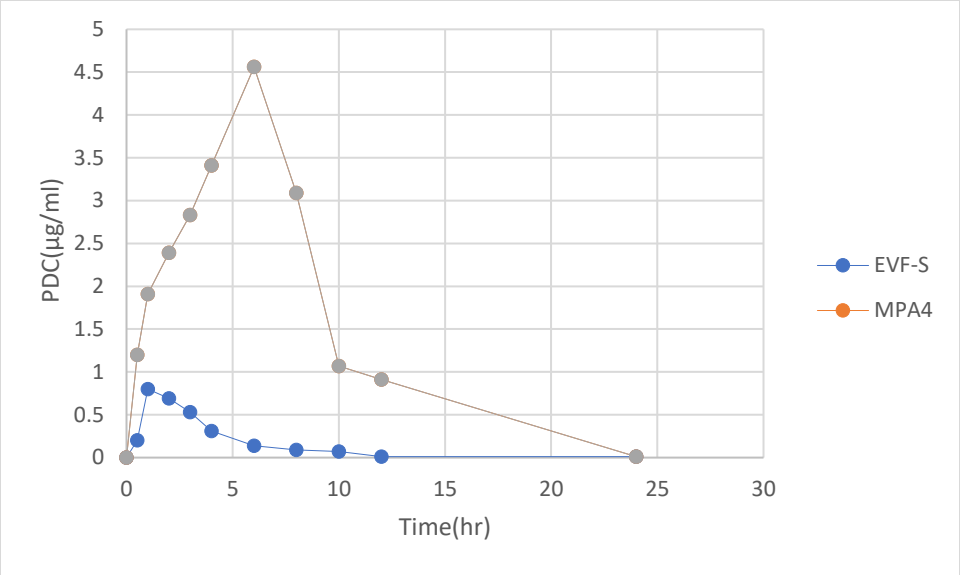


Figure 8: Comparision graph of Pharmacokinetic parameters of Efavirenz optimized MPA4 formulation and Efavirenz suspension

Table No 1: Box-Behnken Design for Maltodextrin based Efavirenz loaded Proniosome (MPA)

Formulation	Maltodextrin(X_1)	Span60 (X_2)	Cholesterol(X_3)	Y_1 (EE) % (W/W)	Y_2 VD (nm)	Y_3 % Drug Released
MPA1	1	1	0	76.17±3.16	300±12.30	97.14±2.30
MPA2	-1	-1	0	71.18±2.13	312±13.20	102.11±1.30
MPA3	0	0	0	79.23±3.44	369±15.12	89.44±2.40
MPA4	0	-1	1	86.59±1.68	275±21.80	99.41±3.30
MPA5	0	1	1	76.93±1.98	404±15.1	97.04±2.11
MPA6	1	0	1	61.62±1.98	303±13.70	93.12±2.31
MPA7	1	-1	0	59.37±1.09	414±21.40	88.47±2.07
MPA8	1	0	-1	60.82±3.38	311±12.10	96.77±2.07
MPA9	0	0	0	71.16±2.35	299±18.12	86.23±2.61
MPA10	-1	0	-1	80.32±2.86	515±20.9	94.12±1.22
MPA11	-1	0	1	82.82±0.42	417±31.9	93.07±1.70
MPA12	-1	1	0	77.92±2.06	501±21.3	92.17±1.33
MPA13	0	1	-1	70.85±2.05	302±23.2	92.33±1.40

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

MPA14	0	0	0	59.23±0.63	314±21.9	100.12±2.31
MPA15	0	-1	-1	80.85±2.05	544±11.2	96.14±1.31

Table No.2: Comparison study of Ex-vivo permeation of Optimized formulation with pure drug

TIME (Mins)	**Amount of Drug Permeated(µg)**	
	Pure Drug	**MPA4**
20	500	1020
40	975	1300
60	850	1495
80	910	1600
100	1200	2100
120	1500	2614

Table No 3:Pharmacokinetic parameters of Efavirenz optimized MPA4 formulation and Efavirenz

Parameter	**EVF-S**	**MPA4**
$T_{1/2}$(hr)	8.36	15.64
T_{max}(hr)	1	6
C_{max}(µg/ml)	0.8	4.56
K_{elm}(h^{-1})	0.82	0.44
AUC(µg.hr/ml)	3.11	36.23
AUMC(µg.hr^2/ml)	12.28	238.90
MRT(hr)	3.94	6.59

CONCLUSION

It is evident from the experiment that; slurry method can be adopted for loading EFV. in maltodextrin based proniosomal formulation. The optimal combination of cholesterol and span-60 in equal proportions of the blend MPA4 is proven to demonstrate high E.E., surface charge and low vesicle size, the solid-state characterization indicates that conversion to molecular and amorphous states has been achieved from crystalline state. Also, the dissolution behaviour (*in- vitro*) and flow properties were as per standards. Apart, the absorption of the drug formulation is better inside the GI tract as concluded from the experiment in rat intestine from *ex-vivo* permeation studies. The better absorption is due to the presence of proniosomes in the formulation; which proves its potential as a carrier of EFV. through oral route. However, more *in-vivo* studies are required to be explored to increase the bioavailability of EFV by the use of proniosomes as carriers.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Singh P, Verma A, Mittal P, Khinchi MJAJoPR, Development. Self Emulsifying Drug Delivery System: An Approach To Enhance Oral Bioavailability. 2013:178-93.
2. Figueroa-Campos A, Sánchez-Dengra B, Merino V, Dahan A, González-Álvarez I, García-Arieta A, et al. Candesartan cilexetil in vitro–in vivo correlation: predictive dissolution as a development tool. 2020;12(7):633.
3. Babadi D, Dadashzadeh S, Osouli M, Abbasian Z, Daryabari MS, Sadrai S, et al. Biopharmaceutical and pharmacokinetic aspects of nanocarrier-mediated oral delivery of poorly soluble drugs. 2021:102324.
4. Ahad A, Raish M, Al-Jenoobi FI, Al-Mohizea AMJCdd. Sorbitane monostearate and cholesterol based niosomes for oral delivery of telmisartan. 2018;15(2):260-6.
5. Baig MR, Shahiwala A, Khan SJAiB, Bioavailability. Sensible Use of Technologies to Increase Solubility and Bioavailability in Formulation Development. 2018;1(1):1-4.
6. Gurrapu A, Jukanti R, Bobbala SR, Kanuganti S, Jeevana JBJAPT. Improved oral delivery of valsartan from maltodextrin based proniosome powders. 2012;23(5):583-90.

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

7. Jangam RP, Thombre AN, Gaikwad NPJAJoP, Technology. A review: proniosomes as a novel drug delivery system. 2017;7(3):166-74.
8. Debnath A, Kumar AJIJP, Eng. Structural and functional significance of niosome and proniosome in drug delivery system. 2015;3(3):621-37.
9. Upadhye S, Rafik INJAjPr. Proniosomes: A novel vesicular drug delivery system. 2020;10(2):260-73.
10. Mogatle S. African traditional medicines-antiretroviral drug interactions: the effect of african potato (Hypoxis hemerocallidea) on the pharmacokinetics of efavirenz in humans: Rhodes University; 2008.
11. Ganta KK, Mandal A, Chaubey BJCb, toxicology. Depolarization of mitochondrial membrane potential is the initial event in non-nucleoside reverse transcriptase inhibitor efavirenz induced cytotoxicity. 2017;33(1):69-82.
12. Chiappetta DA, Hocht C, Taira C, Sosnik AJN. Efavirenz-loaded polymeric micelles for pediatric anti-HIV pharmacotherapy with significantly higher oral bioavailability. 2010;5(1):11-23.
13. Ismail S, Khattab AJJoDDS, Technology. Optimization of proniosomal itraconazole formulation using Box Behken design to enhance oral bioavailability. 2018;45:142-50.
14. Khudair N, Agouni A, Elrayess MA, Najlah M, Younes HM, Elhissi AJJoDDS, et al. Letrozole-loaded nonionic surfactant vesicles prepared via a slurry-based proniosome technology: Formulation development and characterization. 2020;58:101721.
15. Zhou Y, Li W, Chen L, Ma S, Ping L, Yang ZJEt, et al. Enhancement of intestinal absorption of akebia saponin D by borneol and probenecid in situ and in vitro. 2010;29(3):229-34.

NOTE: **"Herewith submitting our article through International e-conference organized by Nirmala College of Pharmacy. So, kindly do the needful**