ejpmr, 2017,4(11),377-383

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

FORMULATION AND CHARACTERISATION OF CHITOSAN BASED LAMIVUDINE NANOPARTICLES

P. Pravalika Reddy*, Dr. B. Madhava Reddy and Dr. D. Jaya Prakash

[1]Malla Reddy Pharmacy College, Maisammaguda, Secunderabad, Telangana.
[2]G. Pulla Reddy College of Pharmacy, Mehdipatnam, Telangana.
[3]University College of Technology, Osmania University, Telangana.

***Corresponding Author: P. Pravalika Reddy**

Malla Reddy Pharmacy College, Maisammaguda, Secunderabad, Telangana.

Article Received on 22/08/2017 **Article Revised on 12/09/2017** **Article Accepted on 03/10/2017**

ABSTRACT

The aim of the present work is to formulate and characterize nanoparticles of lamivudine drug. Lamivudine is an antiretroviral drug and BCS Class-III having high solubility and low Permeability. Nanoparticles of Lamivudine are fabricated by using chitosan polymer and pregelated Sodium alginate by Ionotropic pregelation method and optimization in terms of polymer concentration, cross-linking agent,stirring time and stirring speed. Calcium chloride is also included in the formulation for pregelation of sodium alginate. Different formulations of nanoparticles are prepared using different concentrations of chitosan, Sodium alginate, stirring time and speed. The drug loaded optimized lamivudine nanoparticles showed average particle size of 77.7 nm. Drug entrapment ranged between 67.79%- 89.98%. Invitro release studies revealed that the rate of drug release from NP 2 Formulation is 89.55% in 24 hours. Release of drug followed first order and the mechanism is super case II transport, indicates swelling of polymer with diffusion.

KEYWORDS: Lamivudine, Nanoparticles, Chitosan, Sodium alginate, Calcium chloride, Ionotropic Pre gelation Method.

INTRODUCTION

Novel drug delivery systems present an opportunity for formulation scientists to overcome the many challenges associated with antiretroviral (ARV) drug therapy, thereby improving the management of patients with HIV/AIDS. Current treatments available for human immunodeficiency virus, namely antiretrovirals, do not completely eradicate the virus from the body, leading to life time commitment. Many antiretrovirals suffer drawbacks from toxicity and unpleasant side effects; causing patience non-compliance. To minimize challenges associated with the antiretrovirals, biodegradable nanoparticles used as drug delivery systems hold tremendous potential to enhance patience compliance. Chitosan-alginate (CS/ALG) nanoparticles were prepared by Ionotropic pre-gelation of an alginate core followed by chitosan polyelectrolyte complexation. Alginate (ALG) is a water soluble linear polysaccharide extracted from brown sea weed and is composed of alternating blocks of 1-4 linked α-L-guluronic and β-D-mannuronic acid residues. ALG has been reported to be mucoadhesive, biodegradable, and biocompatible and has potential for numerous pharmaceutical and biomedical applications such as drug delivery system and cell encapsulation.[1,2] Alginate micro and nanoparticles can be obtained easily by inducing gelation with calcium ions.[3,4] Such easy-gelling property can be used to produce a pre-gel consisting of very small aggregates of gel particles, followed by the addition of an aqueous polycationic solution to make a polyelectrolyte complex coating, recently, chitosan (CS) was selected as an alternative cationic polymer. Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV).It is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination.

MATERIALS AND METHODS

Materials: Lamivudine is obtained as a gift sample from Cipla Pharmaceuticals Ltd, Mumbai. Glacial acetic acid is procured from Qualigens Fine Chemicals. Chitosan, Sodium alginate and Calcium chloride are purchased from Finar Reagents, Ahmedabad. All other chemicals were of analytical grade and used as received. Distilled water is used throughout the study. Remi Magnetic stirrer is used.

Method

Preparation of drug loaded sodium alginate nanoparticles

Chitosan-alginate nanoparticles are prepared in a two-step procedure based on the Ionotropic pre-gelation[5-10] of polyanion(sodium alginate) followed by polycationic cross linking with chitosan.7.5 ml of 18mm calcium chloride is added dropwise for 1 hour under magnetic stirring at 800rpm into a beaker containing 100 ml of 0.06%w/v sodium alginate and drug to form an alginate pre-gel. Then 25 ml of different concentrations of chitosan(0.2-0.4%)solution is added dropwise into the pre-gel for 90 min. The particles produced are in nanorange. The drug loaded nanoparticles are recovered by ultracentrifugation at 10000 rpm for 30min and washed with distilled water to obtain nanoparticles.

Table 1: Formulation of nanoparticles with different concentrations of Sodium alginate.

Code	Drug(mg)	Sodium alginate %	CaCl2 MM	Chitosan%	Stirring time (min)	rpm
NP1	100	0.05	18	0.4	90	800
NP2	100	0.06	18	0.4	90	800
NP3	100	0.07	18	0.4	90	800

Table 2: Formulation of nanoparticles with different concentrations of Calcium Chloride.

Code	Drug(mg)	Sodium alginate %	CaCl2 MM	Chitosan%	Stirring time (min)	rpm
NP4	100	0.05	15	0.4	90	800
NP5	100	0.05	18	0.4	90	800

Table 3: Formulation of nanoparticles with different Stirring time using 0.2%Chitosan.

Code	Drug(mg)	Sodium alginate %	CaCl2 MM	Chitosan%	Stirring time (min)	rpm
NP6	100	0.06	18	0.2	45	800
NP7	100	0.06	18	0.2	60	800
NP8	100	0.06	18	0.2	90	800

Table 4: Formulation of nanoparticles with different Stirring time using 0.3%Chitosan.

Code	Drug(mg)	Sodium alginate %	CaCl2 MM	Chitosan%	Stirring time(min)	rpm
NP9	100	0.06	18	0.3	45	800
NP10	100	0.06	18	0.3	60	800
NP11	100	0.06	18	0.3	90	800

Table 5: Formulation of nanoparticles with different Stirring time using 0.4%Chitosan.

Code	Drug(mg)	Sodium alginate%	CaCl2 MM	Chitosan%	Stirring time(min)	rpm
NP12	100	0.06	18	0.4	45	800
NP13	100	0.06	18	0.4	60	800
NP14	100	0.06	18	0.4	120	800
NP15	100	0.06	18	0.4	180	800

Table 6: Formulation of nanoparticles with different Stirring Speed.

Code	Drug(mg)	Sodium alginate %	CaCl2 MM	Chitosan%	Stirring time(min)	rpm
NP16	100	0.06	18	0.4	90	500
NP17	100	0.06	18	0.4	90	600
NP18	100	0.06	18	0.4	90	700
NP19	100	0.06	18	0.4	90	1000

Characterization of Drug Loaded Nanoparticles

Particle Size and zeta potential

The particle size distribution and zeta potential of the drug entrapped nanoparticles is analyzed by Zetasizer nanoZS90 (Malvan Instruments Ltd, UK), the samples are placed in disposable cuvettes for particle size measurements and zeta dip cell is used to find the potential. Each experiment is conducted in triplicate.

Surface Morphology

Shape and Surface morphology of lamivudine loaded Alginate-Chitosan nanoparticles is studied using high-resolution Scanning Electron microscopy (SEM). The samples on conducive carbon point are placed in a specimen holder, Vacuum-dried and sputter coated with platinum using accelerating voltage of 2KV for 90 sec.

Fourier Transform Infra-Red Spectroscopy

Compatibility of drug with excipients is determined by carrying out FTIR studies. Lamivudine loaded Alginate-Chitosan nanoparticles separated from nanoparticulate suspensions are dried by freeze dryer and their FTIR spectrum are obtained by using Fourier Transform Infrared spectrophotometer BRUKER, FTIR- (8400 S Shimadzu) using KBR Pellet method. KBR pellet obtained is scanned in the range of 400-4000cm-1. The

characteristic peaks are recorded for pure drug and nanoparticles.

Differential Scanning Calorimetry

The thermal behaviour of lamivudine and nanoparticles containing drug are examined by using DSC 60, Shimadzu. Sample of about 5mg is placed in aluminium pan and analysed at a scanning temperature range from 50 to 600^0c at the heating rate of 10^0c/min under constant purging of Nitrogen at 20ml/min.

Estimation of Entrapment efficiency

The Entrapment efficiency of nanoparticles is determined by separation of drug loaded nanoparticles from the aqueous medium containing non associated lamivudine by ultracentrifugation at 10000 rpm for 30min and the amount of free lamivudine in the supernatant was measured by UV spectrophotometer at 270 nm. The lamivudine entrapped in the nanoparticles is calculated as

Entrapment efficiency (%) = (Tp – Tf) 100/Tp

Where, Tp is the total Lamivudine used to prepare the nanoparticles and Tf is the total free Lamivudine in the supernatant.

In vitro drug release studies

In vitro drug release studies are carried out by using dialysis bag method. Lamivudine Nanoparticles (weight equivalent to 5mg of drug) is taken in dialysis bag and placed in 500 ml of Phosphate buffer pH 7.4 at 37^0c under continuous magnetic stirring in a beaker. At specified time intervals of 1,2,4,6, 8, 12, 16, 20 and 24 hours, 5ml of aliquots were withdrawn from the medium and replaced with equal volume of phosphate buffer. The concentration of drug is assayed by spectrophotometry at 270 nm.

Determination of drug release kinetics.

To understand the mechanism of drug release, the results of in vitro drug release study of three best formulations were determined using the following mathematical models: zero order kinetics, first order kinetics, higuchi model and Korsmeyer - peppas plot.

RESULTS AND DISCUSSION

Preparation of Lamivudine nanoparticles

Lamivudine nanoparticles are prepared by Ionotropic pre-gelation method. The results of nanoparticles prepared using various factors are as follows:

Sodium alginate concentration: As the Sodium alginate concentration increases entrapment efficiency increases upto 0.06% but after that entrapment decreases and at 0.1% a gel formation is observed. The nanoparticles prepared by Ionotropic pre-gelation method using different concentration are shown in the below table 7 and depicted in Figure 1.

Table 7: Effect of Sodium Alginate on Entrapment efficiency.

Formulation	Sodium Alginate %	Entrapment efficiency
NP1	0.05	75
NP2	0.06	89.98
NP3	0.07	**77**

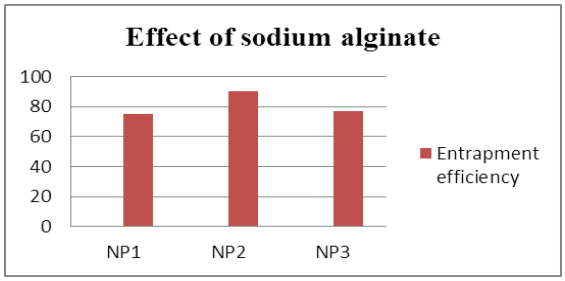

Fig. 1: Effect of Sodium Alginate on Entrapment efficiency.

b) Calcium Chloride: As the concentration of Cacl2 increases entrapment efficiency increased only upto 18mm, after this it decreases is shown in the below table 8 and depicted in Figure 2.

Table 8: Effect of Calcium Chloride on Entrapment efficiency.

Formulation	Sodium Alginate	Calcium Chloride	Entrapment efficiency
NP4	0.05%	15mm	67.8
NP5	0.05%	18mm	74.0

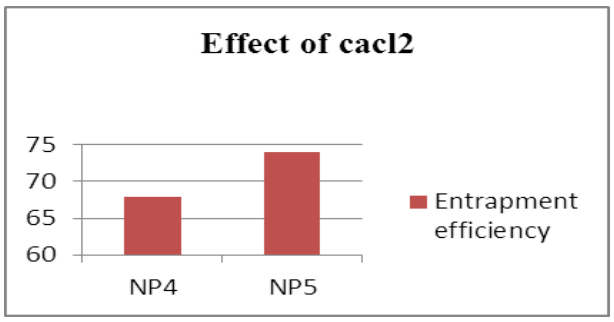

Fig. 2: Effect of Calcium Chloride on Entrapment efficiency.

c) Stirring time: Nanoparticles are prepared by using different stirring time by keeping other parameters constant, if stirring time is less nanoparticle formation does not occur. The following table 9,10,11 shows the effect of Stirring time on drug Entrapment efficiency with different chitosan concentration and depicted in Figure 3,4,5.

Table 9: Effect of Stirring time using 0.2%Chitosan.

Formulation	Stirring time min	Entrapment efficiency
NP6	45	82.33
NP7	60	86.78
NP8	90	88.30

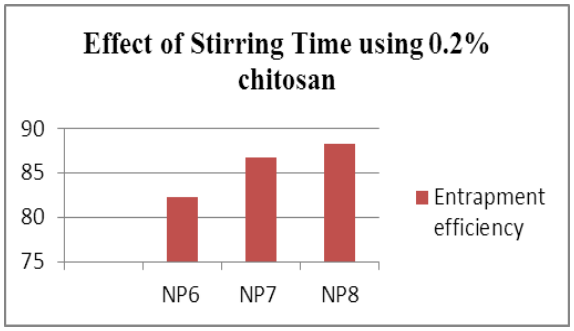

Fig. 3: Effect of Stirring time using 0.2% Chitosan.

Table 10: Effect of Stirring time using 0.3% Chitosan.

Formulation	Stirring time Min	Entrapment efficiency
NP9	45	83.91
NP10	60	85.74
NP11	90	89.70

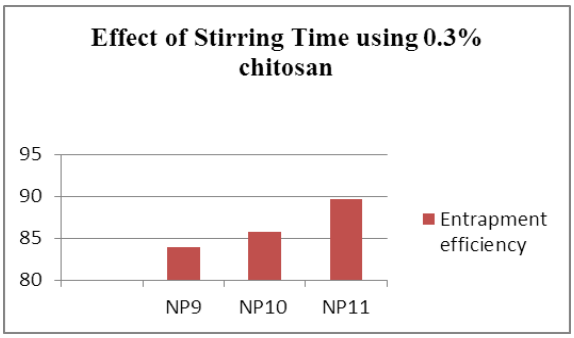

Fig. 4: Effect of Stirring time using 0.3% Chitosan.

Table 11: Effect of Stirring time using 0.4% Chitosan.

Formulation	Stirring time Min	Entrapment efficiency
NP12	45	86.42
NP13	60	87.28
NP14	120	70
NP15	180	62.33

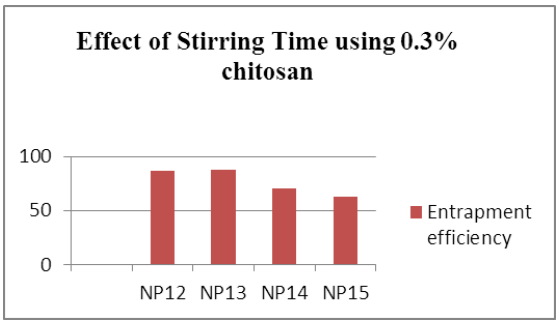

Fig. 5: Effect of Stirring time using 0.4% Chitosan.

d) Stirring Speed: The nanoparticles are prepared at different speeds and below table and figure shows effect of Stirring Speed on Entrapment efficiency. As the Stirring Speed increases, particle size decreases as a result small size nanoparticles are formed. If Stirring Speed is less microparticles are formed.

Table 12: Effect of Stirring Speed on Entrapment efficiency.

Formulation	Speed in rpm	Entrapment efficiency
NP16	500	70.16
NP17	600	82.54
NP18	700	85.00
NP19	1000	87.00

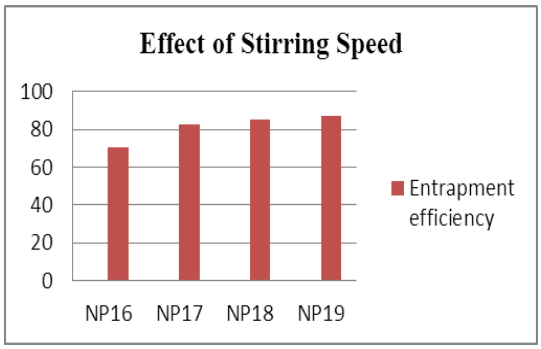

Fig. 6: Effect of Stirring Speed on Entrapment efficiency.

At 400 rpm there is no formation of nanoparticles whereas nanoparticles prepared at 500,600,700,800 and 1000 rpm showed formation of nanoparticles. As the speed increases, the entrapment efficiency increases and reached maximum at 800 rpm but at 1000 rpm the entrapment efficiency is decreased. So, 800rpm is used throughout the study.

Characterization of drug loaded Nanoparticles

SEM Analysis: SEM Analysis shows that the particles with target size can be prepared by Ionotropic pre-gelation method. Lamivudine loaded nanoparticles has spherical shape with size ranging from 100 nm to 1000 nm as shown in Fig 7.

Particle Size determination: The Particle Size of optimized Lamivudine loaded nanoparticle formulation is shown in Fig 8.The mean particle size of the optimized formulation is found to be 77.7nm.

Zeta potential: The zeta potential of -44.5 mv shown in Fig 9 indicates good stability of formulation.

FTIR Analysis: The Characteristic peaks of lamivudine are at 3203.9 cm^{-1} (-OH- stretching),

1612.2 cm^{-1} (-NH-Bending), 1285.5 cm^{-1} (-C=O Stretching), 1160.2 cm^{-1} (C-N –Stretching) and 851.9,786.9 cm^{-1}(C-H Aromatic bending) as shown in Fig 10.In the spectrum of Lamivudine loaded nanoparticles formulation, peaks are obtained at 3238.7 cm^{-1}, 1608.8 cm^{-1}, 1376.9 cm^{-1}, 1203.0 cm^{-1}, 804.9 cm^{-1} and 709.4 cm^{-1}. Because of the presence of polymer some

additional peaks are present as shown in Fig 11.This indicates that no interaction occurred between the drug and the excipients.

Differential Scanning calorimetry

DSC studies are performed to investigate the physical state of the drug in the nanoparticles as it influences the in vitro release of drug from system.DSC thermogram of lamivudine and nanoparticles containing drug are shown in Fig 12, 13. The thermogram of drug showed a sharp melting peak at 149.2 ^{o}c and Lamivudine loaded nanoparticles at 149.1 ^{o}c. It indicates that there is no effect of polymers on the thermal behaviour of the drug.

Drug release and Release Kinetics

The in vitro release pattern of lamivudine loaded nanoparticles prepared by Ionotropic pregelation method for NP2, NP8, NP 11 are shown in Figure 14.It reveals that the rate of drug release from NP 2, NP8, NP 11 ,Formulation is 89.55, 88.30,89.70 % after 24 hours.

To determine the release model the results of in vitro drug release study of optimized formulation are substituted in equations of zero order, first order, higuchi model and Korsmeyer - peppas model and the results are plotted and shown in Figure 15.Among them first order model showed a high R^2 Value of 0.974, indicates the release of drug followed first order release Kinetics. To understand the mechanism of drug release, Korsmeyer - peppas equation is applied to th eoptimised formulations and it showed good linearity and the release exponent is found to be 0.96 as shown in Fig 16.According to this model, if the value of n is >0.85, indicates that the mechanism is super case II transport, indicates swelling of polymer with diffusion.

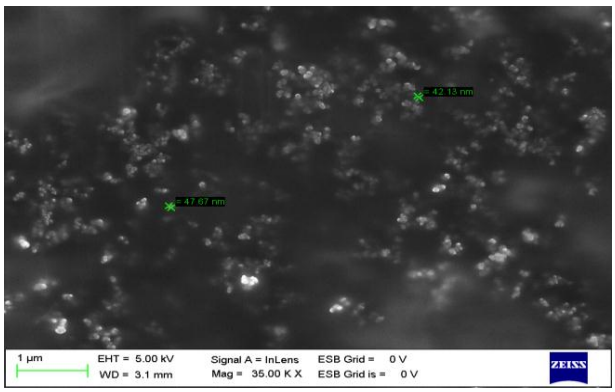

Fig. 7: SEM studies of drug loaded optimized nanoparticles.

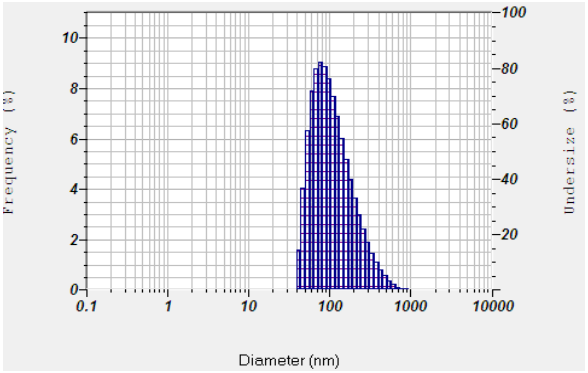

Fig. 8: Particle size distribution of Lamivudine loaded nanoparticles.

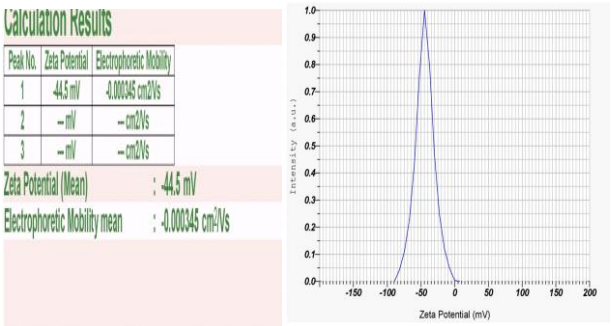

Fig. 9: Zeta potential of drug loaded optimized nanoparticles.

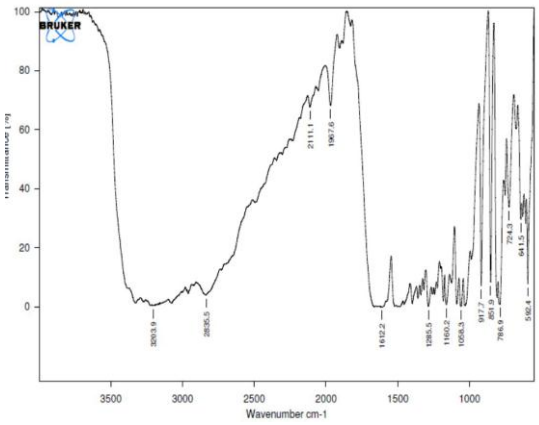

Fig. 10: FTIR spectra of pure Lamivudine.

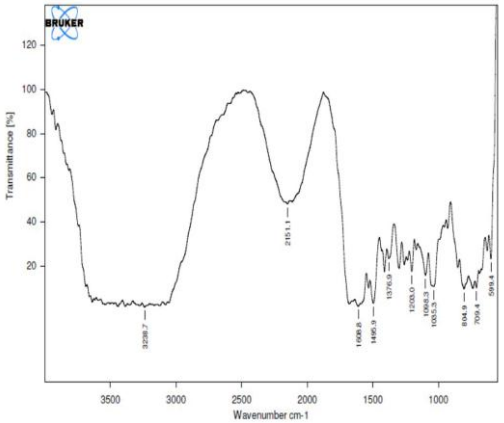

Fig. 11: FTIR of spectra of Lamivudine Nanoparticles.

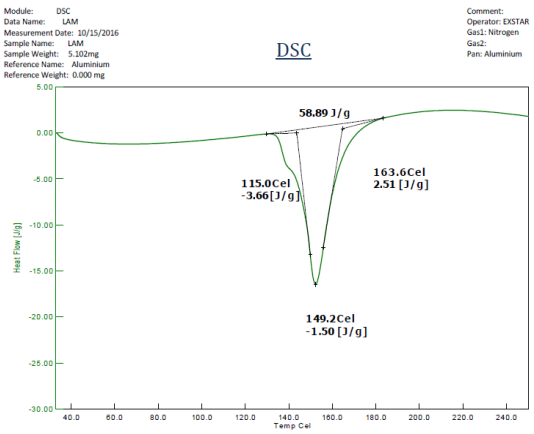

Fig. 12: DSC of Pure Lamivudine.

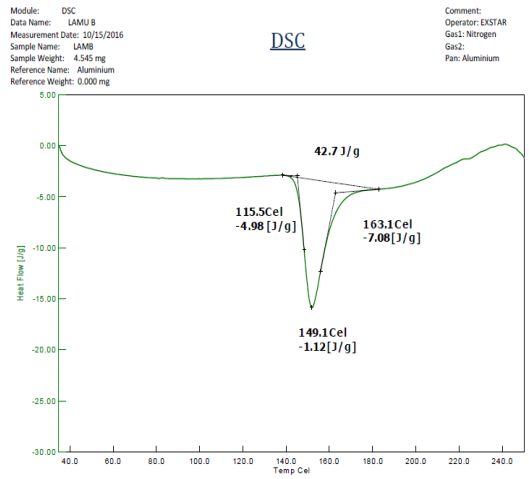

Fig. 13: DSC of Lamivudine NP2 Formulation.

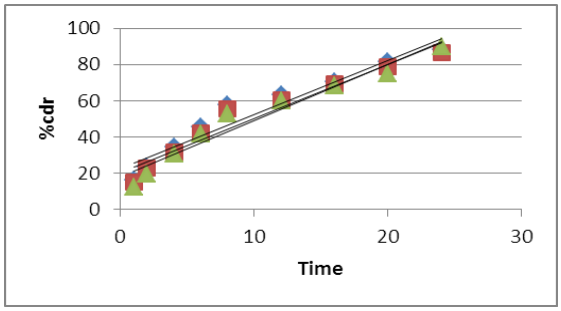

Fig. 14: In vitro drug dissolution profile for optimized NP2, NP8, NP 11 Formulations.

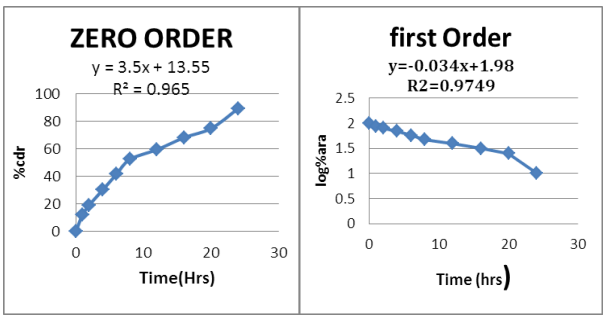

Fig. 15: Drug release kinetics for NP 2 formulation.

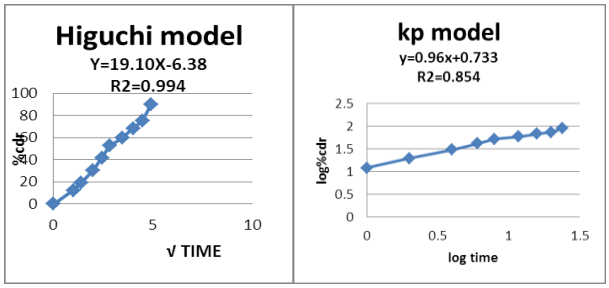

Fig. 16: Model dependent kinetics for optimized formulations.

Formulation	R2(Zero Order)	R2(First Order)	R2(Higuchi model)	R2(KP Model)	N(Release Rate Constant)
NP2	0.965	0.974	0.994	0.854	0.96
NP8	0.952	0.983	0.995	0.995	0.61
NP11	0.959	0.992	0.996	0.828	0.92

CONCLUSION

The method of preparation of lamivudine nanoparticles is found to be simple and reproducible. This method is able to produce discrete, free flowing and uniform sized particles. Among all the formulations, NP2 optimised formulation is selected as the ideal after considering their better drug Entrapment Efficiency and In vitro drug release studies. Particle size analysis showed that the formed particles were in nano size and negative surface charge indicates good stability. All the formulations are able to sustain the drug release for a period of 24 hours. Thus, it may reduce the side effects and improved bioavailability and drug release pattern followed first order and the mechanism is super case II transport, indicates swelling of polymer with diffusion.

ACKNOWLEDGEMENTS

I am very thankful to my guide and co guide for supervising me regarding all the matters of work.

REFERENCES

1. M. Rajaonarivony, C. Vauthier, Development of a new drug carrier made from alginate, J.pharm sciences, September 1993; 82(9): 912–917.
2. Sarmento B, Ribeiro A J, Insulin-loaded nanoparticles are prepared by alginate Ionotropic pre-gelation followed by chitosan polyelectrolyte complexation**,** Journal of nanoscience, Aug 2007; 7(8): 2833-41.
3. Pan Y, Li YJ, Zhao HY, Zheng JM. Bioadhesive polysaccharide in protein delivery system: chitosan

nanoparticles improve the intestinal absorption of insulin *in vivo*. Int. J. Pharm, 2002; 249: 139–147.
4. Mladenovska K, Cruaud O, Richomme P, Belamie E, 5-ASA loaded chitosan-Ca-alginate microparticles: Preparation and physico-chemical characterization. Int. J. Pharm, 2007; 345: 59–69.
5. Dev Ashish, Lamivudine by polylactic acid/chitosan nanoparticles for Anti –HIV drug delivery applications,Carbohydrate Polymers, May 2010; 80: 833-838.
6. Mishra D, Jain N. Glycyrrhizin conjugated chitosan nanoparticles for hepatocyte targeted delivery of lamivudine, Journal of Pharmacy and Pharmacology, Aug 2014; 66(8): 1082-93.
7. Nanoencapsulation of water soluble drug lamivudine using a double emulsion spray drying technique for improving HIV Treatment, Journal of Nanoparticle Research, November 2013.
8. S.Tamizhrasi, A.shukla, Formulation and Evaluation of lamivudine loaded polymethacrylic acid nanoparticles, International Journal of Pharm Tech Research, July 2009; 1(3).
9. Wang bing, Preparation and Cellular uptake of PLGA particles loaded with lamivudine, Chinese Science Bulletin, Nov 2012; 57(31): 3985–3993.
10. E. Ramadan. Transdermal microneedle –mediated delivery of polymeric lamivudine loaded nanoparticles, Journal of Pharmaceutical Technology & Drug Research, 2016; 5(1).