**FORMULATION, DEVELOPMENT AND EVALUATION OF LAMIVUDINE
ENCAPSULATED SOLID LIPID NANOPARTICLES****Mohit Singh Thakur*, Khushi Chouksey, Dr. Kuldeep Ganju****Sagar Institute of Pharmacy and Technology (SIPTec), Bhopal***Corresponding Author's E mail: mohitsinghthakur2016@gmail.com

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ABSTRACT

The aim of this study is to formulate the lamivudine loaded solid lipid nanoparticles in order to enhance the bioavailability and to reduce the dose frequency. The lamivudine loaded solid lipid nanoparticles were prepared by solvent injection method. The formulations were characterized for particle size, zeta potential, entrapment efficiency, drug content and *in vitro* release studies. Particle size was found to be 218.23nm, percentage entrapment efficiency was found to be 79.98±0.47%, Zeta potential was found to be - 42.23 mV and percentage drug content was found to be 75.65±0.85% for the optimized formulation F11. Lamivudine solid lipid nanoparticles using combination of polymers evolved as the optimized formulation and it releases more than 99.74% drug in 24 hrs. The optimized formulation F11 can be considered as a promising sustained drug delivery system of Lamivudine solid lipid nanoparticles providing nearly first order drug release over a period of 24 hrs. Results of this study indicated that solid lipid nanoparticles would be a promising therapeutic system for efficient delivery of the lamivudine and could possibly be advantageous in terms of increased bioavailability of lamivudine.

Keywords: Solid lipid nanoparticles, Lamivudine, Solvent injection technique, Nanoparticles.

INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with size in range of 10-1000 nm in which drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. The drug delivery system using solid lipid nanoparticles came into being about two decades ago and since then lot of work has been done in this field. Solid lipid nanoparticles emerged in 1991 with the objective to provide biocompatibility, storage stability and to prevent the incorporated drug from degradation¹. Solid lipid nanoparticles, colloidal carriers of nanoscopic size (50–1000 nm), made up of solid lipids (high melting fat matrix), are developed to conquer the weaknesses (e.g., polymer degradation and cytotoxicity, lack of a suitable large scale production method, inferior stability, drug leakage and fusion, phospholipid

degradation, high production cost, and sterilization problems) of traditional colloidal carriers, like polymeric nanoparticles and liposomes². Solid lipid nanoparticles show various distinctive features such as low toxicity, large surface area, prolonged drug release, superior cellular uptake as compared to traditional colloidal carriers as well as capability to improve solubility and bioavailability of drugs^{3,4}. The release of drug from solid lipid nanoparticles depends on matrix type and drug location in the formulation. The solid lipid nanoparticles fabricated from biodegradable and biocompatible ingredients are able to incorporate both hydrophilic and lipophilic bioactives and thus turning out to be a viable option for controlled and targeted drug delivery^{4,5}. The solid core of Solid lipid nanoparticles is hydrophobic with a monolayer coating of phospholipids and the drug is usually dispersed or dissolved in the core⁵⁻⁷.

Lamivudine is a synthetic nucleoside analogue which acts as a reverse transcriptase inhibitor. Lamivudine is used for the treatment of chronic hepatitis and human immunodeficiency virus (HIV) infections with a half-life of nearly 5-7 hours. Conventional formulations of Lamivudine are found to have many drawbacks, such as adverse side effects resulting from accumulation of drug in multidose therapy, poor patient compliance, and high cost^{8,9}. Recently, increasing attentions has been focused on the solid lipid nanoparticles (SLNs); because it offers advantages like possibility of controlled drug release, improve drug stability and low toxicity¹⁰. Incorporation of lamivudine in solid lipid nanoparticles results in decrease bone marrow toxicity, increase bioavailability and improve antiviral activity. The active targeting of solid lipid nanoparticles containing entrapped antiretroviral drug is possible approach to overcome the limitations of conventional therapy. Mannose receptors present on the surface of macrophages, and therefore mannose conjugated solid lipid nanoparticles may target the macrophages, which may improve drug bioavailability in the brain, which improve the therapeutic outcomes^{11, 12}. So that there is a need to develop lamivudine nanoparticles to control the drug release. The objective of the present study was to prepare solid lipid nanoparticles of lamivudine to overcome some of these problems.

MATERIALS AND METHODS

Material

Lamivudine was kindly gifted by Ranbaxy Laboratories Limited, Dewas, India. All other reagents, solvents and chemicals were analytical grade and purchased locally.

Method

Determination of λ_{max} of lamivudine

Accurately weighed 10 mg of drug was dissolved in 10 ml of 7.4 pH buffer solution in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 7.4 pH buffer solution prepare suitable dilution to

make it to a concentration range of 2-10 μ g/ml. The λ_{max} of lamivudine was determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer.

Formulation of lamivudine loaded solid lipid nanoparticles

Solid lipid nanoparticles were prepared by using solvent injection technique using ethanol as organic solvent. Soya lecithin, drug and steric acid is dissolved in the ethanol in definite ratio and warmed to 70°C. To the phosphate buffer solution (pH 7.4) a definite amount of tween 80 is added to prepare aqueous phase and kept for stirring which is maintain at 70°C. The organic phase was added drop wise with stirring to the pre warmed aqueous solution with the help of hypodermic needle. The mixture was then sonicated (Ultra sonicator, Bath type, Electronic India) for varying time to obtain nanoparticles. The optimum parameters i.e. tween 80 concentrations in definite ratio and maximum sonication time resulted in maximum entrapment efficiency and controlled release were used for the preparation of solid lipid nanoparticles using similar method. Twelve formulations were prepared by using different concentrations of tween 80 and sonication time to determine the effect of surfactant and sonication time on the potency of the solid lipid nanoparticles ^{13, 14}.

Effect of formulation process variables

The effect of formulation variables such as amount of soya lecithin, steric acid, tween 80, sonication time on the particle size was studied. From the results obtained, optimum level of those variables was selected and kept constant in the subsequent evaluations. However, molecular dispersion of the drug in the stearic acid matrix led to a reduction in the crystallinity of stearic acid.

Table 1: Composition of solid lipid nanoparticles by varying amount of soya lecithin

Components	Formulation code		
	F1	F2	F3
Drug (mg)	50	50	50
Soya lecithin (mg)	15	30	45
Steric acid(mg)	25	25	25
Tween 80(ml)	0.5	0.5	0.5
Sonication time (min)	6	6	6

Table 2: Composition of solid lipid nanoparticles by varying amount of steric acid

Components	Formulation code		
	F4	F5	F6
Drug (mg)	50	50	50
Soya lecithin (mg)	30	30	30
Steric acid(mg)	20	30	40
Tween 80(ml)	0.5	0.5	0.5
Sonication time (min)	6	6	6

Table 3: Composition of solid lipid nanoparticles by varying amount of tween 80

Components	Formulation code		
	F7	F8	F9
Drug (mg)	50	50	50
Soya lecithin (mg)	30	30	30
Steric acid(mg)	30	30	30
Tween 80(ml)	0.5	1.0	1.5
Sonication time (min)	6	6	6

Table 4: Composition of solid lipid nanoparticles by varying sonication time

Components	Formulation code		
	F10	F11	F12
Drug (mg)	50	50	50
Soya lecithin (mg)	30	30	30
Steric acid(mg)	30	30	30
Tween 80(ml)	1.0	1.0	1.0
Sonication time (min)	6	12	15

Preparation of drug loaded solid lipid nanoparticles

One optimized formulation of drug loaded solid lipid nanoparticles were prepared by microemulsion method ¹⁵ and the composition of drug loaded batches are given in table 5.

Table 5: Composition of optimized nano particles formulation

Components	Formulation code
	F11
Drug (mg)	50
Soya lecithin (mg)	30
Steric acid(mg)	30
Tween 80(ml)	1.0
Sonication time (min)	12

Evaluation of nanoparticles

Particle size and zeta potential

Particle size and zeta potential of the solid lipid nanoparticles were measured by photon correlation spectroscopy using a Malvern Zetasizer ^{16, 17}.

Entrapment efficiency

The entrapment efficiency of the drug was defined as the ratio of the mass of formulations associated drug to the total mass of drug 80. Solid lipid nanoparticles entrapped Lamivudine were separated from the free drug by dialysis method. The free drug (unentrapped) in the solid lipid nanoparticles dispersion was sedimented by controlled centrifugation at 2000 rpm for 30 min using Remi centrifuge and the solid lipid nanoparticles dispersion was decanted without disturbing the drug pellet. Solid lipid nanoparticles dispersion was used for estimation of unentrapped drug content. Unentrapped drug content determined using UV spectrophotometry at 270nm ¹⁸.

Drug content

From the prepared solid lipid nanoparticles formulation 1ml of suspension was dissolved in the 10 ml of 7.4 PBS buffer and ethanol mixture. The amount of lamivudine was determined using UV spectrophotometer at 270nm. The placebo formulation prepared similarly to drug loaded solid lipid nanoparticles was used as blank and the total drug content was calculated.

In vitro drug release in gastrointestinal fluids of different pH

The prepared solid lipid nanoparticles delivery system was evaluated for *in vitro* drug release. The drug release studies were carried out using USP XXII paddle type dissolution test apparatus. The dissolution

study was carried out in 900 ml dissolution medium (PBS pH 7.4) which was stirred at 100 rpm maintained at $37 \pm 0.2^\circ\text{C}$.

A weighed quantity of formulation (100 mg) was spread over the surface of dissolution media (900 ml) at $37 \pm 0.2^\circ\text{C}$. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 10ml by PBS (pH 7.4). The samples withdrawn were assayed spectrophotometrically at 270.0 nm for lamivudine and using UV visible spectrophotometer. The release of lamivudine was calculated with the help of standard curve of lamivudine. The drug release for the drug in uncoated formulation and coated formulation were observed ¹⁹.

Drug release kinetics -model fitting of the release data

In order to investigate the mode of release from the nanoparticles, the release data were fitted into zero-order, first-order, Higuchi, Korsmeyer-Peppas equations. The regression equations were calculated and the correlation coefficients were determined. In order to analyze the drug release mechanism, *in vitro* release data were fitted into a zero-order, first-order, Higuchi, Korsmeyer-peppas model. Drug dissolution has been described by kinetic models in which the dissolved amount of drug (Q) is a function of the test time, t or $Q=f(t)$. Some analytical definitions of the Q(t) function are commonly used, such as zero-order, first- order, Higuchi, Korsmeyer–Peppas models ²⁰.

Results:

The λ_{max} of lamivudine was determined and the spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). The spectrum peak point graph of absorbance of lamivudine versus wave length is shown in figure 1.

Figure 1: Wavelength maxima of lamivudine in phosphate buffer pH 7.4

Optimum level of process variables was selected and kept constant in the subsequent evaluations. However, molecular dispersion of the drug in the stearic acid matrix led to a reduction in the crystallinity of stearic acid.

Percentage entrapment efficiency of prepared solid lipid nanoparticles was found in range of 60.23 ± 0.65 to 79.98 ± 0.47 percentage. Drug content of prepared solid lipid nanoparticles was found in range of 65.56 ± 0.74 to 85.65 ± 0.47 percentage. The outcomes of the results are discussed in the table 6. The maximum entrapment efficiency was found in formulation F11. The optimized formulation F11 among other batches was subjected to further studies.

Table 6: Result for entrapment efficiency and drug content of drug loaded solid lipid nanoparticles

Formulation Code	Entrapment Efficiency	Drug Content
F1	65.58 ± 0.32	68.89 ± 0.52
F2	72.32 ± 0.45	76.65 ± 0.47
F3	60.23 ± 0.65	65.58 ± 0.32
F4	72.23 ± 0.21	68.85 ± 0.21
F5	74.45 ± 0.14	82.23 ± 0.45
F6	70.12 ± 0.78	65.58 ± 0.65
F7	68.89 ± 0.42	72.23 ± 0.21
F8	75.65 ± 0.32	85.65 ± 0.47
F9	63.32 ± 0.14	75.56 ± 0.48
F10	65.56 ± 0.52	65.56 ± 0.74
F11	79.98 ± 0.47	85.56 ± 0.89
F12	70.45 ± 0.32	75.65 ± 0.85

% Entrapment efficiency of optimized solid lipid nanoparticle formulation (F-11) was found 79.98 ± 0.47 . It was observed that the percent drug entrapment was slight decrease with increasing the concentration of polymer and on increasing the time of stirring. It is due to the leaching out the drug from solid particle on increasing the mechanical force by stirrer and size reduction. It was clearly shown when formulation was sonicated for 6, 12, 15 min then the % EE was 65.56 ± 0.52 , 79.98 ± 0.47 and 70.45 ± 0.32 . The 12 min was selected as optimized time for stirrer because it provided the required size of drug content $85.56 \pm 0.89\%$ and good % EE i.e 79.98 ± 0.47 . The F-11 formulation was selected as optimized formulation.

Table 7: Particle size and entrapment efficiency of optimized solid lipid nanoparticles

Formulation Code	Particle size (nm)	% Entrapment Efficiency	Zeta potential (mV)	% Drug Content
F11	218.23	79.98±0.47	- 42.23	75.65±0.85

Particle size was found to be 218.23nm, percentage entrapment efficiency was found to be 79.98±0.47%, Zeta potential was found to be - 42.23 mV and percentage drug content was found to be 75.65±0.85% for the optimized formulation F11. The outcomes of the results are tabulated in table 7 and figure 2&3.

Figure 2: Particle size of optimized solid lipid nanoparticles **Figure 3: Zeta potential of optimized solid lipid nanoparticles**

The prepared solid lipid nanoparticles delivery system was evaluated for *in vitro* drug release. The observations of drug release for the drug in uncoated formulation and coated formulation is tabulated in table 9. The release of lamivudine mainly depended upon the polymer concentration. The burst release of lamivudine from solid lipid nanoparticles at initial stage resulted from the dissolution of drug crystals on the surface of solid lipid nanoparticles. on increasing polymer concentration, the release rate of lamivudine from solid lipid nanoparticles decreased drastically. It was found that the amount of drug diffused from all the formulation ranged from 18.85 to 26.65% during first hour. Formulation F11 containing solid lipid nanoparticles showed complete drug release in 24hrs.

Table 9: Cumulative % drug release of lamivudine loaded solid lipid nanoparticles

S. No.	Dissolution medium	Time (hrs)	% Cumulative Drug Release
			Solid lipid Nanoparticles
1	Phosphate Buffer (pH 7.4)	0.1	18.85
2		1	26.65
3		2	38.89
4		3	49.98
5		4	55.65
6		5	68.87
7		6	76.65
8		8	86.65
9		10	92.23
10		12	94.65
11		24	99.74

Table 10: *In vitro* drug release data for F11

S. No.	Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release $\pm$ SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
1	0.5	0.707	-0.301	18.85	1.275	81.15	1.909
2	1	1.000	0.000	26.65	1.426	73.35	1.865
3	2	1.414	0.301	38.89	1.590	61.11	1.786
4	3	1.732	0.477	49.98	1.699	50.02	1.699
5	4	2.000	0.602	55.65	1.745	44.35	1.647
6	5	2.236	0.699	68.87	1.838	31.13	1.493
7	6	2.449	0.778	76.65	1.885	23.35	1.368
8	8	2.828	0.903	86.65	1.938	13.35	1.125
9	10	3.162	1.000	92.23	1.965	7.77	0.890
10	12	3.464	1.079	94.65	1.976	5.35	0.728
11	24	4.899	1.380	99.74	1.999	0.26	-0.585

Figure 4: Cumulative percent drug released vs time (zero order plots)

The *in vitro* release data was applied to various kinetic models to predict the drug release kinetic mechanism. The release constant was calculated from the slope of appropriate plots, and the regression coefficient (r^2) was determined. It was found that the *in-vitro* drug release of solid lipid nanoparticles was best explained by first order kinetics for best formulation F11 as the plots shows highest linearity. The correlation coefficient (r^2) was found 0.997 for F11.

Figure 5: Log cumulative percent drug remaining vs time (first order plots)

Figure 6: Cumulative percent drug release vs root time (higuchi release kinetics)

Figure 7: Log cumulative percent drug released vs log time (peppas plots)

Table 11: Regression analysis data of solid lipid nanoparticles formulation

Formulation	Zero order	First order	Higuchi	Pappas plot
F11	$R^2 = 0.671$	$R^2 = 0.997$	$R^2 = 0.872$	$R^2 = 0.956$

Lamivudine is a hydrophilic drug with half-life of 5–7 h and classified as Class 1 in the biopharmaceutical classification system, denoting that it has high solubility and permeability [21]. SLN are potential delivery system for lipophilic drugs where aqueous solubility of the drug is the limiting factor for its absorption [22]. Moreover, the incorporation of such drugs within SLN is easier due to their affinity for the lipid. On the other hand, entrapment of hydrophilic drug inside the hydrophobic matrix of SLN is a real challenge as the drug has maximum tendency to partition in the water during the fabrication process. Success of the *in vitro* drug release studies recommends product for further *in vivo* studies, which may improve patient compliance. From the results, formulation F11 containing Lamivudine solid lipid nanoparticles using combination of polymers evolved as the optimized formulation and it releases more than 99.74% drug in 24 hrs. The optimized formulation F11 can be considered as a promising sustained

drug delivery system of Lamivudine solid lipid nanoparticles providing nearly first order drug release over a period of 24 hrs.

Conclusion

The method of preparation of solid lipid nanoparticles of lamivudine was found to be simple and reproducible. The slow and constant release of lamivudine from solid lipid nanoparticles maintain constant drug plasma concentration thereby increasing therapeutic efficacy. This study shows that solid lipid nanoparticles could be a useful carrier for lamivudine. The developed formulation overcome and alleviates the drawbacks and limitations of lamivudine sustained release formulations.

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