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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. Total 12 (GSLN 1 to GSLN 12) formulations by hot homogenization (Quantum H-900) method followed by ultrasonication (Sonics U-164) were prepared. The process parameters like type of lipid, surfactant, power input & pulse for ultrasonication were optimized. The SLN dispersion is subjected for characterization which includes particle size, zeta potential, entrapment efficiency, *in vitro* drug release. 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. Compritol 888, Glyceryl monostearate (GMS),Glyceryl distearate (GDS) were obtained from S.D. Fine chemicals, Mumbai. Poloxamer 188 was 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, Compritol 888, GMS and GDS separately were 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 lipids 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 *in vitro* release of Ganciclovir from prepared SLN formulations is performed by dialysis bag diffusion technique. The dialysis membrane was soaked for 12h in water before it is used for drug release studies and is sealed at one end and 5ml of drug loaded SLN was placed and sealed at another end. This is hanged and immersed in a beaker. The release studies were conducted in 0.1N HCl in first 2hours and in 6.8 phosphate buffer in the next 4 hours. 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 analyzed by UV-Visible spectrophotometric method. The % cumulative drug release was calculated (Bruce J. Aungst etal.,1996).

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

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.

4. CONCLUSION

Solid Lipid Nanoparticles of Ganciclovir were prepared to improve the oral bioavailability of the same drug. From the results obtained the GSLN4 was considered as optimized formulation. 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 SLNs.

Figure 1: FTIR for Ganciclovir

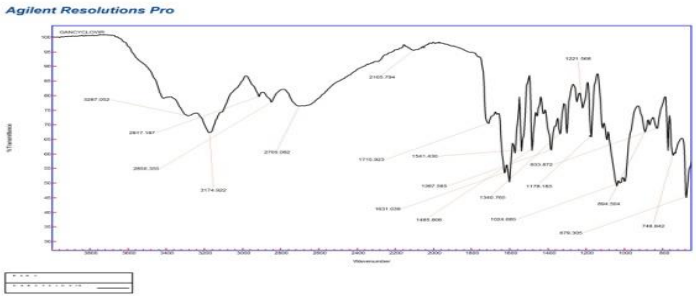


Figure 2: FTIR Spectrum for Compritol 888

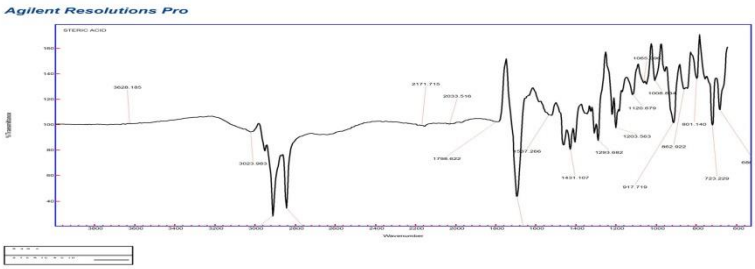


Figure 3: FTIR Spectrum of GMS

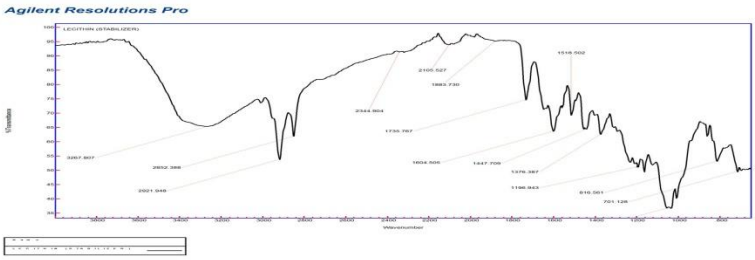


Figure 4: FTIR Spectrum of Poloxamer 188 (Pluronic)

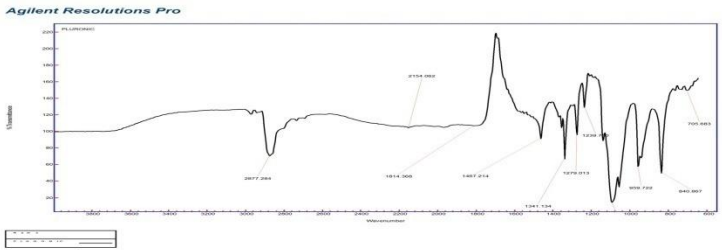


Figure 5: FTIR spectrum of GDS

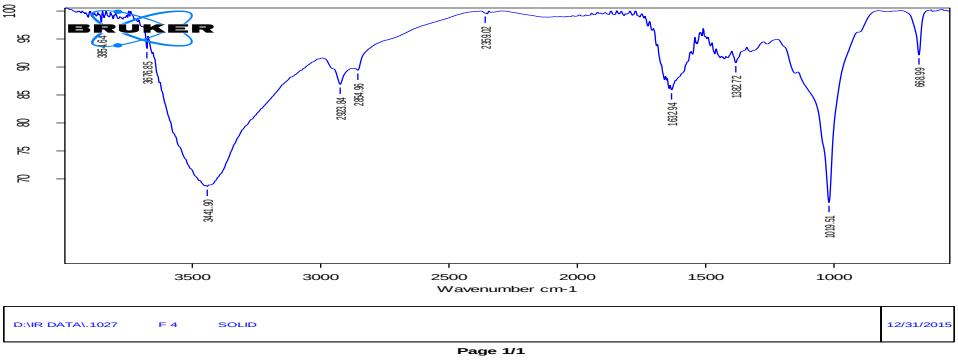


Figure 6: FTIR Spectrum of Admixture

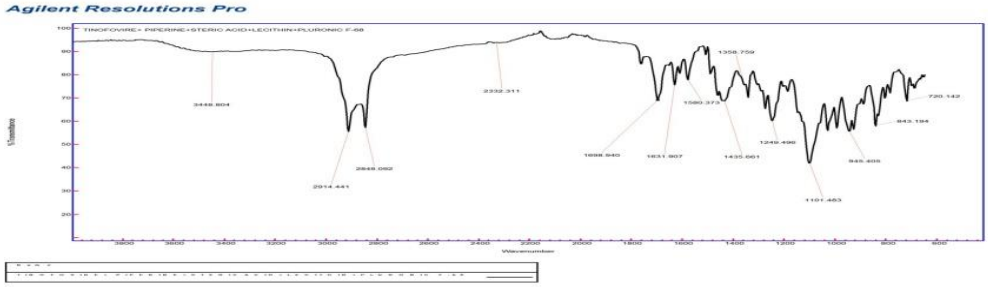


Table 1: FTIR Spectral studies

Name of the functional group	Frequency of Ganciclovir (cm^{-1})	Frequency of Compritol 888 (cm^{-1})	Frequency of GMS (cm^{-1})	Frequency of GDS(cm^{-1})	Frequency of Poloxamer 188(cm^{-1})	Frequency of Admixture (cm^{-1})
C=C & C=N stretching	1485	-	1434	1467	-	1580
C-C stretching	1024	1019	-	801	1279	1249
C-H bending	748	-	828	-	-	720
C-H stretching	2850	-	2939	3023	2877	2848
N-H stretching	-	2359	2310	-	-	2332
C=O stretching	1715	-	1630	-	1814	1698

Table 2: Composition of Ganciclovir Solid Lipid Nanoparticles

Code	Ganciclovir	Compritol 888 (Lipid)	Glyceryl di stearate (Lipid)	Glyceryl monostearate (Lipid)	Tween 80 (Surfactant)	Poloxamer 188 (Surfactant)
GSLN1	50	150	-	-	1%	-
GSLN 2	50	150	-	-	2%	-
GSLN 3	50	150	-	-	-	1%
GSLN 4	50	150	-	-	-	2%
GSLN 5	50	-	150	-	1%	-
GSLN 6	50	-	150	-	2%	-
GSLN 7	50	-	150	-	-	1%
GSLN 8	50	-	150	-	-	2%
GSLN 9	50	-	-	150	1%	-
GSLN 10	50	-	-	150	2%	-
GSLN 11	50	-	-	150	-	1%
GSLN 12	50	-	-	150	-	2%

Table 3: Selection of lipid

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

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 4: Percent Entrapment Efficiency (%EE)

Formulation No.	Percent Entrapment Efficiency (%EE) (n=3)
GSLN1	62.1 ±0.32
GSLN2	59.3 ±0.43
GSLN3	68.2 ±0.26
GSLN4	64.7 ±0.28
GSLN5	51.3 ±0.95
GSLN6	49.2 ±0.92
GSLN7	57.8 ±0.43
GSLN8	54.2 ± 0.53
GSLN9	44.1 ± 0.23
GSLN10	42.6 ± 0.31
GSLN11	48.2 ± 0.63
GSLN12	46.1 ± 0.87

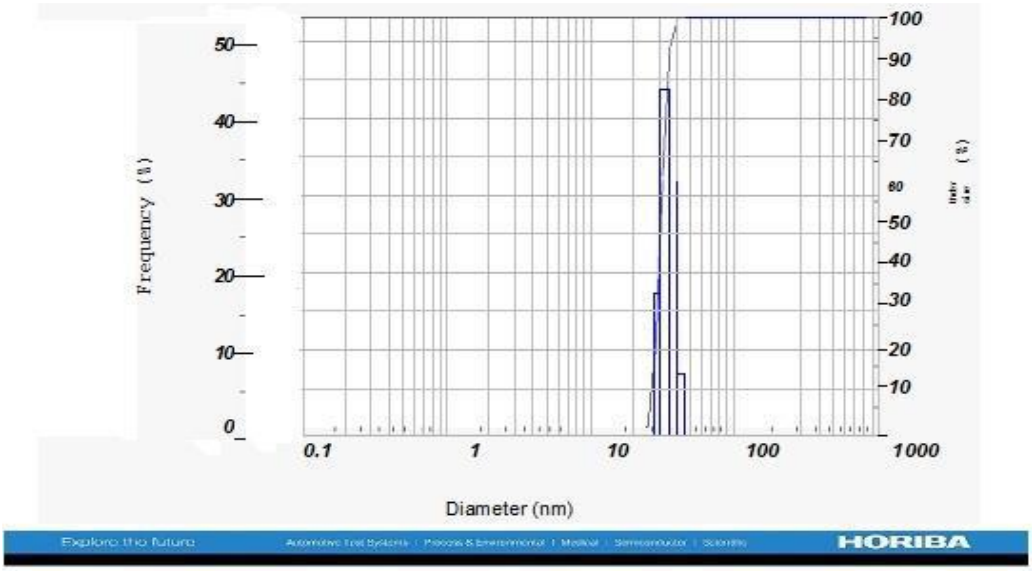


Figure 7: Particle size of GSLN 4

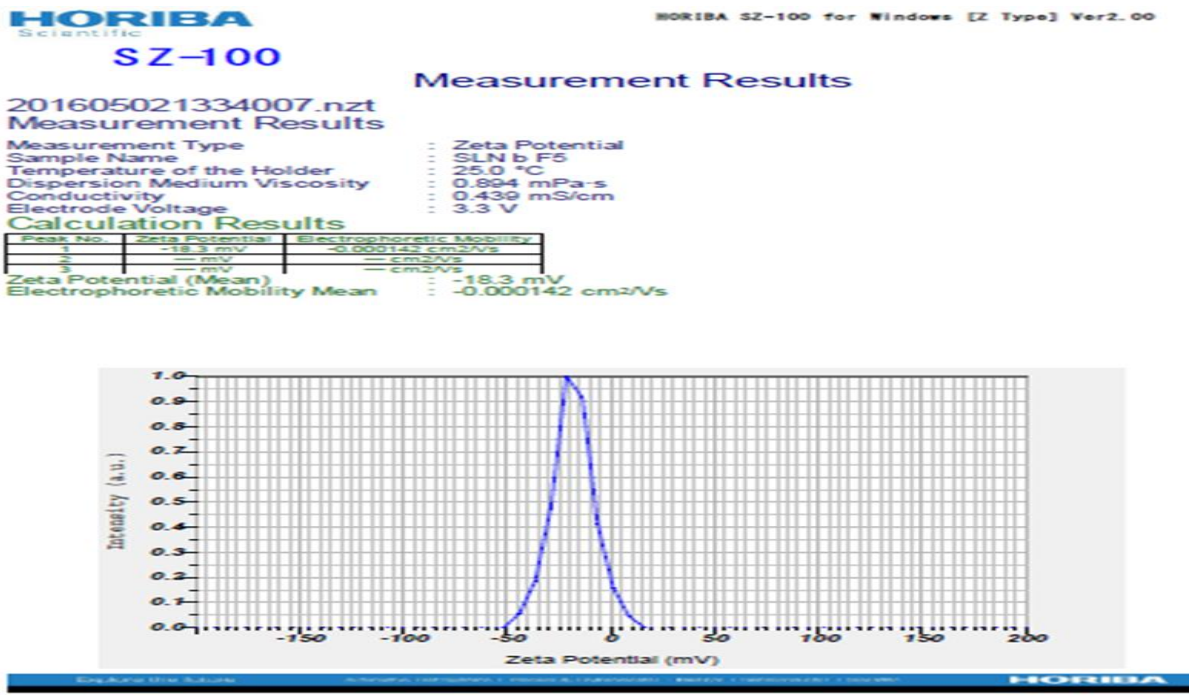


Figure 8: Zeta potential of GSLN 4

Table 5. Zeta potential

Formulation No.	Zeta potential
GSLN 4	-18.3mV
GSLN 8	-25.0mV
GSLN 12	-2.6 mV

Table 6. Particle size

Formulation No.	Particle size
GSLN 4	37.2 nm
GSLN 8	271.5 nm
GSLN 12	348.8 nm

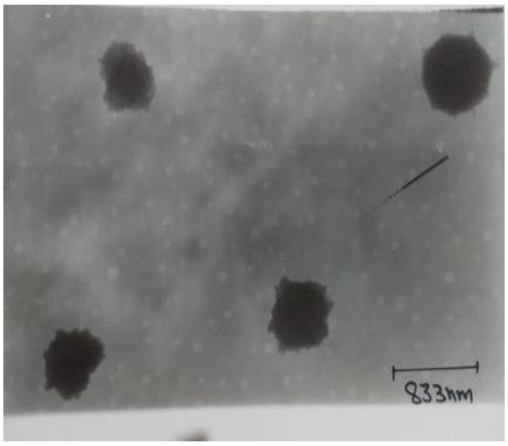
Time (Hrs)	GSLN 1	GSLN 2	GSLN 3	GSLN 4	GSLN 5	GSLN 6	GSLN 7	GSLN 8	GSLN 9	GSLN 10	GSLN 11	GSLN 12
1	28±0.12	34±0.1	37±0.13	44±0.14	28±0.12	32±0.06	36±0.16	41±2.1	26±0.12	32±0.06	36±0.09	39±0.12
2	55±0.14	65±0.11	72±0.1	78.9±0.18	46±0.14	53±0.09	57±0.08	58±0.11	40±0.09	45±0.09	48±0.05	50±0.11
3	60±0.16	70±0.13	77±0.02	83.9±0.11	51±0.16	58±0.12	62±0.06	63±0.09	45±0.15	50±0.12	53±0.07	55±0.15
4	65±0.18	75±0.15	82±0.12	88.9±0.12	56±0.15	63±0.16	67±0.1	68±0.08	50±0.13	55±0.15	58±0.1	60±0.13
5	70±0.19	80±0.17	87±0.09	93.9±0.16	61±0.14	68±0.14	72±0.14	73±0.12	55±0.08	60±0.16	63±0.14	65±0.09
6	75±0.11	85±0.12	92±0.15	**98.9±.2**	66±0.13	73±0.11	77±0.13	**78±0.17**	60±0.12	65±0.12	68±0.13	**70±0.011**

Table 7: *In vitro* Drug release studies

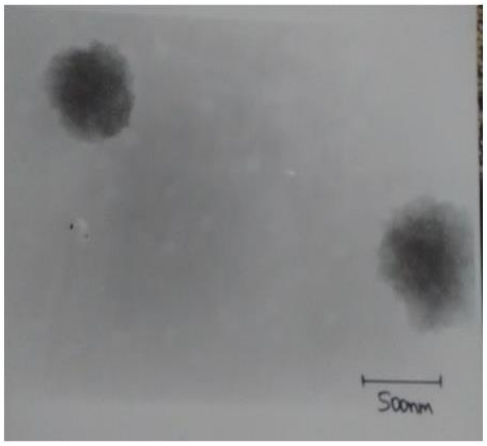
Formulation Number	Zero order		First order		Higuchi's		Koresmeyer's Peppa's		
	K_0	R^2	K_1	R^2	K_H	R^2	n	K_H	R^2
GSLN1	11.39	0.834	0.218	0.934	31.63	0.967	0.134	29.62	0.949
GSLN2	12.75	0.807	0.299	0.946	35.82	0.959	0.119	32.01	0.961
GSLN3	13.78	0.794	0.398	0.964	38.90	0.952	0.471	32.65	0.971
GSLN4	14.35	0.775	0.587	0.961	40.97	0.950	0.415	34.97	0.982
GSLN5	9.78	0.848	0.165	0.938	27.17	0.984	0.454	29.51	0.958
GSLN6	10.75	0.825	0.202	0.937	30.13	0.976	0.434	34.67	0.943
GSLN7	11.17	0.808	0.225	0.937	31.59	0.972	0.400	38.01	0.947
GSLN8	11.00	0.785	0.225	0.931	31.44	0.966	0.341	42.65	0.973
GSLN9	8.85	0.863	0.138	0,941	24.46	0.991	0.447	26.91	0.981
GSLN10	9.32	0.829	0.156	0.929	26.16	0.983	0.380	32.35	0.989
GSLN11	9.57	0.805	0.168	0.920	27.13	0.974	0.380	32.35	0.989
GSLN12	9.71	0.787	0.175	0.914	27.73	0.966	0.342	36.30	0.993

Table 8: *In vitro* Drug release Kinetics

Figure 9: Transmission Electron Microscopy



A) TEM photograph (833nm)



B) TEM photograph (500nm)

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