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Research Article

EXPLORING THE ORAL ABSORPTION ENHANCING EFFECT OF PIPERINE AND CHITOSAN ON TENOFOVIR LOADED SOLID LIPID NANOPARTICLES

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ABSTRACT

The main aim of the present work is to formulate the absorption enhanced Solid lipid Nanoparticles of Tenofovir Disproxil Fumerate by the use of piperine and Chitosan as absorption enhancers. SLNs are prepared by hot homogenization method followed by ultrasonication with Compritol 888, Glyceryl monostearate, Glyceryl distearate as solid lipids. Poloxomer 188 and Tween 80 were used as surfactants. Twelve formulations were prepared (TSLN 1 – TSLN 12) with drug lipid ratio of 1:3 and different concentrations of surfactants (1% and 2%). The formulated Nanoparticles were characterized for particle size, zeta potential, Entrapment efficiency, *in vitro* drug release and Transmission Electron Microscopy (TEM) Studies. Among the prepared formulations TSLN 4 was observed as better formulation with less particle size and zeta potential, highest drug release and better entrapment efficiency. The optimized formulation SLN4 was added with different amounts of chitosan and piperine (2, 4, 6 and 8mg) to form nine formulations (F1-F9). All formulations are subjected for *ex vivo* absorption studies with chicken intestine to prove the permeability enhancement. Among nine formulations F4 shown highest permeability coefficient (10.2 X 10^{-5} cm/sec). Histopathological studies revealed that chitosan is safe for gastro intestinal epithelial cells as it does not disturbs the natural integrity of the same. Thus, Tenofovir Disproxil fumerate loaded SLNs prepared with Chitosan can be clinically promising for enhancing the oral intestinal absorption of the said BCS Class-III drug.

Keywords: Absorption enhancement, Solid Lipid Nano particles (SLN), Tenofovir Disproxil Fumerate

INTRODUCTION

All drugs are classified in to four classes by Biopharmaceutical Classification system (BCS) based on their solubility and permeability. Among the four classes, BCS class-III drugs are characterized by high solubility and low permeability. The oral bioavailability of most of the available drugs is limited by poor permeability which is the characteristic of BCS class-III drugs[1]. The present drug Tenofovir Disproxil Fumerate (TDF) belongs to the same BCS class which is an anti viral drug. TDF is an ester prodrug of Tenofovir which is hydrolyzed to Tenofovir inside the cell and is phosphorylated to the active metabolite, tenofovir diphosphate. It acts by nucleotide reverse trascriptor inhition[2]. This is used in first line treatment of chronic hepatitis B and HIV/AIDS. The delivery of this drug through oral route is limited by poor oral absorption and thereby poor bioavailability. Its absorption is 25% in fasting state and 40% with high fatty meal[3]. So, there is a need to develop a new strategy to improve the absorption of above drug. Addition of bioenhancers like piperine and chitosan may increase the absorption tremendously and at the same time Solid lipid Nanoparticles (SLNs) are such type of delivery system which will correct the limitation of the drug by its small size and ability of targeting.

Solid lipid nanoparticles are widely used in improving the oral bioavailability of drugs belonging to BCS Class-III and IV with poor permeability nature[4]. SLNs are advanced drug delivery tools in which drug is held into the solid lipids like triglycerides, fatty acids, steroids and waxes which is stabilized by some surfactants like Poloxamer, soy lecithin etc[5]. These systems were in the range of 100 – 150 nm and they will act as alternatives to polymeric materials. These SLNs are very similar to the oil in water type of emulsion except in case of liquid lipid which is replaced by solid lipid[6]. These systems may be a promising sustained release approach for delivering a poorly permeable drug.

In the present work SLNs of TDF were formulated with an object of targeting and the optimized formulation is added with above said bioenhancers (Chitosan and Piperine) to increase the absorption and bioavailability of the drug. The resulting absorption enhanced SLNs were subjected for *ex vivo* intestinal absorption study with help of chicken intestine followed by the histopathology studies.

MATERIALS & METHODS

TDF was purchased from Hetero drugs Private Ltd. Hyderabad, Telangana. Compritol 888 was purchased from Hi media, Chennai, which is used as solid lipid. Glyceryl monostearate and distearate were received as gift samples from Orchid chemicals, Chennai, which are also used as solid lipids. Poloxamer 188 was purchased from S.D. Fine Chemicals, Mumbai, which is used as surfactant. Tween 80 was purchased from Yarrow Chemicals

which is also used as surfactant. All the chemical components used in the present work are of analytical grade.

Pre-formulation studies

Possible interactions between active pharmaceutical ingredient and excipients were identified by Fourier Transform Infrared spectrometer (FTIR). Respective FTIR spectra were obtained for all individual ingredients and the admixture. The resultant spectra were interpreted for possible interactions.

Construction of standard calibration curve

Standard calibration curve was plotted for TDF in pH 6.8 phosphate buffer and 0.1N HCl. Working standards of 5, 10, 15, 20 and 25 mcg/ml concentrations were prepared and the absorbance was obtained to plot the concentration on X-axis and absorbance on Y- axis.

Lipid selection

Solubility study of different lipids in drug was performed for lipid selection. Each lipid (Compritol 888, Glyceryl monostearate & Glyceryl distearate) was mixed separately in two test tubes with active ingredient 1:2 and 1:3 ratios. The mixture of lipid and drug were melted in water bath above 5^0C melting point of lipid. The test tubes were observed for miscibility.

Formulation of SLNs of TDF

High pressure homogenization method was used to formulate the solid lipid nanoparticles[7]. To prepare SLNs, an aqueous phase and an oil phase must be prepared. So, oil phase containing lipids like Compritol 888, Glyceryl monostearate and Glyceryl distearate with TDF were added to a measuring beaker and an aqueous phase containing Poloxamer 188 and water were added to another measuring beaker. Beakers containing oil phase and aqueous phase were subjected to increased temperature up to 75^0C for melting followed by addition of two phases with shearing to form a primary emulsion. It is followed by ultrasonication and high pressure homogenization for 3 cycles to produce SLNs[8].

Evaluation

Percent Entrapment Efficiency

The formulated SLNs were evaluated for %EE. To obtain this, about 10ml of SLN dispersion centrifuged in cooling centrifuge which is maintained at 20,000 RPM for 2 hours. The supernatant is analyzed at 261nm (n=3) with the help of UV visible spectrophotometer. This will gives the amount of drug present in the final SLN formulation. The final %EE is calculated by formula given below[9].

%EE = Total amount of drug – Amount of drug present in supernatant X 100 / Total amount of drug

Particle size and Zeta potential

These two parameters gives information about the size of the particle in the dispersion and degree of aggregation of Nanoparticles respectively. This was analyzed by an instrument called HORIBA zeta sizer. A little volume of SLN dispersion is diluted with purified water which is further added to polystyrene cells and placed in particle size analyzer. The sample was kept at 25^0C and the light scattering was seen. The results were read in the instrument.[10]

Transmission Electron Microscopy studies (TEM)

TEM studies are mainly useful for studying the morphology of SLNs. Mixture of phototungstic acid and SLN dispersion were placed on copper grid. Carbon was previously coated on this grid. The resulting sample was micro graphed[11].

***In vitro* dissolution studies**

Dialysis bag diffusion method is used for invitro release studies of prepared SLN formulation. The dialysis membranes was previously soaked in aqueous water for 12h. The one end of the membrane was closed and 5ml of SLN formulation was poured through the opening and closed. This is immersed in measuring beaker by hanging from the top.

The release studies were conducted for 12 hours (first 2hrs in 0.1N HCl and next 10Hrs in 6.8 phosphate buffer). The samples were diluted suitably and analyzed by UV-Visible spectrophotometric method. The % cumulative drug release was calculated[12].

Preparation of absorption enhanced Solid Lipid Nanoparticles of TDF

The optimized SLN formulation with TDF which shown better drug release, less particle size, enough encapsulation efficiency was taken and it is added with different amounts of absorption enhancers (Piperine and Chitosan) and subjected to *Invitro* absorption studies[13].

***In vitro* absorption studies by chicken intestine**

These are also referred as continuous dissolution and absorption studies. It consists of a single basket USP dissolution apparatus with a perfusion apparatus which consists of two tubes (Tube A & Tube B) connected together. Tube A and Tube B are having straight and bent cannulas respectively at their end. This perfusion compartment is attached with chicken everted intestinal segment which is considered as receptor compartment[14]. Chicken intestine was isolated from male white leghom chicks and kept in Krebs ringers solution and it is everted by using glass rod[15-16].

$$Papp\ (cm/sec) = (dQ/dt) \times 1 / (60 \times A \times C_0)$$

Where dQ/dt = the amount of compound traversing through tissue in time t (min), A = exposed area of the tissue, C_0 = Initial concentration of drug in the donor compartment.

Enhancement ratio (ER) = Permeability coefficient of drug with enhancer / Permeability of drug alone

The above study was performed for pure drug suspension as well as best formulation for Tenofovir disproxil fumerate.

RESULTS

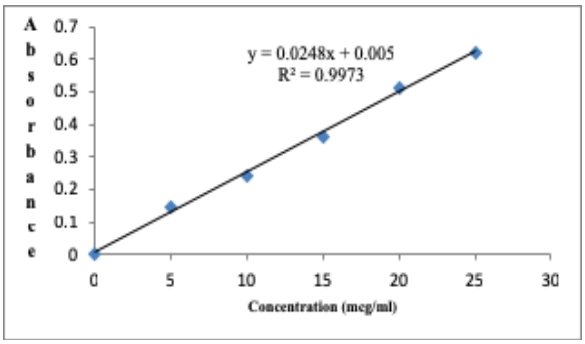

Figure 1: Standard calibration curve of TDF in pH 6.8 Phosphate buffer

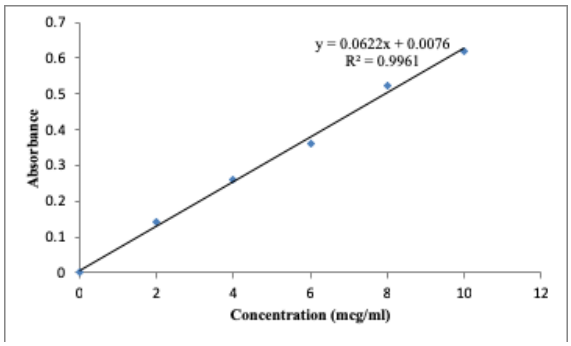

Figure 1: Standard calibration curve of TDF in 0.1 N Hydrochloric acid

Table 1: Selection of lipid

Name of the lipid	Melting point of lipid	Drug : Lipid ratio	
		1:2	1:3
Glyceryl monostearate	55-60^{0}C	Turbid	Clear
Glyceryl disterate	52-55^{0}C	Turbid	Clear
Compritol 888	65-77^{0}C	Turbid	Clear
Stearic acid	69-70^{0}C	Not clear	Turbid

Table 2: Formulation Composition of SLNs of Tenofovir

Code	Tenofovir	Compritol 888 (Lipid)	Glyceryl di stearate (Lipid)	Glyceryl monostearate (Lipid)	Tween 80 (Surfactant)	Poloxamer 188 (Surfactant)
TSLN1	50	150	-	-	1%	-
TSLN 2	50	150	-	-	2%	-
TSLN 3	50	150	-	-	-	1%
TSLN 4	50	150	-	-	-	2%
TSLN 5	50	-	150	-	1%	-
TSLN 6	50	-	150	-	2%	-
TSLN 7	50	-	150	-	-	1%
TSLN 8	50	-	150	-	-	2%
TSLN 9	50	-	-	150	1%	-
TSLN 10	50	-	-	150	2%	-
TSLN 11	50	-	-	150	-	1%
TSLN 12	50	-	-	150	-	2%

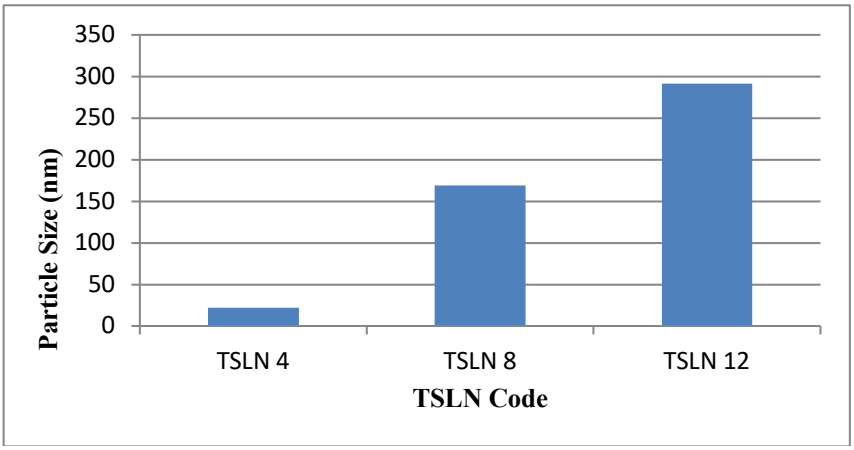

Figure 3: Particle sizes of SLNs

Table 3: Zeta potential of SLNs

Formulation No.	Zeta potential
TSLN 4	-45.6mV
TSLN 8	-25.2 mV
TSLN 12	-26.2 mV

Table 4: *In vitro* drug release for F1 - F6

Time (Hours)	TSLN1	TSLN 2	TSLN 3	TSLN 4	TSLN 5	TSLN 6
1	**30±0.12**	**36±0.1**	**40±0.13**	**45±0.14**	**30±0.12**	**34±0.06**
2	55±0.14	65±0.11	72±0.1	79.8±0.18	46±0.14	53±0.09
3	60±0.16	70±0.13	77±0.02	84.8±0.11	51±0.16	58±0.12
4	65±0.18	75±0.15	82±0.12	89.8±0.12	56±0.15	63±0.16
5	70±0.19	80±0.17	87±0.09	94.8±0.16	61±0.14	68±0.14
6	75±0.11	85±0.12	92±0.15	**99.8±0.13**	66±0.13	73±0.11
12	76±0.32	85±0.22	91±0.01	99.7±0.11	67±0.21	72±0.19

Note: Values represents Mean ± SD; n=3

Table 5: *In vitro* drug release for F7 - F12

Time (Hrs)	TSLN 7	TSLN 8	TSLN 9	TSLN 10	TSLN 11	TSLN 12
1	**38±0.16**	**42±0.1**	**28±0.12**	**34±0.06**	**38±0.09**	**40±0.12**
2	57±0.08	60±0.11	40±0.09	45±0.09	48±0.05	54±0.11
3	62±0.06	65±0.09	45±0.15	50±0.12	53±0.07	59±0.15
4	67±0.1	70±0.08	50±0.13	55±0.15	58±0.1	64±0.13
5	72±0.14	75±0.12	55±0.08	60±0.16	63±0.14	69±0.09
6	77±0.13	**80±0.17**	60±0.12	65±0.12	68±0.13	**74±0.011**
12	76±0.22	81±0.02	60±0.01	65±0.22	68±0.11	**74**±0.02

Note: Values represents Mean ± SD; n=3

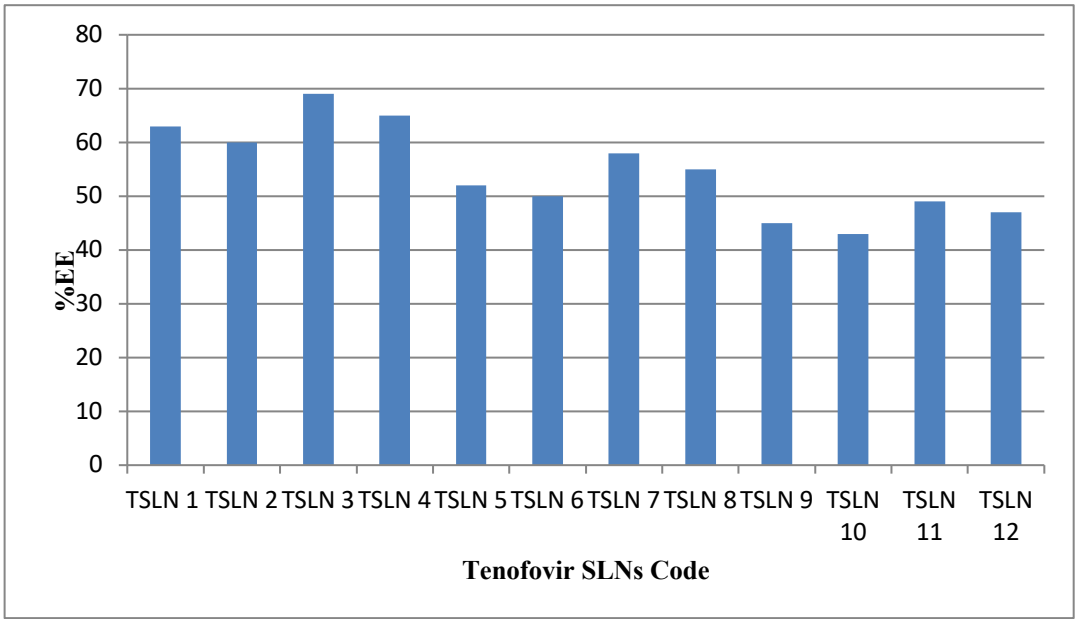

Figure 4: % Entrapment efficiency

Transmission Electron Microscopy (TEM)

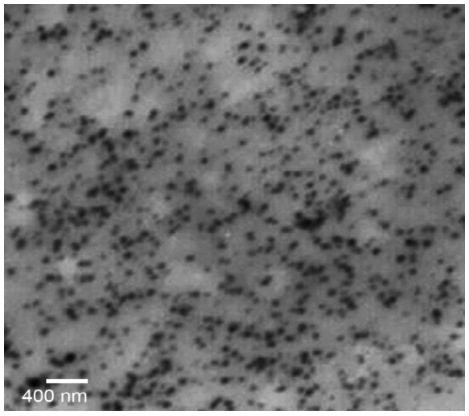

Figure 5: TEM image of TSLN 4 at 400nm

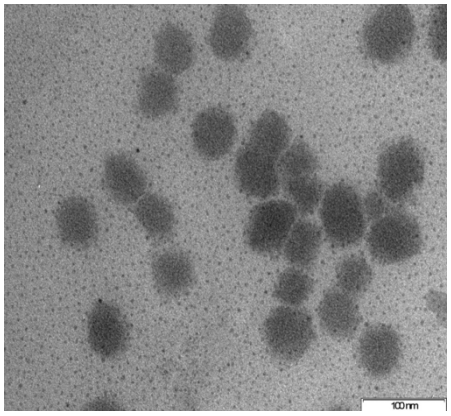

Figure 6: TEM image of TSLN 4 at 100nm

Table 6: Optimized formulation (TSLN 4) added with absorption enhancers and their respective apparent permeability constants

Optimized Formulation number	TDF (mg)	Compritol (mg)	Poloxamer188	Chitosan (mg)	Piperine (mg)	Papp (cm/sec)
F1	50	150	2%	2	-	4.21×10^{-5}
F2	50	150	2%	4	-	6.01×10^{-5}
F3	50	150	2%	6	-	8.61×10^{-5}
F4	50	150	2%	8	-	10.2×10^{-5}
F5	50	150	2%	-	6.5	3.51×10^{-5}
F6	50	150	2%	-	7	5.06×10^{-5}
F7	50	150	2%	-	7.5	7.22×10^{-5}
F8	50	150	2%	-	8	9.31×10^{-5}
F9	50	150	2%	-	-	2.10×10^{-5}

Figure 9: Histopathology of F 8

DISCUSSION

The pre-formulation studies for Tenofovir Disproxil fumerate were conducted. The FTIR studies of drug with polymers and absorption enhancers were conducted. The spectrums of FTIR shown that no functional group is missed in the admixture of drug and excipients which were present in the individual ingredients. So, there is no interaction between drug and polymers for the formulation of Solid Lipid Nanoparticles.

The drug release from the formed SLNs follow biphasic release pattern. About 30-45% of incorporated drug was released in the first two hours followed by a slower release of the drug up to 12 hours. The amount of drug released in SLN 1 – SLN 4, SLN 5 – SLN 8 AND SLN 9 – SLN 12 is different. This may be attributed to the presence of different types of lipids i.e., compritol 888, glyceryl monosterate and glyceryl disterate. It is also due to the presence of different surfactants like tween 80 and Poloxamer with varying concentrations i.e., 1% and 2% concentrations.

Among the first four formulations i.e., SLN 1- SLN4, the formulation SLN 4 shown the highest release (upto 100%) which may be due to the presence of surfactant Poloxamer 188 (2%). The surfactant Poloxamer 188 shows better drug release than the tween80 (in F1&F2) due to the higher HLB value of Poloxamer 188 (greater than 24) than tween 80 (HLB 15). Further the higher percentage of surfactant higher is the drug release.

The formulations SLN1 – SLN 4 shows higher entrapment efficiency which is attributed to the presence of lipid Compritol 888. It is superior to other lipids in terms of entrapment efficiency due to its relaxed nature. Further, Poloxamer 888 causes slight increase in viscosity of external phase thereby diffusion speed of drug is reduced towards external phase. So, among all formulations SLN 3 shows highest % entrapment efficiency due to the presence of Polaxomer and due to the presence of less concentration (1%).

The particle size and zeta potential of SLN 4 for both drugs was performed. Both shown the nanoparticulate range as mentioned in results. The zeta potential values of both drugs reveals that the particles in the dispersion were in non aggregated state.

The optimized formulation of both drugs (i.e., SLN4) was taken and it is added with different amounts of chitosan and piperine. For all the formulations of TDF (F1 – F9) absorption studies by using chicken intestine were performed by using everted sac method. The results reveal that the permeation coefficient of F4 prepared with TDF containing 8mg of chitosan shown highest permeability coefficient (10.2×10^{-5} cm/sec). On the other hand the permeation coefficient of F8 containing 8mg of piperine showed a permeability coefficient of 9.31×10^{-5} cm/sec which is lesser than F4. bio-enhancing effect of chitosan is more when compared to Piperine.

CONCLUSION

The present research work "exploring the oral absorption enhancing effect of piperine and chitosan on tenofovir loaded solid lipid nanoparticles" investigated the possibility of enhancement of absorption of poorly absorbed drug TDF through gastrointestinal mucosa. Tenofovir Disproxil Fumerate is a poorly absorbed drug with high solubility in the Gastro intestinal lumen. Its absorption is limited by the tight junctions of epithelial cells. Absorption enhancers can overcome this sort of absorption limitations. So, Piperine and Chitosan are two natural absorption enhancers which will make the penetration of this drug through gastrointestinal epithelial cells.

The target drug is formulated in the form of Solid Lipid Nanoparticles (SLNs) by the use of Piperine and Chitosan as absorption enhancers. The *ex vivo* absorption studies proved that the prepared SLNs have shown better absorption than the pure drug. The SLNs prepared by Piperine shown irreversible damage to epithelial cells. Whereas the SLNs prepared by Chitosan shown reversible damage to epithelial cells which is desirable.

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