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DESIGN AND CHARACTERIZATION OF SOLID LIPID NANOPARTICLES OF GANCICLOVIR FOR IMPROVED ORAL ABSORPTION

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ABSTRACT

Ganciclovir is a BCS Class-III drug is used in first line therapy for immunocompromised people for prevention & treatment of infections caused by cytomegalovirus. Delivery of this drug by oral route is limited as a result of its low oral bioavailability. Thus, design of novel oral delivery system in the form of solid lipid Nanoparticles (SLN) overcomes these drawbacks. Different types (Piperine & Chitosan), compositions (Piperine- 5mg to 20mg; Chitosan – 200mg to 350 mg) & combinations (Piperine alone, Chitosan alone. Piperine & chitosan combinations) of absorption enhancers were mixed with drug and prepared SLN 1 to SLN 12 formulations by hot homogenization (Quantum H-900) method followed by ultrasonication (Sonics U-164). The process parameters like type of lipid, surfactant, power input & pulse for ultrasonication were optimized. Formulations from SLN1 to SLN4 contain piperine, SLN5 to SLN8 contains chitosan and SLN9 to SLN12 contains combination of piperine & chitosan as absorption enhancers in different compositions. The SLN dispersion is subjected for characterization which includes particle size, zeta potential, entrapment efficiency, *in vitro* drug release, *ex vivo* studies. Among all formulations, SLN 8 is found to be optimized formulation as it is confirmed as safest formulation without rupturing the epithelial cells.

1. INTRODUCTION:

Biopharmaceutical Classification System (BCS) classified all the drugs based on the permeability and solubility into four classes. Drugs which are characterized by high solubility and low permeability fall under BCS Class – III drugs. The oral bioavailabilities of most of the drugs are limited by their low permeability which belongs to the same class. Ganciclovir is an anti viral drug belongs to the same BCS classification. The core objective of the present study is to design & characterize solid Lipid Nanoparticulate drug delivery systems to enhance oral bioavailability of the antiviral drug Ganciclovir (Sam Maher et al., 2015). This drug is used in first line therapy for immunocompromised people for prevention & treatment of infections caused by cytomegalovirus. Ganciclovir is

primarily delivered through intravenous route which suffers from several drawbacks like high cost, skilled person for administration which finally leads to patient incompliance. Delivery of this drug by oral route is limited as a result of its low oral bioavailability. Thus, design of novel oral delivery system in the form of solid lipid Nanoparticles (SLN) overcomes these drawbacks. Solid Lipid Nanoparticles (SLN) are nanoparticles invented 20 years ago and are prepared from a lipid matrix which is a solid at body and room temperature, stabilized by suitable surfactants. The researchers identified a fact that the use of solid lipids instead of liquid oils may provide the controlled drug release. This is because of the lower mobility of the drug in solid matrix compared to liquid oil. SLNs. Contribute their widespread in treating the pulmonary diseases, cancers, central nervous system related diseases, cardiovascular system related diseases, osteoporosis, diabetes etc. Despite from advantages, several challenges like burst release, inefficient release remains to be resolved in SLNs for better delivery of drugs (Schipper NGM et al.,1997).

2. MATERIALS & METHODS

2.1 Materials

Ganciclovir drug is obtained as a gift sample from Hetero drugs Pvt.Ltd. Jadcherla, Telangana. Chitosan is obtained from S.D. Fine chemicals, Mumbai. Piperine is obtained from Yucca enterprise, Mumbai. Poloxamer 188 and soya lecithin were obtained as gift samples from Orchid chemicals, Chennai. Steraic acid is obtained from Hi-media, Chennai. All the chemicals used in the study were of Pharmaceutical grade.

2.2 Methods

2.2.1 Preparation of Ganciclovir Solid Lipid Nanoparticles: High Pressure Homogenization (HPH) method is used to prepare the Solid Lipid Nanoparticles (SLN) of TDF. HPH is a powerful technique for the large scale production. It has been used for years for the preparation of nanoemulsions and SLN. One of the sub method of HPH is Hot Homogenization method which is used for the present formulation. In hot HPH, lipid and drug are melted in the presence of surfactant at the same temperature. This mixture is sheared by hot shear device, to form a pre emulsion (Pre em). The hot Pre em was cooled to recrystallize in order to generate SLN. In this lipid phase containing TDF, Stearic acid, piperine and (or) chitosan is taken in a beaker and in other hand Poloxamer 188 with water is taken. Both were heated at 75^0C (above the melting point of lipid). Then the aqueous phase is added to lipid phase gradually by shearing to obtain a primary emulsion. This is subjected to ultrasonication at 400 watts power and 90%pulse for 15 minutes followed by subjecting it to High Pressure Homogenization at 750 bars pressure for 3 cycles. The resultant dispersion is cooled at 18^0C to generate the SLN (JH Hochman etal.,1994).

2.2.2 Formulation Optimization:

The formulation optimization was done by preparing different bathes by varying the parameters. The parameters studied were type of surfactant and its amount, energy input & pulse for ultra sonication, time of ultrasonication.

2.2.2.1 Selection of Lipid:

Suitable surfactant selection was done by solubility study of lipid in drug. Drug and lipid were mixed in two different ratios 1:2 & 1:3 in different test tubes. The mixture of lipid (Compritol & stearic acid) and drug were melted above 5^0C melting point of lipid using water bath. The test tubes were observed for miscibility and clarity whose results were tabulated below (ShineyTakatsuka et al.,2006).

2.2.2.2 Selection of surfactant: The selection of proper surfactant for the preparation of SLN can be done by taking

3 types of surfactants. They are Tween 80, Span 20 & Poloxamer 188. All the surfactants were taken in 1.0, 1.5 & 2.0 % w/v and prepared the SLN dispersion by keeping all other parameters constant. The prepared dispersions were checked for entrapment efficiency (n=3). Among the three surfactants Poloxamer 188 showed better entrapment efficiency (Schipper NGM et al., 1997).

2.2.2.3 Optimization of ultrasonication for energy input and pulse: The energy input & pulse required for the stable SLN dispersion can be optimized by preparing the formulations with 250, 400, 750 watts power and 30, 60 and 90% pulse. All the prepared formulations were checked for entrapment efficiency. The formulation prepared by 400 watts power and 90% pulse shown better entrapment efficiency than other types of formulations.

2.3 Evaluation studies:

2.3.1 Percent Entrapment Efficiency (%EE): The prepared SLN were evaluated for percent drug entrapment efficiency. To obtain the %EE, 10ml of the prepared SLN dispersion is taken in a centrifuge tube and it is placed in Remi cooling centrifuge. The centrifuge is rotated at 20,000 RPM for 2 hours. The resultant supernatant is taken and analyzed at 261nm (n=3) by UV visible spectrophotometer to obtain the amount of drug present in the dispersion. The %EE is calculated by the following mathematical expression (Varma MV et al., 2003).

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

2.3.2 Particle size and Zeta potential:

The particle size & Zeta potential is the vital characterization parameter for Nanoparticles. The particle size decides whether the prepared formulation is in nano size or not. The zeta potential explains about the degree of aggregation of Nanoparticles. The average particle size and zeta potential of best formulation was analysed by HORIBA (zeta sizer). A small volume of SLN dispersion is diluted with high purity water which is again filled in polysterene cells and subjected to particle size analyser at a wavelength of 632nm. The scattering of light on the sample was monitored at 173^0 angle at a temperature of 25^0C. the values of particle size and zeta potential were obtained from the software present in the instrument.

2.3.3 Transmission Electron Microscopy (TEM for morphological characterization):

The formulated Solid Lipid Nanoparticles were characterized for their morphological study by using Transmission Electron Microscopy (Zeiss EVOMa 15). The SLN dispersion was mixed with phosphotungstic acid (0.02% w/v) and kept aside in room temperature (for 5minutes) to obtain the equilibration. A drop of this preparation is placed on a copper grid which is coated with carbon. Draining of excess liquid is done and dried at room temperature. The prepared sample is micrographed at 200kv on a digital TEM station.

2.3.4 *In vitro* release studies:

The *invitro* release of Ganciclovir from prepared SLN formulations is carried out by the dialysis bag diffusion technique. The dialysis membrane was soaked for 12h in water before it is used for release studies. The dialysis bag was sealed in one end and 5ml of drug loaded SLN was placed and sealed at another end. This is hanged and immersed in a beaker containing 100ml of 6.8 phosphate buffer. The contents of the beaker were stirred at 100rpm at 37 ± 0.5^0C. The aliquots of the sample were withdrawn at regular intervals of time (every 1hr) and replaced with same amount of fresh medium. The samples were diluted suitably and analysed by UV-Visible spectrophotometric method. The % cumulative drug release was calculated(Bruce J. Aungst etal.,1996).

2.3.5 *In vitro* absorption studies (Continuous dissolution and absorption studies):

The *in vitro* absorption studies are also referred as continuous dissolution and absorption studies. The system consists of a single basket USP dissolution apparatus 2. A perfusion apparatus consisting of two tubes (Tube A & Tube B) connected together is attached to the dissolution vessel. Tube B has a bent cannula and Tube A has a straight cannula at their end. The dissolution vessel which is considered as donar compartment is filled with 900 ml of pH 6.8 phosphate buffer. The perfusion apparatus is attached with an everted intestinal segment of chicken in between the two ends of cannulas.

2.3.5.1 Isolation of Everted Chick intestine:

Male white leghorn chicks were bought from the local market weighing between 500gm and 600gm. On the other hand the Krebs Ringer's solution was prepared by adding 6.3 gm of Sodium Chloride, o.35 gm of potassium chloride, 0.14gm calciumchloride, and 0.16gm of potassium dihydrogen phosphate, 0.15 gm of magnesium sulphate, 2.1 gm of sodium carbonate and 5gm of glucose in one liter of distilled water (Jonathan M et al.,2009). For isolation of intestine the chick is altered by making a median incision on the abdomen and small intestine was isolated. The lumen was carefully cleared for mucous by rinsing with a pH6.8 phosphate buffer solution. A 6cm intestinal segment was removed and transferred to oxygenated Krebs Ringer's solution. It was washed with Krebs Ringer's solution. The proximal part of intestine was turned back and ligated on a glass rod and everted sac is formed.

2.3.5.2 Protocol

Dissolution studies: Type of dissolution apparatus: USP type –I, Dissoltion medium: p 6.8 phosphate buffer, Volume of dissolution medium: 900ml, Paddle speed: 75rpm, Time interval for sampling withdraw from dissolution (donor) compartment :every 1 hr, Temparature of dissulotion mediu: 37°±0.5°C, SLN Dispersion: 100ml is tied to the bottom of rotating paddle (contains 50mg of drug)

Absorption studies:

Name of animal intestine taken; Hen, Part of intestine used : duodenum Solution used for cell viability - Krebs ringer solution, Medium taken in absorption compartment - Krebs ringer solution, Volume of medium taken in absorption compartment - 30ml. Time interval for sampling withdraw from absorption (receiver) compartment – every 1hr 10 minutes. From the above experiment apparent permeability coefficient (Papp) can be determined from the following formula.

Papp (cm/sec) = (dQ/dt) x 1 / (60 x A x 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 and Ganciclovir.

2.3.6 Histopathological Studies:

Histopathological studies of everted intestine (which is exposed to 24 hrs absorption studies) was performed by cutting down it to pieces and were flushed with normal saline and dipped in 10% neutral buffered formalin solution at the end of the experiment and the pieces were processed by paraffin technique. The cut pieces of 5-µm thickness were stained with Haematoxylin-Eosin method and were focused under fluorescent microscope and observed for the damage of epithelial cells on the intestinal mucosa if any.

3. RESULTS & DISCUSSION

3.1 Preformulation studies:

The preformulation studies for both drugs Tenofovir Disproxil fumerate and Ganciclovir were conducted. The FTIR spectral studies drug with polymers and

absorption enhancers were conducted. The spectrums of FTIR shown that no functional group is mixed in the admixture of drug and polymers which were present in the individual ingredients. So, there is no interaction between drug and polymers for the formulation of Solid Lipid Nanoparticles.

3.2Particle size: The particle size and zeta potential of SLN 8 was performed. It shown the nanoparticulate range as mentioned in results (27nm). The zeta potential values (-18.3) reveal that the particles in the dispersion were in non aggregated state.

3.3Percent entrapment efficiency: The entrapment efficiency of all the prepared formulations was studied. The %EE of first four formulations is less when compared to SLN 5 – SLN8. The reason is assumed that the presence of piperine, the lipid is unable to hold the drug in its matrix beacause of its nature of making more pores on any structure. Where in the latter case they contained the chitosan which is not having any effect on entrapment of drug as like that of piperine

3.4 *In vitro* drug release studies:

The invitro drug release studies reveals that SLN 1 –SLN 4 the drug release is more when compared to SLN 5 – SLN 8 owing to the reason that the first four formulations consists of the piperin as an absorption enhancer which enhances more pores in the nano particle structure due to which the release. Where as in the latter case absorption enhancer is chitosan where it doesn't forms pores as much as that of piperine, so the less release than first four formulations. The SLN 9- SLN 12 showed more release than the first eight formulations because of the presence of both enhancers (Piperine, Chitosan) which made more number of pores on nano particle structure. Different kinetic models like zero order, first order, Higuchi's, korsemeyer, Hixson-crowel plots were plotted.

3.5TEM (Transmission Electron Microscopy) Analysis:

The TEM analysis (Zeiss EVOMa 15) of the best formulation (SLN8) reveals that the Nanoparticles are in round shape with smooth morphological structure. The TEM images of best formulation (SLN 8) were shown in figure 10.

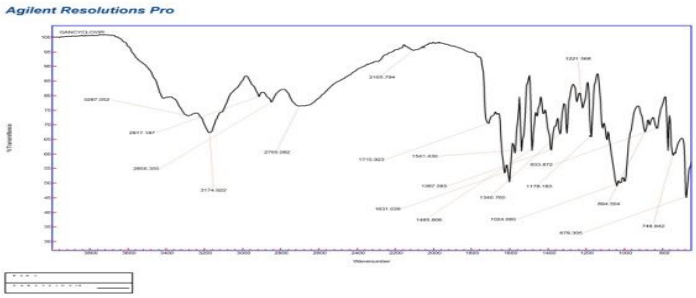
3.6Absorption studies: Simultaneous dissolution and absorption studies for the best formulation is performed by using chicken intestine with everted sac method. The results shown that there is a two fold increase in the absorption of best formulation (SLN 8) when compared to pure drug suspension. The permeability coefficient (Papp) of prepared best formulation is higher than the pure drug suspension which was shown in results. This confirms the absorption enhancement of poorly permeable drug with SLN.

3.7Histological studies: Histopathological studies for formulations with piperine, chitosan and combinations of chitosan & piperine were performed. The results revealed that the formulations with piperine caused a irreversible damage to epithelial cells where as formulation with chitosan, maintained epithelial cell integrity.

4. CONCLUSION

Solid Lipid Nanoparticles of Ganciclovir were prepared with different absorption enhancers to improve the oral bioavailability of the same drug. From the results obtained the SLN8 which contains Chitosan as an absorption enhancer shown safer drug release and enhanced absorption when compared to the pure Ganciclovir drug without disturbing the natural epithelial cell integrity. This formulation also showed better entrapment efficiency and drug release. Hence, Ganciclovir, an anti viral drug used for immunocompromised patients shows better bioavailability when it is formulated in the form of Novel Drug Delivery Systems like SLN with absorption enhancers.

Figure 1: FTIR for Ganciclovir:



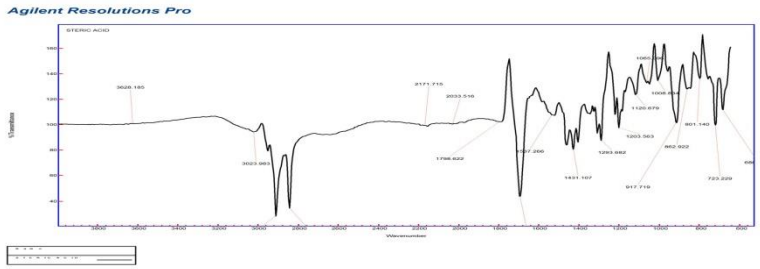


Figure 2: FTIR Spectrum for Stearic acid and Figure 3: FTIR Spectrum of Lecithin.

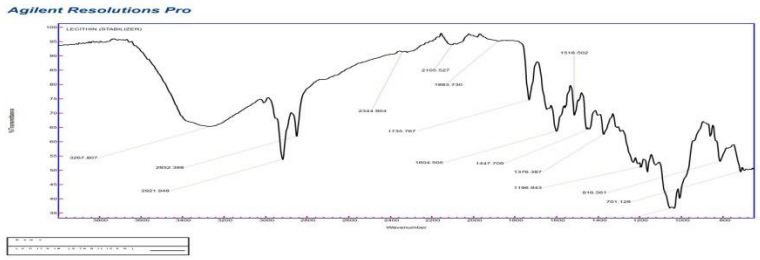


Figure 4: FTIR Spectrum of Poloxamer 188 (Pluronic)

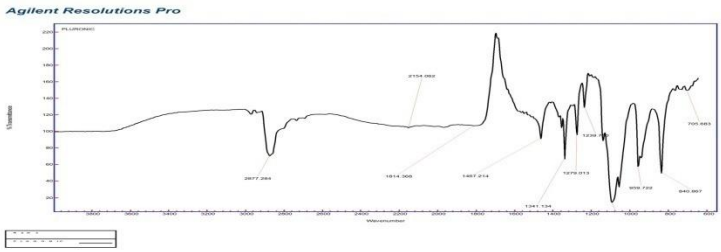


Figure 5: FTIR spectrum of Chitosan:

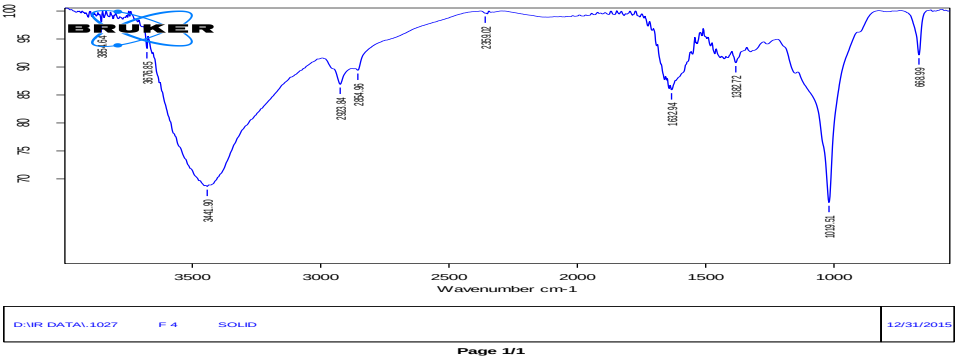


Figure 6: FTIR Spectrum of Piperine:

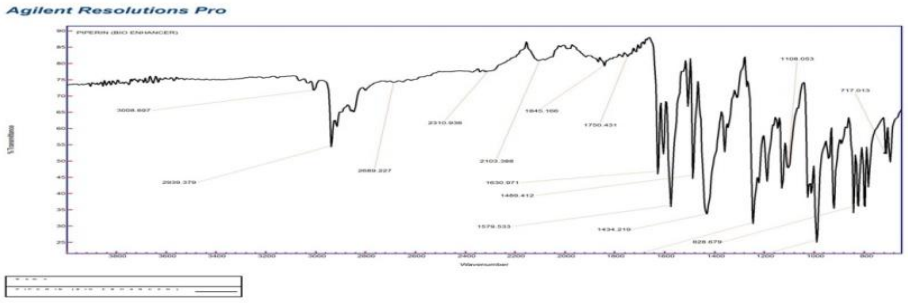


Figure 7: FTIR Spectrum of Admixture:

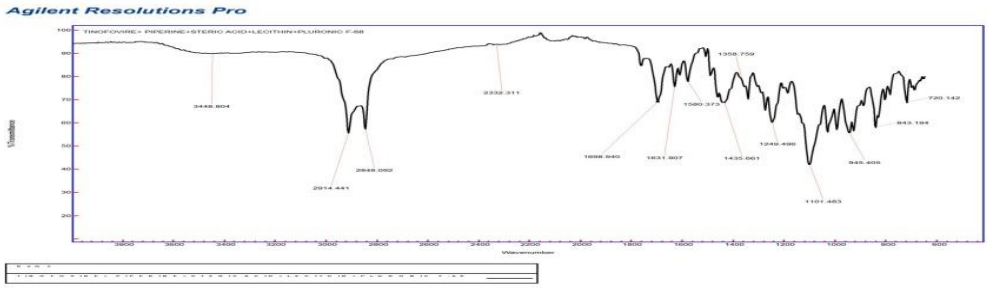


Table 1: FTIR Spectral studies

Name of the functional group	Frequency of Ganciclovir (cm^{-1})	Frequency of chitosan (cm^{-1})	Frequency of Piperine (cm^{-1})	Frequency of Stearic acid(cm^{-1})	Frequency of Poloxamer 188(cm^{-1})	Frequency of lecithin(cm^{-1})	Frequency of Admixture (cm^{-1})
C=C & C=N stretching	1485	-	1434	1467	-	1447	1580
C-C stretching	1024	1019	-	801	1279	816	1249
C-H bending	748	-	828	-	-	-	720
C-H stretching	2850	-	2939	3023	2877	2921	2848
N-H stretching	-	2359	2310	-	-	2344	2332
C=O stretching	1715	-	1630	-	1814	1735	1698

Table 2: Composition of Ganciclovir Solid Lipid Nanoparticles

Formulation No.	API (Tenofovir/Ganciclovir)mg	Lipid(Stearic acid)mg	Surfactant (Poloxamer) %W/V for 100 ml SLN	Piperine(absorption enhancer) mg	Chitosan (Absorption enhancer) mg
SLN 1	50	1000	2%	5	-
SLN 2	50	1000	2%	10	-
SLN 3	50	1000	2%	15	-
SLN 4	50	1000	2%	20	-
SLN 5	50	1000	2%	-	200
SLN 6	50	1000	2%	-	250
SLN 7	50	1000	2%	-	300
SLN 8	50	1000	2%	-	350
SLN 9	50	1000	2%	5	200
SLN 10	50	1000	2%	10	250
SLN 11	50	1000	2%	15	300
SLN 12	50	1000	2%	20	350

Table 3: Selection of lipid

3.2 Formulation optimization:

Name of the lipid	Melting point of lipid	Drug Lipid ratio	
		1:2	1:3
Compritol	65^0C	++	++
Stearic acid	69^0C	+	+++

\+ Not clear
++ Turbid
+++ Clear

Table 4: Selection of surfactant:

S.NO.	Surfactant system	%w/v for 30ml of dispersion	%Entrapment efficiency (n=3)
1	Tween 80	1.0	78.7±0.64
2	Tween 80	1.5	80.0±0.47
3	Tween 80	2.0	81.2±0.51
4	Span 20	1.0	74.9±0.32
5	Span 20	1.5	79.3±0.17
6	Span 20	2.0	80.2±0.41
7	Poloxamer 188	1.0	81.9±0.72
8	Poloxamer 188	1.5	84.1±0.32
9	Poloxamer 188	2.0	85.7±0.45

Table 5: Optimization of ultrasonication for energy input and pulse:

S.NO.	Power (Watts)	Pulse (%)	Percent Entrapment
1	250	30	70.2±0.12
2	250	60	72.3±0.17
3	250	90	74.3±0.57
4	400	30	83.1 ±0.23
5	400	60	85.1±0.71
6	400	90	86.1±0.25
7	750	30	71.1±0.43
8	750	60	71.3±0.61
9	750	90	72.3±0.57

3.3 Evaluation studies

Percent Entrapment Efficiency (%EE):

$$\%EE = \frac{\text{Total amount of drug – amount of drug present in supernatant}}{\text{Total amount of drug}} \times 100$$

Table 6: Percent Entrapment Efficiency (%EE)

Formulation No.	Percent Entrapment Efficiency (%EE) (n=3)
SLN1	65.4 ±0.21
SLN2	69.6 ±0.34
SLN3	74.4 ±0.62
SLN4	78.3 ±0.82
SLN5	86.8 ±0.59
SLN6	88.9 ±0.29
SLN7	84.4 ±0.34
SLN8	87.8 ± 0.35
SLN9	70.6 ± 0.32
SLN10	72.7 ±0.13
SLN11	76.3 ±0.36
SLN12	79.5 ±0.78

Table 7: Particle size and Zeta potential:

Formulation No.	Particle size	Zeta potential
SLN 8	27 nm	-18.3 mV

Figure 8: Particle size analysis of SLN8

SZ-100

201604271104000.nsz

Measurement Results

Measurement Type	: Particle Size
Sample Name	: SLN a
Scattering Angle	: 173
Temperature of the Holder	: 25.2 °C
Dispersion Medium Viscosity	: 0.892 mPa·s
Transmission Intensity before Meas.	: 923
Distribution Form	: Standard
Distribution Form(Dispersity)	: Monodisperse
Representation of Result	: Scattering Light Intensity
Count Rate	: 1388 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	27.0 nm	11.0 nm	22.9 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	27.0 nm	11.0 nm	22.9 nm

Cumulant Operations

Z-Average	: 1894.6 nm
PI	: 0.188

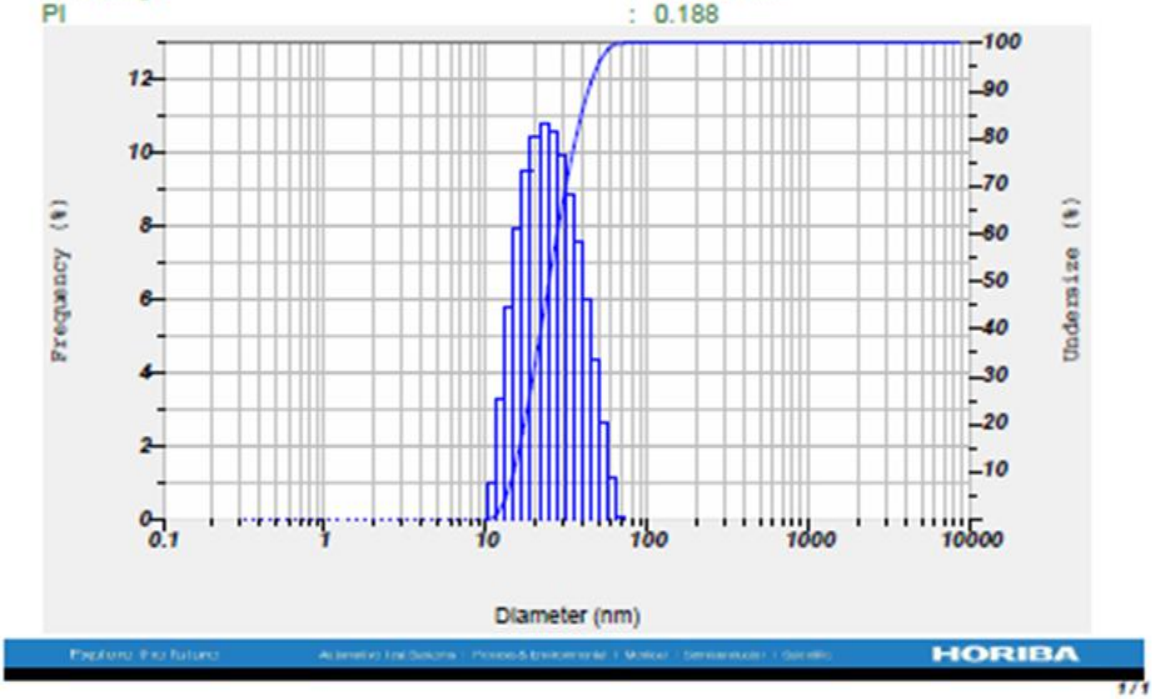


Figure 9: Zeta potential of SLN8

HORIBA Scientific

HORIBA SZ-100 for Windows [Z Type] Ver2.00

SZ-100

Measurement Results

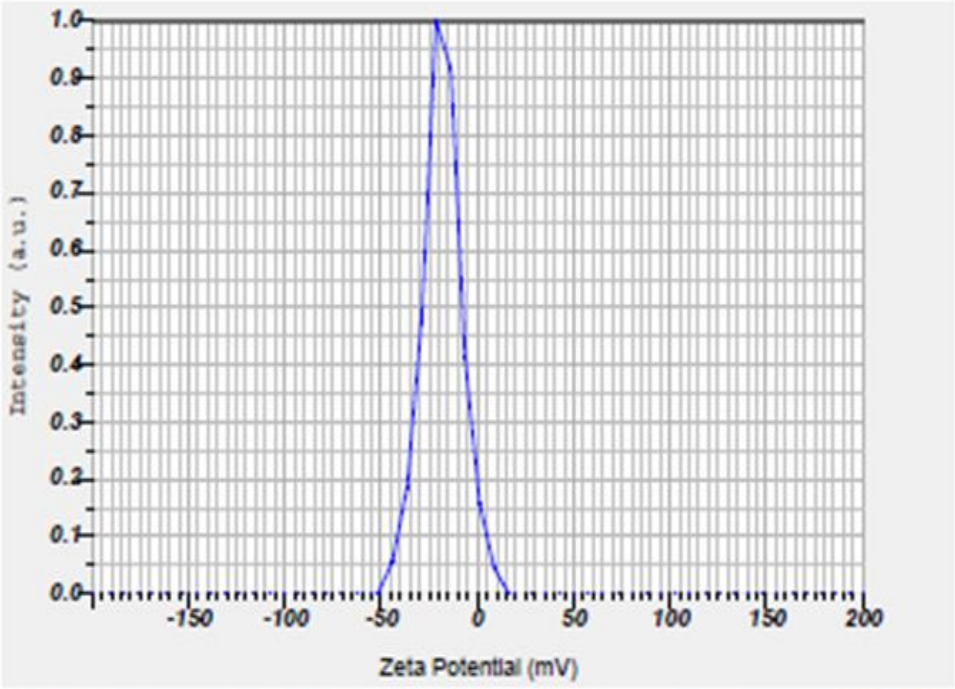
201605021334007.nzt
Measurement Results

Measurement Type	:	Zeta Potential
Sample Name	:	SLN b F5
Temperature of the Holder	:	25.0 °C
Dispersion Medium Viscosity	:	0.894 mPa·s
Conductivity	:	0.439 mS/cm
Electrode Voltage	:	3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-18.3 mV	-0.000142 cm2/Vs
2	— mV	— cm2/Vs
3	— mV	— cm2/Vs

Zeta Potential (Mean) : -18.3 mV
Electrophoretic Mobility Mean : -0.000142 cm²/Vs

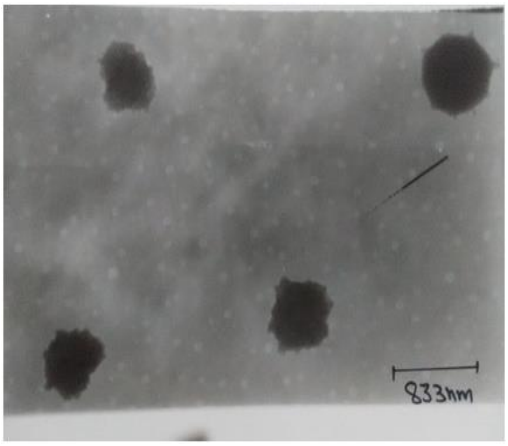


HORIBA

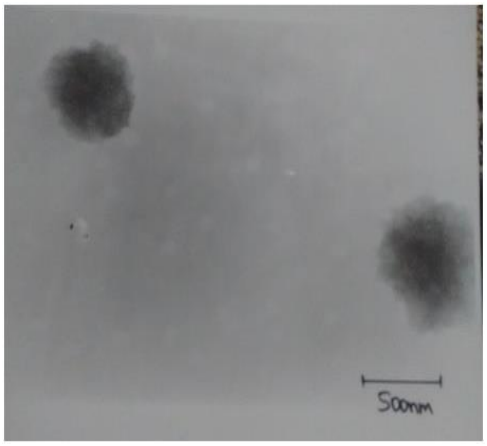
Time (Hrs)	Percentage cumulative drug release (n=3)											
	SLN 1	SLN 2	SLN 3	SLN 4	SLN 5	SLN 6	SLN 7	SLN 8	SLN 9	SLN 10	SLN 11	SLN 12
1	12.2±0.41	14.9±0.5	17.1±0.5	18.2±12	6.4±0.46	5.2±0.12	7.9±0.54	11.2±0.21	19.9±0.26	21.7±0.21	21.2±0.5	22.5±0.32
2	16.5±0.12	18.3±0.2	21.7±0.43	22.5±0.32	8.8±0.27	9.9±0.42	11.8±0.12	15.3±0.31	23.3±0.12	25.5±0.31	25.4±0.42	26.6±0.23
3	19.2±0.32	21.9±0.5	24.9±0.76	25.6±0.5	11.2±0.72	12.3±0.76	14.5±0.32	18.4±0.43	26.5±0.52	28.2±0.52	28.2±0.31	29.3±0.31
4	22.9±0.54	24.7±0.43	27.1±0.24	28.8±0.43	14.7±0.14	15.8±0.32	17.8±0.63	21.8±0.75	29.8±0.42	31.6±0.63	31.7±0.54	32.7±0.42
5	25.8±0.76	27.4±0.65	30.4±0.65	31.3±0.52	17.6±0.31	18.5±0.83	20.9±0.31	24.9±0.41	32.3±0.53	33.3±0.51	34.9±0.31	35.3±0.61
6	28.3±0.43	30.7±0.32	33.8±0.63	34.8±0.13	20.8±0.96	21.2±0.21	23.2±0.28	27.1±0.51	35.7±0.51	36.8±0.62	37.5±0.62	38.9±0.62
7	31.2±0.23	33.2±0.76	36.6±0.76	37.6±0.52	23.9±0.5	24.9±0.61	26.9±0.72	30.2±0.65	38.2±0.75	39.4±0.76	40.7±0.31	41.2±0.51
8	34.2±0.65	36.8±0.21	39.3±0.32	40.2±0.31	26.4±0.61	27.2±0.32	29.8±0.42	33.9±0.31	41.4±0.31	42.9±0.87	43.2±0.31	44.1±0.56
9	37.8±0.12	39.3±0.72	42.8±0.5	43.9±0.92	29.2±0.82	30.4±0.71	32.3±0.73	36.2±0.51	34.7±0.42	45.4±0.92	46.4±0.26	47.5±0.12
10	40.1±0.23	41.2±0.32	44.8±0.21	45.5±0.21	31.9±0.21	33.6±0.92	34.8±0.72	38.6±0.76	46.9±0.93	47.2±0.21	48.6±0.82	49.2±0.41
22	72.5±0.59	76.2±0.65	78.2±0.43	80.9±0.21	63.4±0.87	65.3±0.12	69.4±0.49	77.9±0.31	75.3±0.31	78.2±0.43	80.8±0.23	82.4±0.42
23	75.9±0.98	78.8±0.21	80.6±0.76	84.3±0.65	65.0±0.91	67.9±0.12	76.3±0.63	79.8±0.71	78.7±0.63	82.1±0.12	85.3±0.31	86.9±0.51
24	78.2±0.21	79.2±0.87	82.3±0.41	86.9±0.31	68.5±1	72.4±0.62	79.2±0.63	83.6±0.31	85.2±0.63	86.3±0.54	88.6±0.31	89.3±0.31

Table 8: *In vitro* Drug release studies

Figure10: Transmission Electron Microscopy



A) TEM photograph (833nm)



B) TEM photograph (500nm)

Table 9: *In vitro* absorption studies (Continuous dissolution and absorption studies):

Formulation No.	Permeability coefficient (Papp) cm/sec	Enhancement ratio
Ganciclovir pure drug suspension	$0.396x10^{-5}$	----
SLN 8	$2.021x\ 10^{-5}$	5.103

Histopathological Studies:

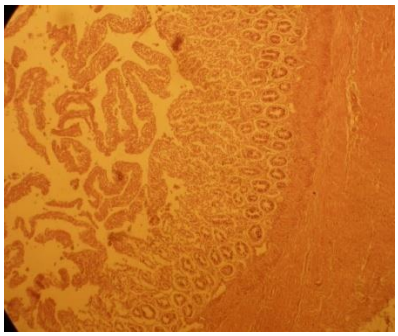


Fig 11: Control

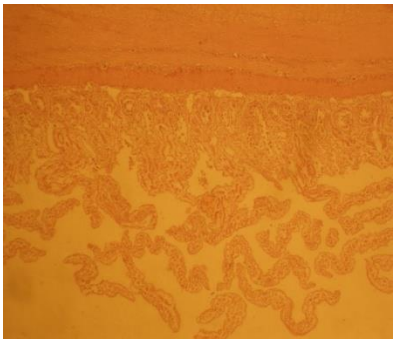


Fig 12: SLN 4 (with Piperine alone)

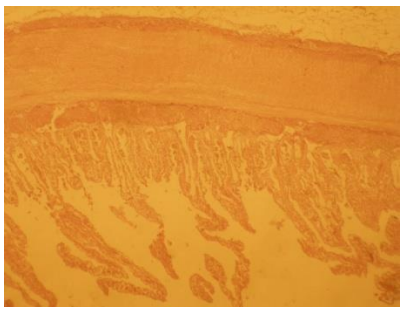


Fig 13: SLN 8 (with Chitosan alone)

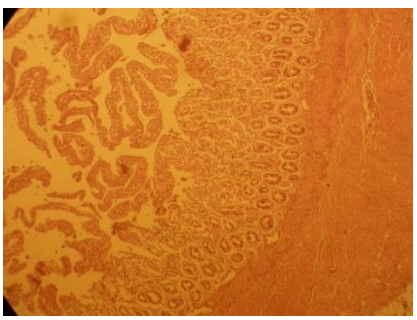


Fig 14: SLN 12 (Piperine + Chitosan)

REFERENCES

1. Sam Maher, Marc Davocelle, Sinead Ryan, Siobhan McClean, Impact of amino acid replacements on invitro permeation enhancement and cytotoxicity of the intestinal absorption promoter, melittin; International Journal of Pharmaceutics 387 (2010) 154-160.
2. Jonathan M. Miller, ArikDahan, Quasi-equilibrium analysis of the ion-pair mediated membrane transport of low-permeability drugs; Journal of controlled release; 137(2009) 31-37.
3. M. RavindraBabu, P. Ravi Prakash, N. Devenna; Intestinal drug transporters; Creative Journal of Pharmaceutical research; 02 (2015) 88-99.
4. Dimple pabla, Intestinal permeability enhancement of levothyroxine sodium by straight chain fatty acids studied in MDCK epithelial cell line; European Jpournal of Pharmaceutical Sciences 40 (2010) 466-472.
5. Myungjookang, jaeyoulcho, Byung Ho Shim, Bioavailability enhancing activities of natural compounds from medicinal plants; Journal ofs Medicinal plants research 13 (2009) 1204 – 1211.
6. Shiney Takatsuka, Takeo Kitazawa, Enhancement of intestinal absorption of poorly absorbed hydrophilic compounds by Simultaneous use of mucolytic agent and non-ionic surfactant; EJP&B, 62 (2006) 52-58.
7. Hochman; Mechanism of absorption enhancement and tight junction Regulation; JCR; 29 (1994) 253-267.
8. Schipper, chitosans as absorption enhancers for poorly absorbable drugs; Journal of pharmaceutical research 14 (1997) 923-929.
9. Buur A, Bundgaard H, Falch E; Prodrugs of 5- Fluorouracil; Acta Pharm. Suec 23 (1986);205-216.
10. Varma MV, P-glycoprotien inhibitors and their screening; a perspective from bioavailability enhancement; Journal of Pharmacology research 48 (2003) 347-359.
11. Viega, Oral bioavailability and hypoglycaemic activity of tolbutamide/cyclodextrin inclusion complexes; International Journal of Pharmaceutical sciences 202 (2000) 165-171.
12. Bruce, Enhancement of the intestinal absorption of peptides and non-peptides; Journal of controlled release 41 (1996) 19-31.
13. Myung Joo Kang, Bioavailability enhancing activities of natural compounds from medicinal plants; School of Bioscience and Biotechnology; 16 December, 2009; 200-701.
14. Dimple pabla; Intestinal permeability enhancement of levothyroxine sodium by straight chain fatty acids studied in MDCK epithelial cell line; European Jpournal of Pharmaceutical Sciences 40 (2010) 466-472.
15. Young; “Chemical laboratory information profile, Oleic acid; Journal of chemical education; 79:24.
16. Shiney Takatsuka; Enhancement of intestinal absorption of poorly absorbed hydrophilic compounds by Simultaneous use of mucolytic agent and non-ionic surfactant; European Journal of Pharmaceutics & Biopharmaceutics 62 (2006) 52-58.
17. Young, “Chemical laboratory information profile, Oleic acid; Journal of chemical education; 79:24
18. Martena, “Medium chain triglyceride ;International airy journal 16(11): 1374-1382.
19. Stonge, MP; Jones; “Physiological effects of medium chain triglycerides; Potentialagents in the prevention of obesity”; The journal of nutrition 132(3).
20. Meneses, High resolution 31PNMR of extracted phospholipidin; The journal of lipid Reasearch 29(5);679-689