



## **Formulation, Characterization and *in-vitro* Evaluation of Famciclovir Loaded Solid Lipid Nanoparticles for Improved Oral Absorption**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors CKT and PKR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SKS and AKP managed the analyses of the study and literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Famciclovir loaded Solid Lipid Nanoparticles (SLNs) using triglycerides as solid lipids were successfully prepared using the double emulsion-solvent evaporation technique. Formulation parameters like amount and type of lipid and level of surfactants affected the nanoparticle characters. It was observed that nanoparticle characters like average particle size and distribution, drug content, entrapment efficiency and release pattern were dependent on these formulation variables. The optimized formulations depicted the desired characters of low particle size, in the range of 140-170 nm in case of Glyceryl monostearate (GMS) and glyceryl distearate (GDS) SLNs and 250-340 nm in case of glyceryl behenate (GB) SLNs and entrapment efficiencies in the range of 35-48%. In vitro drug release was extended upto 8 h and the release profile was explained by the Baker-Lonsdale model for spherical particles. Morphological examination by Scanning Electron

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Microscope (SEM) and Transmission Electron Microscope (TEM) displayed homogenous solid, spherical and non-porous particles. The formulations depicted good redispersibility after lyophilization and presence of residual solvents in the formulations within the prescribed limits suggested suitability of the preparation technique. Freeze-dried formulations were found to be stable in terms of particle size and drug loading even after 6 months of storage at refrigerated conditions.

*Keywords: Famciclovir; solid lipid nanoparticles; bioavailability; release kinetics; etc.*

## 1. INTRODUCTION

NDDS offer many advantages over conventional delivery systems with enhanced desired therapeutic effects and lowered or no side effects. Recent trend shows that microparticulate and nanoparticulate drug delivery systems have played a significant role in meeting the challenges associated with conventional delivery systems.

Since oral route is the most convenient route of drug administration, nanoparticles often find their applications in oral drug delivery. The advantages offered by nanoparticulate systems in oral drug delivery are improvement in bioavailability of drugs either by increasing the drug solubility, permeability or by overcoming the first-pass effect and P-gp efflux and improved stability of drugs in the GIT [1]. Among the nanoparticulate delivery systems, SLNs have shown good potential in oral drug delivery [2-4]. Composed of biocompatible solid lipids and emulsifiers, SLNs have the ability to incorporate both hydrophilic and lipophilic drugs, and are potential bioavailability enhancer vehicle for various Class II, III and IV drugs. Various absorption mechanisms are proposed for these drug carriers which lead to enhanced oral bioavailability of the drug entrapped into them. These include absorption of the intact nanoparticles through Peyer's patches and M-cells in the intestine or by facilitating lymphatic uptake [5,6]. Moreover, SLNs generally contain lipophilic or hydrophilic surfactants as stabilizers, some of which have been reported to inhibit P-gp mediated efflux [7].

Famciclovir was the first Food and Drug Administration (FDA) approved drug with significant activity against CMV and is still the primary drug of choice for these infections. Initial treatment with Famciclovir involves daily continuous i.v. infusions for several days, followed by oral or i.v. maintenance therapy. The i.v. therapy suffers from limitations like patient inconvenience associated with i.v. administration,

higher cost, incidence of needle-related infections and sepsis. Oral treatment is of choice but poor bioavailability (<10%) requires frequent administration of large dose per day (four capsules of 250 mg administered each time, thrice in a day). Famciclovir is a BCS-class III drug having high solubility and low permeability due to its hydrophilic nature. It is mainly transported by paracellular route, where the limited surface area and the tight junctions present between the adjacent cells restrict the transport of the drugs. Few reports also suggest P-gp mediated efflux of Famciclovir from the enterocytes back to the intestinal lumen. Furthermore, poor oral bioavailability of Famciclovir is associated with greater inter-subject variability of plasma concentrations, development of drug resistance and drug wastage. These shortcomings necessitate the need for better oral delivery systems for Famciclovir, particularly for increased absorption and selective distribution [8,9].

In the present study, solid lipid nanoparticulate delivery systems for Famciclovir have been proposed to overcome the drawbacks of its low and erratic oral absorption. Due to the combined advantages from different carrier systems such as lipo/niosomes and polymeric nanoparticles and feasible scalability, SLNs are very good drug delivery systems to be explored for oral drug delivery. In this work, SLNs were prepared using different lipids, namely GMS, GDS and GB and studies were undertaken for selection and optimization of critical formulation variables. The selected optimized formulations were further studied for bioavailability. Among the different lipids, the least particle size can be achieved using GMS and highest with GB. This can be explained based on the lipophilicity of the lipid used. It has been found that the average particle size of SLN dispersions increase with higher melting lipids [10,11]. In the structure of the lipids, it can be seen that GMS has two strong hydrophilic hydroxyl groups and GDS has one hydroxyl group, where as there is no such group in GB. Instead, GB comprises of three long

aliphatic chains as substituents on the glycerol moiety, rendering it the most lipophilic of all the lipids used. The more lipophilic the lipid is, the more viscous solution it makes in the organic solvent and as a result the particle size of the nanoparticles formed is more due to increased resistance during emulsion formation. The hydroxyl groups in GMS and GDS could be involved in the better emulsification of the system and encapsulation of drugs into SLNs. [12,13]

## 2. MATERIALS

Famciclovir (assay 99.6% w/w) was obtained as a gift sample from Ranbaxy Laboratories Limited (Gurgaon, India). Ultrapure water (Milli-Q Plus, Millipore®, India) was used throughout the analysis. HPLC grade methanol was purchased from Merck. All buffer salts were of analytical grade and procured from SD Fine Chemicals Limited (Gujarat, India). Excipients such as lactose monohydrate, croscarmellose sodium, microcrystalline cellulose, colloidal silicon dioxide and magnesium stearate were obtained from IPCA labs Limited (Mumbai, India) as gift samples. Glyceryl monostearate (GMS), glyceryl distearate (GDS) and glyceryl behenate (GB) were obtained as gifts from Gatefosse (France). Poloxamer 188 (PF-68) and d-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS) were obtained from BASF Inc. (Germany) while soy lecithin, Lipoid S75 from Lipoid (Germany). Commercial product Ganguard® capsules (Ranbaxy Laboratories Limited, India), labelled to contain 250 mg of Famciclovir, were purchased from local market. Famciclovir loaded SLNs were prepared in-house.

### 2.1 Preparation of Solid Lipid Nanoparticles

In the present research, SLNs were formed using w/o/w double emulsion method as it is the most suited method for loading of hydrophilic drugs into the nanoparticulate structure. Nanoparticle formation through this process involves critical steps of formation of stable primary emulsion, formation of secondary emulsion and solvent evaporation from the double emulsion.

A two-step emulsification process was followed to prepare SLNs [14,15]. Internal phase was prepared by dissolving Famciclovir with and without surfactant in water, which was emulsified with the aid of a probe sonicator (1 min, 20W), in the organic phase containing the lipid along with soy lecithin dissolved in DCM and acetone. This

primary (w/o) emulsion was re-emulsified using ultrasonication (3 min, 20W) into an aqueous solution of a surfactant (PF-68 or TPGS), to produce a w/o/w double emulsion. In all the batches, the ratio of aqueous to organic phase for primary w/o emulsion was 1:5 and in the secondary emulsion, the ratio of primary emulsion and outer aqueous phase was 6:10. The organic solvents were then allowed to evaporate first for 3 h at room temperature under magnetic stirring for solidification and hardening of nanoparticles, and then for another 30 min in a rotary evaporator at 30°C. SLNs were isolated by centrifugation at 17500 rpm for 30 min.

The nanoparticles separated by centrifugation were resuspended in water containing sucrose (10% w/w) as cryoprotectant and freeze dried to obtain solid particles. For this, the formulations were frozen overnight in a deep-freezer at -20°C and freeze dried under vacuum (0.1 mbar, -53°C) until free-flowing powder was obtained. The product was then transferred to glass container, sealed with parafilm and stored under refrigerated conditions.

### 2.2 Characterization of Nanoparticles

#### 2.2.1 Drug loading and entrapment efficiency [16,17]

To determine the drug loading (DL) of individual formulation, accurately weighed amount of freeze-dried product was transferred to a conical flask and disrupted by addition of suitable amount of DCM and subjected to ultrasonication (15 min, 25°C). A known quantity of water was then added to the mixture and the contents were stirred overnight to allow Famciclovir to diffuse into the aqueous layer. The aqueous phase was then retrieved from the flask and tested for its Famciclovir content using the HPLC method reported in Analytical and Bioanalytical Methods section. DL was then determined by using the equation 1.

$$DL (\%w/w) = \frac{\text{Amount of drug in the product (mg)}}{\text{Amount of product taken (mg)}} \times 100 \quad (1)$$

EE was determined by indirect method [11]. Accurately weighed SLNs were added to known volume of water and shaken on vortex mixer for 30 s. The clear supernatant obtained after centrifugation was taken and the drug content was analysed in it using the HPLC method described in Analytical and Bioanalytical Methods section. The EE was determined by using the equation 2.

$$\% \text{ EE} = \frac{(\text{Total drug added} - \text{Drug in supernatant})}{(\text{Total drug added})} \times 100 \quad (2)$$

### 2.2.2 Particle size, size distribution, shape and morphology [18-20]

The average particle size, size distribution, PDI and zeta potential of each formulation were analysed by PCS (Zetasizer) using the DLS technique. Freeze dried samples were appropriately diluted with high purity water, filled in disposable polystyrene cells and subjected to particle size analyser operating at wavelength of 632 nm and light scattering was monitored at a 173° angle at a temperature of 25°C. Values of zeta potential and PDI were directly obtained from the software provided with the instrument.

The morphological characterization of the different nanoparticles was done using SEM and TEM. For SEM analysis, a drop of SLN suspension was dried overnight on an aluminium stub under vacuum. This was then sputter-coated using a thin gold-palladium layer under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. These coated samples were then scanned and photomicrographs were taken at an acceleration voltage of 20 kV. For TEM, SLN suspension was mixed with equal volume of 0.02% w/v phosphotungstic acid and kept for 5 min at room temp for equilibration. A drop of this preparation was then placed on a carbon coated copper grid, excess liquid removed and dried at room temperature. The sample was then micrographed at 200kV on a digital TEM station.

### 2.2.3 In Vitro release studies

Famciclovir release from different SLN formulations was evaluated by the dialysis bag diffusion technique [21,22]. Dialysis membrane (Spectrapor, cut off -12500 Da) was soaked in water for 12 h before use for experiment. 2 mL of drug loaded SLN dispersion was filled in the dialysis bag, sealed on both ends and immersed in a beaker containing 100 mL of release medium (pH 6.8 phosphate buffer). The contents of the beaker were stirred at 100 rpm which were maintained at a temperature  $37 \pm 0.5^\circ\text{C}$ . At time intervals of 5, 10, 15, 20, 30 min followed by 1, 1.5, 2, 4, 6, 8 and 12 h, an aliquot of the sample was withdrawn from the release medium and replaced with the same amount of fresh medium. All samples were suitably diluted and drug release was estimated using the HPLC method as described in Analytical and Bioanalytical

Methods section. Cumulative % drug release was calculated and drug release kinetics was studied by subjecting the data to various mathematical models (zero order, first order, Higuchi, Korsmeyer-Peppas and Baker-Lonsdale). The best fit on the release data was decided based on the value of  $R^2$ . The data upto 60% of drug release was used for Peppas model fitting. Time taken for 50% drug release ( $T_{50\%}$ ) was also determined based on best fit model equation.

### 2.2.4 Residual solvent analysis

Analysis of the residual solvents was carried out in accordance with USP [23], on an Agilent 6890 gas chromatograph (Agilent Technologies, USA, available at Amol Pharmaceuticals, Jaipur) equipped with a flame ionization detection system. A DB-5 capillary column (30 m × 0.32 mm i.d.; film thickness 0.25 μm) was used. GC conditions used were: oven temperature of 40°C for 20 min, then raised at a rate of 10°C min<sup>-1</sup> to 240°C, and maintained at 240°C for 20 min. The injector was maintained at 140°C (split mode, ratio 1:5), detector at 250°C and helium was used as the carrier gas (35 cm s<sup>-1</sup>). Head space samples were prepared in 10 mL vials filled by 10 mL of dimethyl formamide in which 20 mg of drug was dispersed. The head space conditions were: equilibration time 30 min at 100°C; pressurization time 2 min; loop fill time 1 min.

The sequence of injections for analysis was as follows: blank, working standards (six injections for system suitability) and test samples (one vial injection per preparation). Quantitation was based upon external standardization for each residual solvent detected in the sample corrected by sample weight versus the corresponding peak area from an equal volume of the working standard, using the equation 3.

$$\text{ppm} = \frac{(\text{Area of sample})}{(\text{Area of standard})} \times \frac{(\text{Weight of standard})}{(\text{weight of sample})} \times (\text{Dilution of sample}) / (\text{Dilution of standard}) \times 10^6 \quad (3)$$

### 2.2.5 Thermal study

Thermal analysis of the SLNs was carried out to assess the physical state of the entrapped drug in the formulations. Thermal analysis was performed using a differential scanning calorimeter (Perkin Elmer, USA), Model: DSC-4000 with integrated thermal analyser; cooling assembly intracooler and integrating software: Pyris Series – DSC 4000. Around 2 mg of finely

pulverized pure drug sample was taken and sealed in non-hermetic aluminium pan with lid and placed in the test holder, while an empty sealed aluminium pan was used as the reference. Inert environment was maintained during analysis by purging nitrogen gas at flow rate of 30 mL min<sup>-1</sup>. Thermogram was acquired at temperature range of 30°C to 300°C with a heating rate of 10°C min<sup>-1</sup> and the melting temperature was recorded. The thermograms obtained for nanoparticulate formulations were compared with those of pure drug, excipients and physical mixture.

### 2.2.6 Stability of formulations

The stability of both SLN dispersions and freeze-dried particles was evaluated after storage at different conditions, RT (25±5°C) and refrigerated (5±2°C). The nanoparticles were evaluated for particle size, PDI and DL at time intervals of 7, 15 days followed by 1, 3 and 6 months.

### 2.2.7 Histopathological evaluation for local toxicity

The histopathological evaluation was carried out by an experienced histopathologist. The intestines of the control group (pure drug) and the test (SLNs treated) group were removed 4 h after oral gavage administration of drug solution, washed using saline and immersed in a 10% aqueous solution of formalin. A transverse section was prepared, stained using

hematoxylineosin, and examined under light microscopy.

## 3. RESULTS AND DISCUSSION

### 3.1 Drug Loading and Encapsulation Efficiency

A significant gain in drug entrapment from 19.4% to 34.54% was seen when GMS amount was increased from 25mg to 100mg (Fig. 1). Similar pattern was seen with GDS batches. For batches with GB level of 100mg, entrapment could not be determined due to agglomerate formation. Increase in lipid content in the formulations increase the possible sites for drug encapsulation resulting in higher EE values [24,25]. As compared to GMS and GDS, SLNs prepared with GB showed lower EE, particularly at lower level, due to the lack of hydrophilic moiety in the structure of GB (Fig. 1). The hydroxyl groups in GMS and GDS could be involved in the better emulsification of the system and encapsulation of Famciclovir into SLNs.

Presence of surfactant along with drug lead to increased interaction of the drug with the lipid phase during the formation of primary emulsion, preventing drug partitioning to the external aqueous phase during formation of secondary emulsion and later solvent evaporation [26, 27]. By including PF-68 or TPGS in the internal phase, a remarkable 2 folds increase in EE of Famciclovir was observed in case of GMS nanoparticles and 1.5 times in case of batches with GDS and GB.

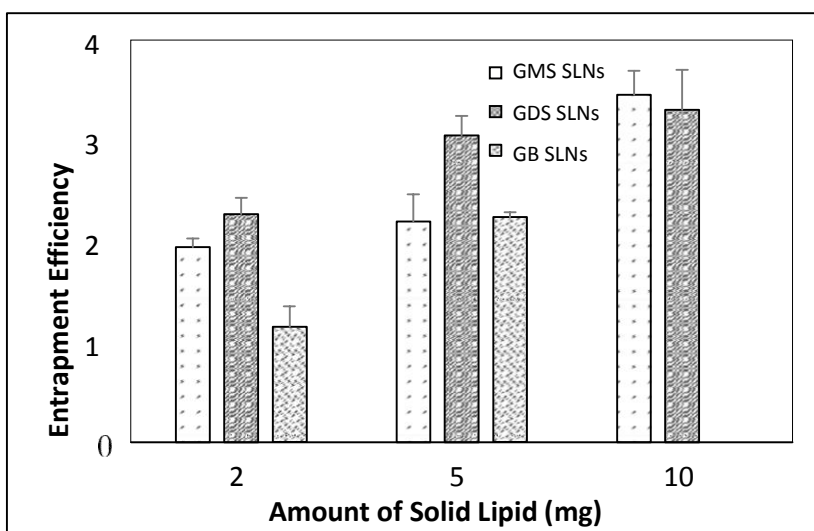


Fig. 1. Influence of type and amount of lipid on EE of famciclovir in SLNs

### 3.2 Particle Size, Shape and Surface Morphology

In batches prepared with different quantities of GMS, the particle size increased from 134.00 to 478.60 nm with increasing quantity of GMS from 25 mg to 200 mg. Similar trend of increasing particle size with increasing lipid amount was observed in SLNs prepared using GDS and GB. Batches prepared with GDS level of 200 mg (FAM/SLN/GDS/10) and GB levels of 100 mg and 200 mg (FAM/SLN/GB/9 and FAM/SLN/GB/10) were not evaluated further for drug loading and entrapment due to visual observation of agglomeration during emulsion formation and very high particle size.

The observation of an increase in particle size of SLNs with increase in lipid content is well in agreement with previous reports of other researchers. Such effect was probably caused by the increasing viscosity of dispersed phase (organic phase solution) causing high resistance against the shear forces during the emulsification, resulting in a poorer dispersibility of the lipid solution into the outer aqueous phase [24,25]. Coarse emulsions were obtained at higher lipid concentrations, which lead to the build of bigger particles during the diffusion process during solvent evaporation. Another reason for such a phenomenon may be the inability of surfactant in the outer phase (which is at fixed concentration) to stabilize the interfacial tension generated by

higher amounts of lipid, leading to an increased particle size and agglomeration [26,27].

Among the different lipids used, the least particle size was observed in batches prepared using GMS and highest was obtained with GB (Fig. 2). This can be explained based on the lipophilicity of the lipid used. It has been found that the average particle size of SLN dispersions increase with higher melting lipids which was also established in this study. In the structure of the lipids, it can be seen that GMS has two strong hydrophilic hydroxyl groups and GDS has one hydroxyl group, where as there is no such group in GB. Instead, GB comprises of three long aliphatic chains as substituents on the glycerol moiety, rendering it the most lipophilic of all the lipids used. The more lipophilic the lipid is, the more viscous solution it makes in the organic solvent and as a result the particle size of the nanoparticles formed is more due to increased resistance during emulsion formation [25,28,29].

The shape and surface characteristics of the SLNs were investigated using SEM and TEM. The SEM images (Fig. 3) indicated that the nanoparticles displayed spherical shape and absence of drug crystals on the surface. TEM images confirmed the nanometer size and the internal globular structure of the SLNs depicting a solid solution matrix model formation, without any aggregation. (Fig. 4).

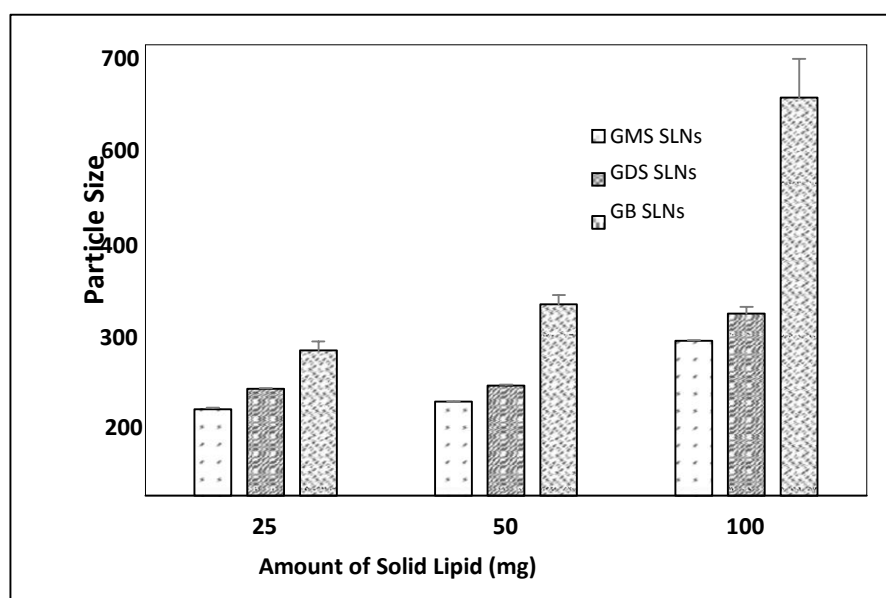
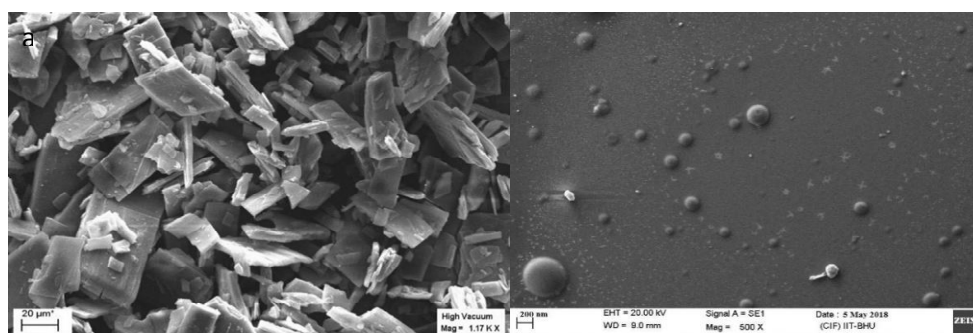
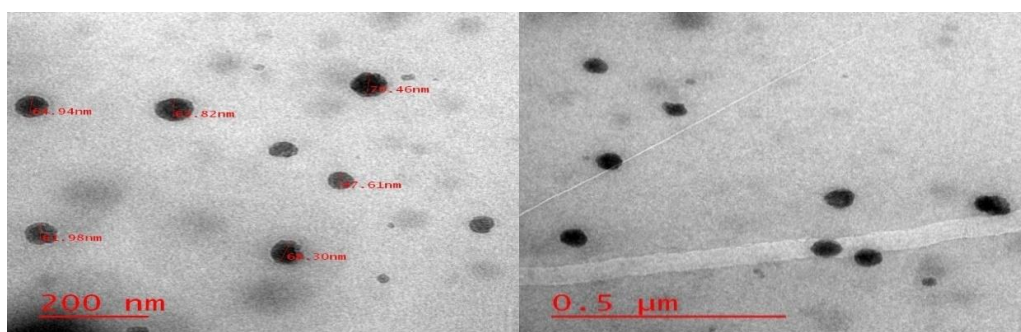


Fig. 2. Effect of amount and type of lipid matrix on particle size of SLNs



**Fig. 3. Surface morphology of Famciclovir a) and Famciclovir loaded SLNs by SEM**



**Fig. 4. TEM images of famciclovir loaded SLNs**

### 3.3 *In Vitro* Drug Release

*In vitro* release profile of Famciclovir from SLN formulations prepared using different lipids and pure drug is shown in Fig. 5. In case of pure drug, complete diffusion through the dialysis membrane occurred within 0.5 h, while the release of drug encapsulated in SLNs showed a biphasic pattern and was extended upto 4-8 h. All SLN batches displayed an initial burst release of approximately 50-65% in 1 h, which may be due to the weakly bound drug present on the surface of the nanoparticulate matrix. Drug release was also affected by the type and amount of lipid matrix used in formulation of SLNs. Among the different lipids used, formulations prepared with GDS and GB displayed the most extended drug release of 8 h (Fig. 5). The reason may be because of their longer carbon chains or greater lipophilicity as compared to GMS. This finding is in accordance with previous reports by other researchers [30,31]. Comparative release profiles of formulations prepared with varying the concentrations of lipids used are shown in Fig. 5. A decrease in drug release was observed with increase in concentration of lipid. Increase in lipid concentration caused increased particle size, leading to lesser surface area and thus causing slower drug release.

Factors contributing to a fast release are the large surface area, a high diffusion coefficient due to small molecular size, and a short diffusion distance for the drug from outer surface region of the nanoparticle. In later phase, the entrapped drug was released in a slow fashion, extending the release upto 4-8 h, which can be attributed to the slow diffusion of drug from the lipid matrix. The drug release could not be extended beyond 8 h due to the hydrophilicity of drug.

To elucidate the release kinetics, the dissolution data was fitted into various mathematical models and it was seen that the data was best fitted into the Baker-Lonsdale model. This model appropriately describes the release of drugs from spherical matrices by diffusion mechanism [32]. The release rate constant and T50% values were calculated based on this best fit model while the mechanism of release was given by the 'n' value obtained on fitting the data into the Korsmeyer-Peppas model. All the kinetic parameters for the drug release from the SLNs is presented in Table 4. Values of 'n' in Korsmeyer-Peppas model indicated that the drug release from the nanoparticles follow Fickian transport ( $n < 0.5$ ), i.e., release was mainly because of diffusion of drug from the nanoparticulate matrix.

Table 1. Formula and characters of GMS based famciclovir loaded SLNs

Batch Code	GAN (mg)	GMS (mg)	Stabilizers (%w/v)				Mean Particle Size (nm) $\pm$ SD	Poly Dispersity Index $\pm$ SD	Zeta Potential (mV)	Drug Loading (%w/w) $\pm$ SD	Entrapment Efficiency (%) $\pm$ SD
			Internal (same as external)	Middle (lecithin)	External (PF 68)	External (TPGS)					
FAM/SLN/GMS/1	50	50	0	0.10	0.10	-	148.32 $\pm$ 11.40	0.41 $\pm$ 0.02	-64.0	8.82 $\pm$ 0.69	22.73 $\pm$ 4.59
FAM/SLN/GMS/2	50	50	0	0.10	0.25	-	145.53 $\pm$ 0.13	0.31 $\pm$ 0.05	-62.0	8.62 $\pm$ 0.55	21.96 $\pm$ 2.71
FAM/SLN/GMS/3	50	50	0	0.10	0.50	-	132.14 $\pm$ 6.70	0.27 $\pm$ 0.01	-55.8	6.65 $\pm$ 0.03	21.11 $\pm$ 3.14
FAM/SLN/GMS/4	50	50	0	0.10	1.00	-	100.03 $\pm$ 1.00	0.25 $\pm$ 0.01	-50.1	5.25 $\pm$ 0.06	16.75 $\pm$ 1.27
FAM/SLN/GMS/5	50	50	0	0.00	0.25	-	424.30 $\pm$ 32.67	0.53 $\pm$ 0.06	-34.2	8.89 $\pm$ 0.14	14.97 $\pm$ 5.15
FAM/SLN/GMS/6	50	50	0	0.05	0.25	-	228.20 $\pm$ 0.57	0.29 $\pm$ 0.01	-42.6	7.77 $\pm$ 0.67	20.13 $\pm$ 1.38
FAM/SLN/GMS/7	50	50	0	0.20	0.25	-	124.05 $\pm$ 0.92	0.06 $\pm$ 0.01	-72.9	5.72 $\pm$ 0.27	11.26 $\pm$ 2.48
FAM/SLN/GMS/8	50	25	0	0.10	0.25	-	134.00 $\pm$ 2.21	0.27 $\pm$ 0.08	-62.8	8.33 $\pm$ 0.23	19.40 $\pm$ 0.85
FAM/SLN/GMS/9	50	100	0	0.10	0.25	-	240.10 $\pm$ 0.71	0.39 $\pm$ 0.01	-58.9	7.49 $\pm$ 0.18	34.54 $\pm$ 2.40
FAM/SLN/GMS/10	50	200	0	0.10	0.25	-	478.60 $\pm$ 14.99	0.47 $\pm$ 0.02	-52.0	6.91 $\pm$ 1.26	36.29 $\pm$ 2.46
FAM/SLN/GMS/11	50	50	0.10	0.10	0.25	-	165.03 $\pm$ 1.51	0.27 $\pm$ 0.01	-66.1	11.47 $\pm$ 0.20	42.07 $\pm$ 1.42
FAM/SLN/GMS/12	50	50	0.25	0.10	0.25	-	143.06 $\pm$ 1.36	0.24 $\pm$ 0.01	-61.8	11.08 $\pm$ 0.34	44.66 $\pm$ 2.48
FAM/SLN/GMS/13	50	50	0	0.10	-	0.01	163.94 $\pm$ 2.06	0.23 $\pm$ 0.01	-65.2	9.45 $\pm$ 1.31	20.50 $\pm$ 1.89
FAM/SLN/GMS/14	50	50	0	0.10	-	0.02	229.90 $\pm$ 27.58	0.21 $\pm$ 0.02	-61.5	8.11 $\pm$ 1.40	15.48 $\pm$ 1.84
FAM/SLN/GMS/15	50	50	0	0.10	-	0.05	329.20 $\pm$ 40.73	0.39 $\pm$ 0.01	-50.4	7.91 $\pm$ 1.03	14.23 $\pm$ 1.17
FAM/SLN/GMS/16	50	50	0.01	0.10	-	0.01	157.41 $\pm$ 0.58	0.11 $\pm$ 0.01	-58.2	10.28 $\pm$ 1.32	36.32 $\pm$ 0.84
FAM/SLN/GMS/17	50	50	0.02	0.10	-	0.01	216.95 $\pm$ 0.41	0.39 $\pm$ 0.04	-54.1	10.55 $\pm$ 0.83	37.86 $\pm$ 0.72

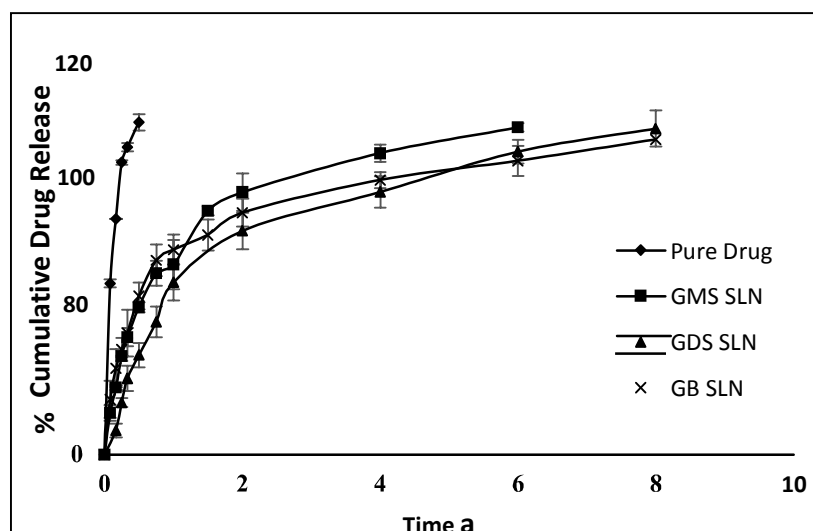


Table 2. Formula and characters of GDS based famciclovir loaded SLNs

Batch Code	GAN (mg)	GDS (mg)	Stabilizers (%w/v)				Mean Particle Size (nm) $\pm$ SD	Poly Dispersity Index $\pm$ SD	Zeta Potential (mV)	Drug Loading (%w/w) $\pm$ SD	Entrapment Efficiency (%) $\pm$ SD
			Internal (same as external)	Middle (lecithin)	External (PF 68)	External (TPGS)					
FAM/SLN/GDS/1	50	50	0	0.10	0.10	-	184.68 $\pm$ 6.40	0.28 $\pm$ 0.06	-51.4	8.94 $\pm$ 1.74	32.76 $\pm$ 4.28
FAM/SLN/GDS/2	50	50	0	0.10	0.25	-	170.57 $\pm$ 1.37	0.25 $\pm$ 0.00	-49.0	8.79 $\pm$ 1.03	30.54 $\pm$ 1.92
FAM/SLN/GDS/3	50	50	0	0.10	0.50	-	148.52 $\pm$ 2.14	0.16 $\pm$ 0.02	-45.3	8.11 $\pm$ 1.23	24.86 $\pm$ 2.59
FAM/SLN/GDS/4	50	50	0	0.10	1.00	-	135.16 $\pm$ 0.74	0.10 $\pm$ 0.02	-40.0	7.52 $\pm$ 1.05	22.96 $\pm$ 3.94
FAM/SLN/GDS/5	50	50	0	0.00	0.25	-	Primary emulsion not formed				
FAM/SLN/GDS/6	50	50	0	0.05	0.25	-	192.14 $\pm$ 3.58	0.39 $\pm$ 0.06	-45.0	9.03 $\pm$ 0.68	26.47 $\pm$ 2.86
FAM/SLN/GDS/7	50	50	0	0.20	0.25	-	167.05 $\pm$ 1.17	0.34 $\pm$ 0.01	-52.7	7.33 $\pm$ 0.74	21.68 $\pm$ 2.33
FAM/SLN/GDS/8	50	25	0	0.10	0.25	-	165.34 $\pm$ 1.39	0.27 $\pm$ 0.02	-59.7	12.1 $\pm$ 2.01	22.68 $\pm$ 1.65
FAM/SLN/GDS/9	50	100	0	0.10	0.25	-	282.15 $\pm$ 10.52	0.28 $\pm$ 0.01	-49.2	9.82 $\pm$ 1.70	33.06 $\pm$ 3.96
FAM/SLN/GDS/10	50	200	0	0.10	0.25	-	Agglomeration seen				
FAM/SLN/GDS/11	50	50	0.10	0.10	0.25	-	177.07 $\pm$ 1.12	0.24 $\pm$ 0.01	-48.6	11.52 $\pm$ 0.91	45.97 $\pm$ 1.98
FAM/SLN/GDS/12	50	50	0.25	0.10	0.25	-	167.08 $\pm$ 2.15	0.29 $\pm$ 0.01	-46.7	10.85 $\pm$ 1.10	47.62 $\pm$ 0.99
FAM/SLN/GDS/13	50	50	0	0.10	-	0.01	187.19 $\pm$ 3.14	0.30 $\pm$ 0.03	-56.1	9.80 $\pm$ 1.28	29.96 $\pm$ 1.61
FAM/SLN/GDS/14	50	50	0	0.10	-	0.02	275.40 $\pm$ 4.04	0.32 $\pm$ 0.03	-53.1	8.54 $\pm$ 1.29	26.02 $\pm$ 1.54
FAM/SLN/GDS/15	50	50	0	0.10	-	0.05	434.40 $\pm$ 25.65	0.50 $\pm$ 0.07	-40.4	6.82 $\pm$ 0.82	24.90 $\pm$ 1.65
FAM/SLN/GDS/16	50	50	0.01	0.10	-	0.01	161.25 $\pm$ 5.64	0.40 $\pm$ 0.01	-54.6	9.52 $\pm$ 0.50	38.16 $\pm$ 0.89
FAM/SLN/GDS/17	50	50	0.02	0.10	-	0.01	169.88 $\pm$ 3.34	0.26 $\pm$ 0.01	-50.0	8.80 $\pm$ 0.96	40.96 $\pm$ 2.27

**Table 3. Formula and characters of GB based famciclovir loaded SLNs**

Batch Code	GAN (mg)	GB (mg)	Stabilizers (%w/v)				Mean Particle Size (nm) $\pm$ SD	Poly Dispersity Index $\pm$ SD	Zeta Potential (mV)	Drug Loading (%w/w) $\pm$ SD	Entrapment Efficiency (%) $\pm$ SD
			Internal (same as external)	Middle (lecithin)	External (PF 68)	External (TPGS)					
FAM/SLN/GB/1	50	50	0	0.10	0.10	-	431.03 $\pm$ 6.22	0.50 $\pm$ 0.01	-62.2	11.07 $\pm$ 1.03	24.87 $\pm$ 0.58
FAM/SLN/GB/2	50	50	0	0.10	0.25	-	358.00 $\pm$ 6.80	0.40 $\pm$ 0.04	-54.7	10.89 $\pm$ 1.27	24.90 $\pm$ 1.29
FAM/SLN/GB/3	50	50	0	0.10	0.50	-	296.80 $\pm$ 4.42	0.36 $\pm$ 0.03	-50.5	9.55 $\pm$ 0.83	22.37 $\pm$ 0.52
FAM/SLN/GB/4	50	50	0	0.10	1.00	-	268.36 $\pm$ 4.38	0.52 $\pm$ 0.01	-46.2	7.91 $\pm$ 0.73	18.42 $\pm$ 0.39
FAM/SLN/GB/5	50	50	0	0.00	0.50	-	Primary emulsion not formed				
FAM/SLN/GB/6	50	50	0	0.05	0.50	-	393.02 $\pm$ 64.06	0.40 $\pm$ 0.05	-47.2	10.52 $\pm$ 1.63	17.27 $\pm$ 2.15
FAM/SLN/GB/7	50	50	0	0.20	0.50	-	282.48 $\pm$ 3.29	0.33 $\pm$ 0.02	-52.9	8.32 $\pm$ 2.18	15.68 $\pm$ 1.61
FAM/SLN/GB/8	50	25	0	0.10	0.50	-	225.02 $\pm$ 13.86	0.32 $\pm$ 0.04	-57.7	12.25 $\pm$ 0.59	11.48 $\pm$ 2.03
FAM/SLN/GB/9	50	100	0	0.10	0.50	-	618.05 $\pm$ 59.65	0.96 $\pm$ 0.06	Lumps formed after freeze drying		
FAM/SLN/GB/10	50	200	0	0.10	0.50	-	Agglomeration seen				
FAM/SLN/GB/11	50	50	0.10	0.10	0.50	-	278.34 $\pm$ 3.63	0.33 $\pm$ 0.04	-49.3	12.01 $\pm$ 0.59	32.16 $\pm$ 0.68
FAM/SLN/GB/12	50	50	0.25	0.10	0.50	-	253.59 $\pm$ 0.99	0.45 $\pm$ 0.09	-45.9	12.09 $\pm$ 0.18	36.32 $\pm$ 0.37
FAM/SLN/GB/13	50	50	0	0.10	-	0.01	374.28 $\pm$ 14.29	0.38 $\pm$ 0.04	-64.4	10.80 $\pm$ 1.96	19.96 $\pm$ 2.72
FAM/SLN/GB/14	50	50	0	0.10	-	0.02	401.20 $\pm$ 19.38	0.57 $\pm$ 0.02	-61.5	9.45 $\pm$ 0.28	17.02 $\pm$ 2.45
FAM/SLN/GB/15	50	50	0	0.10	-	0.05	560.25 $\pm$ 8.39	0.472 $\pm$ 0.07	-47.9	7.82 $\pm$ 1.28	14.90 $\pm$ 0.85
FAM/SLN/GB/16	50	50	0.01	0.10	-	0.01	338.66 $\pm$ 6.71	0.22 $\pm$ 0.05	-62.5	11.52 $\pm$ 1.04	34.16 $\pm$ 1.89
FAM/SLN/GB/17	50	50	0.02	0.10	-	0.01	378.86 $\pm$ 2.46	0.27 $\pm$ 0.03	-59.2	10.40 $\pm$ 1.69	33.39 $\pm$ 1.72



**Fig. 5.** *In vitro* drug release profile of Famciclovir from SLNs prepared using different lipids (Each data represents average of three separate determinations)

### 3.4 Residual Solvent Analysis

Residual solvent analysis was done to quantify the amount of DCM and acetone remnant in the formulations. The solvents used in the manufacture process, if not completely removed, should be reduced to a concentration which is safe to be administered [33]. According to ICH guidelines, DCM belongs to the category of Class 2 solvents and has the maximum permissible concentration of 600 ppm, while acetone is a Class 3 solvent and a level of 5000 ppm is considered acceptable. The results of residual solvent analysis by gas chromatography yielded concentrations in the range of 205.35-208.83 ppm for DCM and 249.41-283.75 ppm for acetone, much below the permissible limits, suggesting the suitability of the solvent evaporation method utilized for the preparation of SLNs.

### 3.5 Thermal Study

The DSC thermogram of pure drug further confirmed that the drug used in this study exists in the crystalline monohydrate form (Fig. 4). The thermogram depicted an endothermic loss upto 110°C followed by conversion to anhydrous form which has a sharp endothermic peak at around 254°C and the drug was found to decompose at its melting temperature, since no peak was obtained on repeated measurement of the same sample. The absence of peak at 254°C in the SLNs suggest that the drug is dispersed in the

SLNs in an amorphous state. Such an effect could be attributed to the molecular level dispersion of the drug within the lipid matrix. Similar loss of crystallinity of drug when formulated into SLNs has also been seen in previous studies [34-35]. Thermal analysis revealed a molecular level dispersion of drug within the SLNs without any chemical or physical interaction between drug and excipients. This suggested the suitability of the preparation technique for formulating stable SLNs.

### 3.6 Stability of Formulations

All the nanosuspensions were found to be stable for one week at room temperature and 3 months at refrigerated condition, with no significant change in particle size distribution and EE. Those prepared with GB showed slight aggregation by 3-4 days at room temperature and 15 days at 5°C. However, the aggregation was re-dispersed with ultrasonic treatment (30 s, 20W).

The dried formulations stored at room temperature displayed good redispersibility, no significant difference in particle size upto 1 month of storage, but an increasing trend of particle size and decreasing trend of DL beyond 1 month was observed. This could be due to leaching out of drug from the SLN matrix due to instability of lipids at room temperature. The formulations at refrigerated temperature were found to be stable

with respective to size and DL even after 6 months of storage (Table 5).

### 3.7 Histopathological Evaluation for Local Toxicity

Photomicrographs of the intestinal mucosa of rat exposed to pure drug Famciclovir and SLNs are

shown in Fig. 7. As indicated, the epithelium of each group was undamaged, and the villus structure was intact. There was no significant difference in arrangement and structure of nuclei and cells between Famciclovir pure drug and Famciclovir loaded SLNs, indicating that SLNs have no significant immediate local toxicity in the intestinal tract.

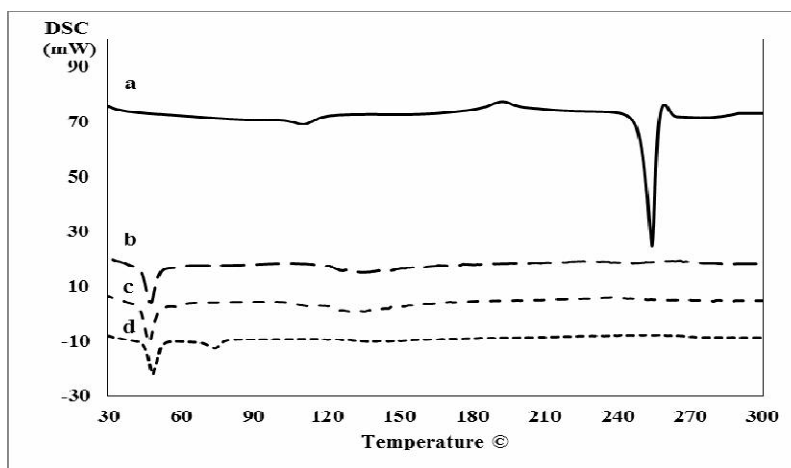


Fig. 6. DSC thermogram obtained for a) Famciclovir b)FAM/SLN/GMS/11, c) FAM/SLN/GDS/11 and d) FAM/SLN/GB/12

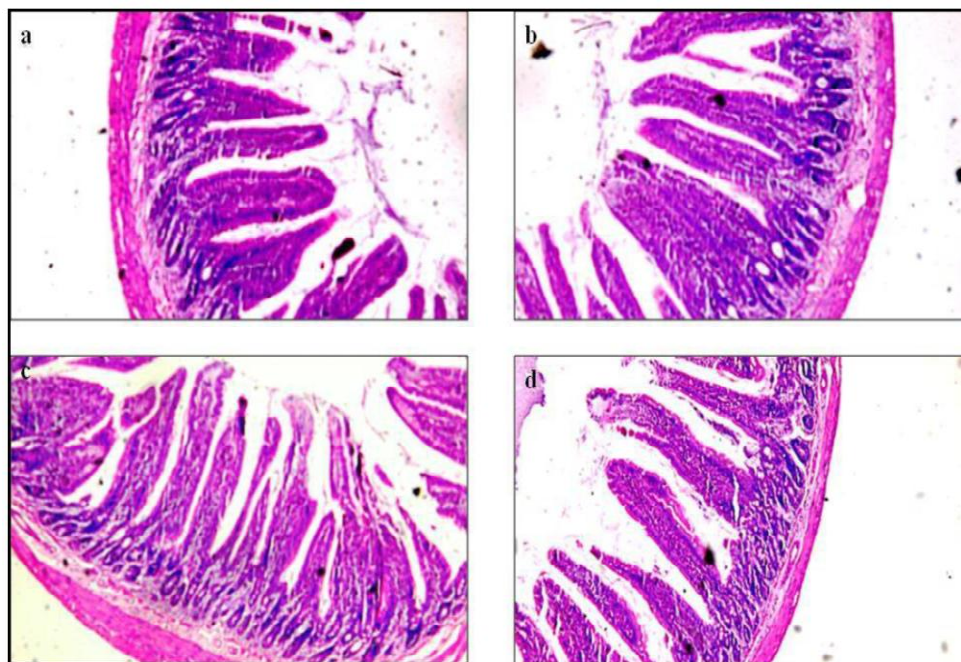


Fig. 7. Photomicrographs of rat intestine at 4 h after oral gavage administration of a) pure drug, b) GMS SLNs, c) GDS SLNs and d) GB SLNs at dose of 50 mg kg<sup>-1</sup>. (original magnification ×10)

**Table 4. Model dependent and model independent mathematical parameters of the in vitro release data**

Batch Code	Best fit model parameters (Baker Lonsdale)		T50% (hr)	'n' value (Peppas model)	f1	f2
	k	R2				
FAM/SLN/GMS/2	0.190	0.9903	0.290	0.408		
FAM/SLN/GMS/11	0.105	0.9882	0.525	0.339	20.28a	44.91a
FAM/SLN/GMS/12	0.083	0.9870	0.661	0.368	26.97a	39.00a
FAM/SLN/GMS/13	0.125	0.9673	0.440	0.319		
FAM/SLN/GMS/16	0.083	0.9433	0.661	0.291	9.61b	59.42b
FAM/SLN/GMS/17	0.063	0.9786	0.881	0.323	23.82b	42.00b
FAM/SLN/GDS/2	0.153	0.9723	0.360	0.466		
FAM/SLN/GDS/11	0.049	0.9549	1.126	0.505	32.80c	34.38c
FAM/SLN/GDS/12	0.046	0.9529	1.201	0.474	51.45c	34.18c
FAM/SLN/GDS/13	0.216	0.9942	0.255	0.366		
FAM/SLN/GDS/16	0.125	0.9852	0.440	0.336	12.88d	54.66d
FAM/SLN/GDS/17	0.074	0.9654	0.741	0.387	32.17d	34.16d
FAM/SLN/GB/3	0.218	0.9503	0.253	0.420		
FAM/SLN/GB/11	0.083	0.9648	0.661	0.304	11.10e	56.90e
FAM/SLN/GB/12	0.063	0.9507	0.881	0.290	33.04e	34.87e
FAM/SLN/GB/13	0.125	0.9512	0.440	0.331		
FAM/SLN/GB/16	0.083	0.9368	0.661	0.304	11.06f	57.34 f
FAM/SLN/GB/17	0.083	0.9554	0.661	0.314	15.52f	50.76 f

Calculated by taking references as aFAM/SLN/GMS/2, bFAM/SLN/GMS/13, cFAM/SLN/GDS/2, dFAM/SLN/GDS/13, eFAM/SLN/GB/3, fFAM/SLN/GB/13

**Table 5. Stability of freeze dried SLNs stored at different temperature conditions (1 month and 6 months data)**

Batch Code	Initial		1-month				6 months			
			25°C ± 2°C		5°C ± 3°C		25°C ± 2°C		5°C ± 3°C	
	Particle Size (nm)	DL (% w/w)	Particle Size (nm)	DL (% w/w)	Particle Size (nm)	DL (%w/w)	Particle Size (nm)	DL (%w/w)	Particle Size (nm)	DL (%w/w)
FAM/SLN/GMS/11	174.78 ± 1.79	11.47 ± 0.20	200.70± 7.29	10.99± 0.85	172.54 ± 2.83	11.23 ± 1.03	242.50± 5.28	8.77 ± 0.64	180.2 ± 2.18	10.94 ± 0.78
FAM/SLN/GMS/17	171.98 ± 0.93	10.55±0.83	176.20 ± 5.52	9.74 ± 0.32	166.04 ± 3.63	9.94 ± 1.04	238.94± 2.78	8.84 ± 0.79	176.5 ± 2.56	10.13 ± 0.84
FAM/SLN/GDS/11	192.14 ± 2.75	11.52±0.91	206.38 ± 3.07	9.84 ± 1.05	215.46 ± 2.29	11.05 ± 0.39	307.20± 3.42	9.94 ± 0.84	203.78± 3.23	11.04 ± 1.01
FAM/SLN/GDS/17	184.42 ± 2.01	8.80 ± 0.96	200.50 ± 2.38	7.69 ± 0.21	189.94 ± 3.81	7.98 ± 0.51	286.60± 8.34	6.01 ± 0.39	178.35± 2.21	7.54 ± 0.63
FAM/SLN/GB/12	295.20 ± 6.37	12.09 ± 0.18	347.80 ± 3.97	10.81± 0.89	300.42 ± 5.30	12.14 ± 1.36	382.00± 3.83	9.69 ± 0.12	307.00 ± 4.32	11.69 ± 0.76
FAM/SLN/GB/17	396.20 ± 6.22	10.40±1.69	447.42 ± 3.95	9.97 ± 0.31	422.00± 10.82	10.08 ± 0.67	489.20± 4.81	8.18 ± 0.63	438.40± 9.21	9.63 ± 0.38

*Each data represents the average of three independent determinations*

#### 4. CONCLUSION

Famciclovir-loaded SLNs prepared by double emulsification technique showed controlled drug release when compared to free drug. Specific designed formulations were selected considering the advantages of small particle size, narrow size distribution, extended release, good in vitro stability and biocompatibility. The histological evaluation depicted no change in architecture of cells showing absence of local toxicity of SLNs on the intestine. Thus, the pharmacokinetic studies confirmed that SLNs are suitable carriers for effective oral delivery of drugs which may address to drug-specific limitations like poor bioavailability. Collectively, these results indicate that SLNs are promising delivery systems to be developed to enhance the oral bioavailability of Famciclovir, so that the dose of the current therapy can be reduced, the inconvenience of i.v. administration can be avoided and overall, the patient compliance be improved.

#### DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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