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Research Article

Development and Evaluation of Niosomal Formulation of Famciclovir

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ABSTRACT

Niosomes are one of the best and most effective among these carriers system.Because of the presence of hydrophilic, lipophilic and amphiphilic moieties in the structure, these niosomes can accommodate various drug molecules with a wide range of solubility. So, these may act as a depot, releasing the drug in a defined manner. Rationale behind the present study is to improve the oral bioavailability of Famciclovir by preparing niosomes. Encapsulation of Famciclovir in lipophilic vesicular structure may be expected to enhance the dissolution, oral absorption and prolong the existence of the drug in the systemic circulation. The niosomal dispersions were formulated using various combinations of cholesterol and spans. The formulations were evaluated in-vitro and in-vivo and compared with the marketed preparation of Famciclovir.

Key-words: liposomes, niosomal dispersion, famciclovir, *in vivo* study

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Introduction

The concept of targeted drug delivery is designed for attempting to concentrate the drug in the target tissues of interest while reducing the relative concentration of the medication in the remaining tissues, where drug is not needed. As a result, drug is much localised on the targeted site and the surrounding tissues are not affected by the drug. Also, loss of drug does not happen due to localisation of drug, leading to getting maximum efficacy of the medication. For this purpose and intention, various different carriers have been used for controlled targeting of drug, such as immunoglobulin, resealed erythrocytes, serum proteins, synthetic polymers, liposomes, microspheres, nanoparticles and niosomes.[1]

Niosomes are one of the best and most effective among these carriers system. Niosomes (non-ionic surfactant vesicles) which are obtained on hydration are microscopic lamellar structures formed upon combining non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class with cholesterol.[2] These non-ionic surfactants thereby form a closed- bilayer vesicle in aqueous media based on its amphiphilic nature by using some energy like heat, physical agitation to form this structure. In the bilayer structure, the hydrophilic heads remain in contact with the aqueous solvent and the hydrophobic parts are oriented away from the aqueous solvent. The properties of these vesicles can be changed by varying the composition of the vesicles, their size, or lamellarity,the tapped volume, or surface charge and the concentration of the surfactant, etc. Various forces act inside the vesicle, e.g., van der Waals forces amongst the surfactant molecules, and repulsive forces that emerge from the electrostatic interactions among charged groups of surfactant molecules. Also, entropic repulsive forces of the head groups of the surfactants and short-acting repulsive forces are present and these are the forces which are responsible for maintaining the vesicular structure of niosomes. The stability of niosomes are affected by type of surfactant, the nature of the encapsulated drug,the temperature at which stored, presence of detergents, presence/absence of membrane spanning lipids, importantly interfacial polymerisation of surfactant monomers *in situ*, inclusion of charged molecule. Because of the presence of hydrophilic, lipophilic and amphiphilic moieties in the structure, these niosomes can accommodate various drug molecules with a wide range of solubility[3]. So, these may act as a depot, releasing the drug in a defined manner. The therapeutic performance of the drug molecules can also be improved by delayed clearance from the circulation and restricting effects to target cells.[4] any surfactant used for the preparation of niosomes should be biodegradable, biocompatible and most importantly non-immunogenic.

The drug delivery systems which use colloidal particulate carriers such as liposomes or niosomes have a particular advantage over conventional dosage forms because these particles can act as drug reservoir, and any modification of the particle composition or the surface can adjust drug release rate and/or the affinity for the target site in the body.

RATIONALE

Famciclovir is a BCS class II drug i.e. with low solubility. The US Food and Drug Administration (FDA) guidelines reported that a drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of the aqueous media over the pH range of 1 to 7.5 and the drug is considered highly permeable when the extent of absorption in human is determined to be 99% of the administered dose in comparison to an intravenous reference dose.

Famciclovir is a selective antiviral agent, active against Herpes simplex viruses I and II, Cytomegalovirus, Varicella zoster, Epstein barr virus. Intravenous administration offers therapeutically effective blood levels, however long term administration of I/V Famciclovir is associated with patient inconvenience, higher costs (40% higher costs than oral route), and incidences of catheter related infections and sepsis. Orally administered Famciclovir is preferred to i.v drug from perspective of patient compliance, convenience and cost, but oral bioavailability of FAN is low requiring administration of large amount per day to reach effective plasma concentration.

Due to its lipophilic nature (log P = 1.09) it has poor solubility characteristics, which restricts its transcellular diffusion. Famciclovir is transported across the intestinal epithelium by paracellular route. Rationale behind the present study was to improve the oral bioavailability of Famciclovir by preparing niosomes.

Formulation Development:

Preparation of Niosomes: Niosome were prepared by using the commonly used methods i.e. Film Hydration Method and Reverse evaporation method, the systematic presentation of both methods were given below. The

surfactants, cholesterol, their ratio, and the amount drug taken for formulation development with the formulation code is given in the Table.1

Table 1: List of surfactants and their molar ratios used in formulation

Formulation code	Surfactant type	Cholesterol	Ratio of surfactant and Cholesterol	Amount of drug
NF1	Span40	29mg	1:1	20 mg
NF2	Span40	19mg	2:1	20 mg
NF3	Span40	14mg	3:1	20 mg
NF4	Span40	23mg	3:2	20 mg
NF5	Span60	29mg	1:1	20 mg
NF6	Span60	19mg	2:1	20 mg
NF7	Span60	14mg	3:1	20 mg
NF8	Span60	23mg	3:2	20 mg

Surfactant and cholesterol were dissolved in diethyl ether:chloroform (1:1) in a round bottom flask Organic solvent was removed using rotary evaporator(60ºC,250 rpm). This yields a thin layer deposited on the wall of the flask. Dried surfactant film was hydrated with aqueous phase at 60°C with gentle agitation.This was followed by sonication for one hour. Niosomes were examined under a microscope.

Results:

***In-vitro* evaluation of niosomes:**

Entrapment efficiency: After preparing niosomal dispersion, unentrapped drug was separated by centrifugation and the drug remained entrapped in niosomes was determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 and analysing the resultant solution by appropriate assay method for the drug. Where,

Entrapment efficiency (EF) = (Amount entrapped /total amount) x 100

Formulation code	Entrapment efficiency
NF1	17%
NF2	21%
NF3	10%
NF4	23%
NF5	16%
NF6	23%
NF7	11%
NF8	25%

Particle size determination: Niosomes formation was confirmed by optical microscopy and TEM.

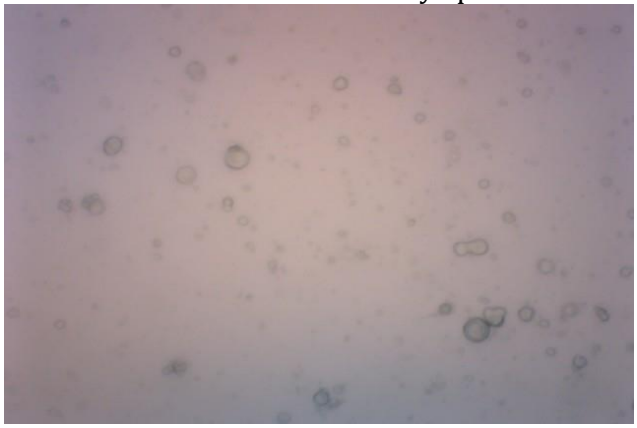

Figure1: TEM analysis of optimised formulation

Transition temperature analysis of niosomes:

A small amount of freeze-dried NSVs or pure semisolid surfactant (Span40, Span60) was sealed in a 40-µl-aluminium crucible. A second crucible containing the equivalent amount of PBS (pH 7.4) was sealed as the reference cell. The temperature of the pans was raised from 40-400ºC, at a rate of 10ºC/min. The heat flow calibration was performed with indium. The reproducibility of the thermograms was determined by repeating the temperature cycle three times for each sample.

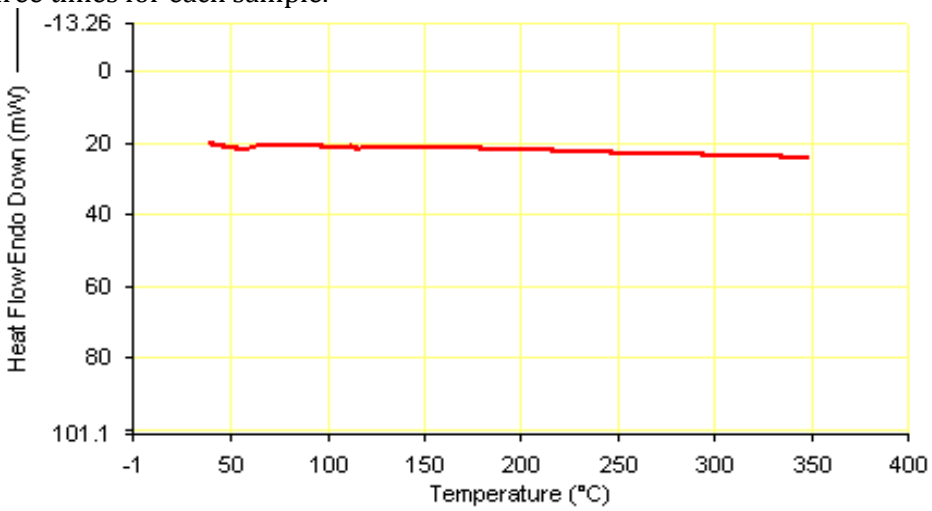

Figure 2:DSC thermogram of Drug loaded formulation (NF8)

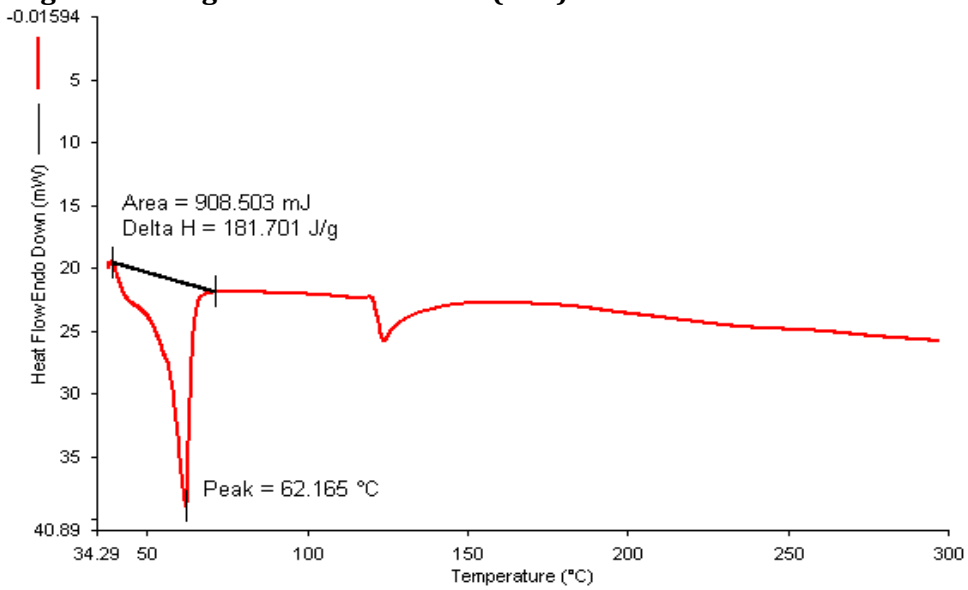

Figure 3: DSC thermogram of plain niosomes(3:2)

***In-vitro* drug release performance**

The study was performed by using dialysis bag method. The vesicle suspension was pipetted into a bag made up of the tubing and sealed. The bag containing the vesicles was placed in 200 ml of buffer solution in a 250 ml beaker with constant shaking at 25°C or 37°C. 5ml samples were withdrawn at regular time intervals (0, 2,4,6,8, 12, 16, 20 and 24 hours) and same amount of phosphate buffer was replaced. Then samples were analyzed for the drug content by UV at 254nm.

Calculation of % release: It was calculated by following formula:

% release = cons. in sample x 250 x 100/Total amount of drug

Table 2*: In vitro* dissolution performance of optimized formulations Release kinetic study

Time (hrs)	NF8 (% CR)	NF4 (% CR)
0	0	0
2	8.08±0.08	12.45±0.12
4	19.5±0.09	25.99±0.02
6	28.3±0.15	36.1±0.06
8	35.6±0.03	45.43±0.13
12	53.3±0.17	65.34±0.15
16	71.0±0.14	83.9±0.11
20	89.1±0.09	92.41±0.08
24	92.2±0.05	98.29±0.07

Kinetic analysis of the *in vitro* release profile of optimized formulation was done in order to determine the release order.

Table 3: Showing all parameters needed for in vitro release kinetic analysis of optimized formulation Stability studies

Time(hr)	Squre root of time	Log t	% drug released	Fraction drug release	log% drug released	% drug remaining	Log % drug remaining
0	0	0	0	0	0	100	2
2	1.4142	0.3010	8.08	0.0808	0.9074	91.92	1.9634
4	2	0.6020	19.5	0.195	1.2900	80.5	1.9057
6	2.449	0.7781	28.3	0.283	1.5230	71.7	1.8555
8	2.828	0.9030	35.6	0.356	1.6493	64.4	1.8088
12	3.464	1.0791	53.3	0.533	1.7803	46.7	1.6693
16	4	1.2041	71	0.71	1.8920	29.0	1.4623
20	4.472	1.3010	89.1	0.891	1.9498	10.9	1.0374
24	4.898	1.3802	92.2	0.922	1.9503	7.8	0.8920

Niosomes were stored at different temperature for 3 months. Then the consistency and concentration of famciclovir were investigated. For the estimation of drug content HPTLC method was used.

Stability studies as per ICH guidelines

Three packs of niosomes were subjected to these studies. The samples were subjected to 40 ± 2ºC and 75 ± 5 % RH. At the end of 0, 30, 60, 90 days, samples were withdrawn, and analysed using HPTLC. A graph was plotted between log% drug remaining vs time**.** The slope of the straight line from the graph was determined and degradation rate constant (K) was calculated by using the equation:

Slope = -K/2.303

Table 4: Degradation of famciclovir in niosomes at 40 ± 2ºC and 75 ± 5 % RH

Time (days)	Drug content(mg)	% drug remaining	Log % drug remaining
0	20	100	2
30	20	99.34	1.9971
60	20	98.67	1.9941
90	20	96.72	1.9855

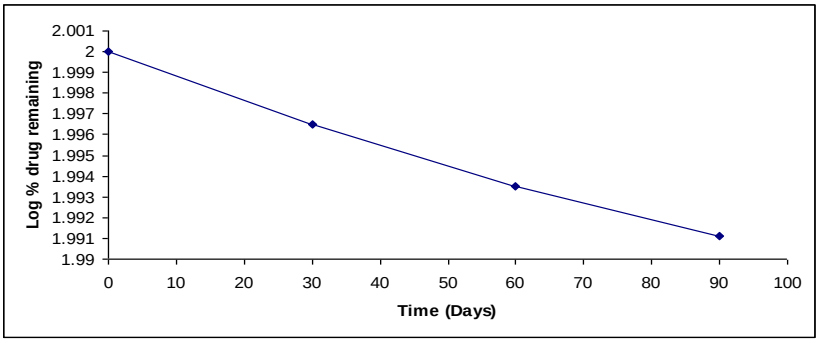

Figure 4: Degradation kinetics of famciclovir in niosomal dispersion at 40 ± 2ºC and 75± 5 % RH Accelerated stability studies according to WHO for the determination of shelf life.

***Iin vivo* study**

The ultimate test of success of formulation depends on its in vivo performance. The in vivo studies were conducted in a suitable animal model. Approval to carry out *in vivo* study was obtained from Lloyd Institute, Institutional Animal Ethics Committee and their guidelines were followed for the studies (173/CPCSEA, 28 Jan 2000; Form no 522). The animals used for in vivo experiments were adult Wistar female albino rats obtained from Animal House, Lloyd campus, Greater Noida, India. The niosomal formulation NF8 that showed the highest release profile of drug *in vitro* studies was subjected to *in vivo* studies. The blood sampling was carried out for around 24 hours and 8 samples were taken from each animal in the group. For 200 gm rat, famciclovir dose will be 44.64 mg.

Preparation of doses from different formulations

Six tablets with label claim of 250 mg were taken, crushed in mortar, and mixed with 50 ml of double distilled water. each group contained six rats. First group was treated with this tablet. Each rat in this group was given 1.0 ml using oral feeding needle.

Niosomal dispersion (NF8)

Second group was treated with the niosomal dispersion (NF8). Based on the average weight (200g) of each rat in this group a dose famciclovir (2ml) was constituted into 4ml working formula. The formulation was then given to the rats orally with the help of feeding needle.

The rats were anesthetized using diethyl ether and blood samples (500 µl) were withdrawn from the tail vein of rat at 0 (pre-dose), 1, 2, 4, 6, 8, 12 and 24hours in micro centrifuge tubes. Tubes were stored at room temperature, 25±2°C and relative humidity (55±5%) for 30 minutes. The clotted blood was then centrifuged at 5000 rpm for 30 min. The serum was separated and stored until drug analysis was carried out using HPTLC method. The collected serum was extracted with ethyl acetate for analysis and centrifuged at 5000 rpm for 5 min and dried at room temperature. After evaporating the ethyl acetate solid residue was reconstituted using 100µl mobile phase and then analyzed by HPTLC method.

The drug from each collected serum was extracted with acetonitrile as a protein precipitating agent. The samples were analysed.

Pharmacokinetic and statistical analysis

Pharmacokinetic parameters were calculated by using non-compartmental analysis using HPTLC analysis. All pharmacokinetic parameters ($t_{max,}$ C_{max}, AUC_{0-t}, $AUMC_{0-t}$ and MRT_{0-t}) were calculated individually for each subject in the group and the values were expressed as mean ±SD.

Pharmacokinetic Study:

Studies shows concentrations of Famciclovir in plasma samples collected over 24 h from rats after oral administration of a single 250-mg Famciclovir tablet and niosomal dispersion (NF8). The maximum concentration was approximately 1.2101 ± 0.4µg.mL^{-1} indicating that this method is suitable for therapeutic drug monitoring.

The pharmacokinetic profile of the Famciclovir after oral administration of Famciclovir tablet and niosomal dispersion (NF8) was plotted.

Table 5: Concentration values of Famciclovir tablets in rat serum after post oral treatment

Time	Concentration (µg/ml)							
(hrs)	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean	SD
0	0	0	0	0	0	0	0	0
1	0.562	0.544	0.499	0.518	0.526	0.524	0.528	0.0217
2	0.94	0.891	0.921	0.896	0.928	0.911	0.914	0.0188
4	1.28	1.282	1.266	1.221	1.258	1.271	1.263	0.0022
6	0.68	0.678	0.689	0.671	0.618	0.647	0.664	0.0265
8	0.203	0.195	0.191	0.181	0.175	0.208	0.192	0.0126
12	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0

Table 6: Concentration values of famciclovir niosomal dispersion (NF8) in rat serum after post oral treatment.

Time	Concentration (µg/ml)							
(hrs)	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean	SD
0	0	0	0	0	0	0	0	0
1	0.528	0.533	0.526	0.513	0.52	0.536	0.524	0.0085
2	1.37	1.334	1.342	1.335	1.332	1.354	1.3426	0.0148
4	0.968	0.983	0.972	0.986	0.989	0.979	0.9796	0.0082
6	0.885	0.871	0.871	0.868	0.872	0.875	0.8734	0.0059
8	0.618	0.625	0.591	0.602	0.609	0.631	0.609	0.0149
12	0.599	0.589	0.589	0.563	0.556	0.581	0.5792	0.0166
24	0.015	0.021	0.016	0.018	0.019	0.0134	0.0178	0.00279

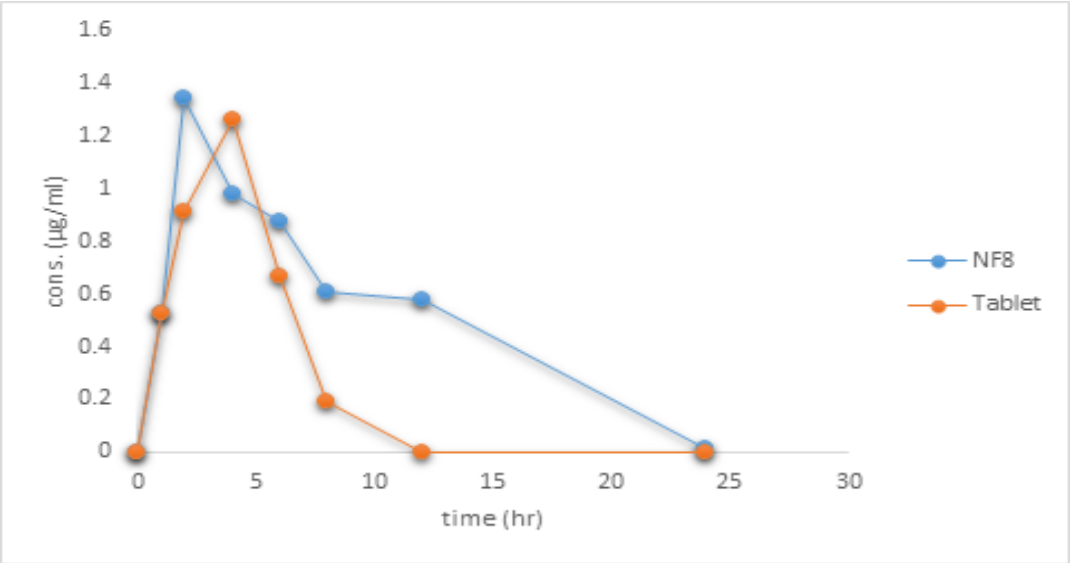

Figure 5: Famciclovir concentrations in rat serum after Oral administration of Tablet and niosomal dispersion.

Discussion

The drug was identified by UV analysis, FT-IR analysis and DSC study. The exhibited absorption maxima at 252-256nm in the range 200-400nm was same as mentioned in literature. The FT-IR spectra and DSC thermogram was found to be same as standard drug. On the basis of these drug sample was found to be authentic.

Entrapment efficiency

Data shows entrapment efficiencies of various niosomal formulations composed of 1:1, 2:1, 3:1, 3:2 molar ratios of Span 40:CH and Span60:CH molar ratios. Results shows that the incorporation of CH into niosomes significantly increased the drug entrapment efficiency up to an optimum Span:CH ratio (3:2). Cholesterol alters the fluidity of chains in bilayers and, reduces the gel to liquid phase transition of surfactant bilayers [5]. It also increases the microviscosity of niosomal membrane conferring more rigidity resulting in higher stability hence greater drug retention. However Further increase of CH content to reach 1:1 Span:CH molar ratio significantly reduced the entrapment efficiency as it starts disrupting the regular bilayered structure leading to lowering of drug entrapment levels.

Sorbitan esters are commonly used and easily available surfactants used for the formulation of niosomes. As per literature the length of alkyl chain is an important factor in determining the niosomal membrane permeability and that long chain surfactants produce high drug entrapment into vesicles. That is why Span40 and Span60 were used in this study. Results showed that the entrapment efficiencies for niosomes prepared using Span60 were significantly higher than those prepared using Span40 with the same molar ratios of Span**:**CH. This might be due to the higher phase transition temperature[6] of Span 60. Long chain surfactant produces high entrapment and this may be due to the hydrophobicity of the alkyl chain of the sorbitan esters . Hence, Span60 having longer saturated alkyl chain (C18) compared to Span 40 (C16) produced niosomes with higher entrapment efficiency. In addition, the length of the alkyl chain influences the HLB value of the surfactant mixture which in turn directly affects the drug entrapment efficiency. The HLB values for Span 40 and Span 60 are 6.7 and 5, respectively, the lower the HLB of the surfactant the higher will be the drug entrapment efficiency[7].

Photomicroscopic and transmisson electron microscopy analysis

The photomicrograph of optimized formulation (span60) (3:2)) was studied. During experiments it was found that niosomes prepared using Span 60 were slightly larger in size than those prepared using Span 40. Span 60 has a longer saturated alkyl chain compared to Span 40, which could increase the size of the vesicle.

Differential scanning calorimetry (DSC):

DSC thermograms of famciclovir, surfactant, cholesterol and drug-loaded niosomes composed of Span 60:CH (3:2) and (2:1)molar ratio were studied. DSC thermogram of plain niosomes composed of (3:2) Span 60:CH molar ratio showed an endothermic peak at 250.71ºC. DSC thermogram of famciclovir loaded niosomes of the same Span 60:CH molar ratio showed disappearance of the melting endotherm of famciclovir and broadening of the endothermic peak at 250.71ºC.

The absence of the melting endotherm of famciclovir and shifting and/or broadening of the endotherms of the surfactant bilayers of niosomes suggests significant interaction of famciclovir with bilayer components and can account for the enhanced entrapment of famciclovir into these formulations[8].

In vitro drug release studies:

The increase of CH molar ratio significantly reduced the efflux of famciclovir, showing cholesterol membrane stabilizing ability and space filling action. Cholesterol is known to increase the rigidity of niosome[9] and abolish the gel-to-liquid phase transition of niosomal systems resulting in niosomes that are less leaky. This decreases the drug release from niosomes. T24h of famciclovir was found to be 89.2% and 82.23% for 2:1 and 3:2 Span 40:CH molar ratios, respectively. The T24h for 2:1 and 3:2 Span 60:CH molar ratios were found to be 79.4% and 78.67%, respectively. Niosomal formulations prepared using Span 60 showed a significantly slower rate of drug release compared to Span 40. This can be explained by the fact that niosomes exhibit an alkyl chain length-dependent release. The higher was the chain length, the lower was the release rate.

Stability studies:
The shelf life of niosomsal dispersion was found to be 1.58. This result shows that formed niosomal dispersion is stable.

***In-vivo* studies:**
The $AUC_{0\rightarrow\infty}$of niosomal formulation ($AUC_{0\rightarrow t}$of 12.811±2.12 µg/ml/h) was found to be two fold higher compared to orally administered drug suspension ($AUC_{0\rightarrow t}$of 6.329±1.56µ/ml/h). Also, the statistic calculation provided a significant difference between both values ($P < 0.05$).
After oral administration, the plasma levels of famciclovir reached a peak of 1.263 ± 0.4µg/ml at 4±0.32 h, while after oral administration of niosomal formulation, it reached a peak of 1.343 ± 0.3µg/ml at 2.051± 0.3 h. The effective drug concentration (>0.69µg/mL in plasma) was maintained for at least 8 hours through administration. The MRT and $AUC_{0\rightarrow t}$ values may reflect the sustained release effect of the niosomal formulation. This sustained release effect was also investigated by the *in vitro* release study. A possible explanation for this sustained release effect is that niosomes act as a carrier and a slow release vehicle. This sustained release effect can improve the bioavailability of drugs with slow and limited absorption and narrow absorption windows. The significant increase of Cmax values may be owing to enhanced absorption of the free drug included in the tested unpurified niosomal formulation (containing both the free and niosomal drug). This finding may be a result of the influence of span 60 as a penetration enhancer on the permeability of gastrointestinal membrane. The *in vivo* data have also demonstrated significantly higher bioavailability (2 times) of famciclovir after oral administration through niosomal dispersion.

Conclusion
The oral route is the most common and preferred route of drug delivery system because it is a most convenient route and provides several advantages. About 90% of the drugs are administered through this route. Upto 40% of hydrophobic drug candidates fail to reach market because they have to be administered at higher dose, have poor aqueous solubility, hydrophobicity and exhibits low bioavailability although exhibiting potential pharmacodynamic activities. Keeping above all these things in mind, the objective of the present study was to develop and characterize an optimal oral stable niosomal formulation using minimum surfactant concentration.

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