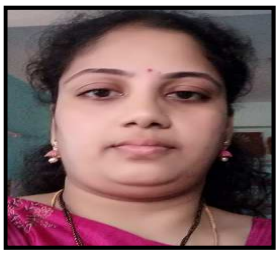
Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com



PREPARATION AND CHARACTERISATION OF LAMIVUDINE AND STAVUDINE LOADED NANOPARTICLES BY NANOPRECIPITATION METHOD

P. Pravalika Reddy*[1], B. Madhava Reddy[2], D. Jaya Prakash[3]

[1*]Department of Pharmaceutical Analysis, Malla Reddy Pharmacy College, Maisammaguda, Secunderabad, Telangana, India.

[2]G. Pulla Reddy College of Pharmacy, Mehdipatnam, Telangana.

[3]Neil Gogte Institute of Technology, Peerzadiguda, Telangana, India.

ABSTRACT

The aim of the present work was to formulate and characterize the lamivudine and stavudine loaded nanoparticles. Nanoparticles of Lamivudine and stavudine were prepared by using nanoprecipitation method using Eudragit RL 100 and Poloxamer 407 as polymers. Nanoparticles were prepared by varying parameters like drug to Polymer ratio, centrifugation time to optimize them. The drug loaded optimized lamivudine nanoparticles showed particle size in the range of 80-90nm. Drug entrapment efficiency ranged from 62.8%-81.4%. *In vitro* release studies revealed that the rate of drug release from NP 1 optimised formulation was found to be 96.88% in 24 hours which can overcome the side effects associated with the conventional dosage forms. Release of drug followed first order and the mechanism is super case II transport, indicates swelling of polymer with diffusion.

KEYWORDS

Lamivudine, Stavudine, Nanoparticles, Nanoprecipitation Method, FTIR and DSC studies.

Author for Correspondence:

Pravalika Reddy P,
Department of Pharmaceutical Analysis,
Malla Reddy Pharmacy College, Maisammaguda,
Secunderabad, Telangana, India.

Email: pravalika.jntu@gmail.com

INTRODUCTON

Lamivudine and stavudine are nucleoside reverse transcriptase inhibitors (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1), phosphorylated intracellularly to its active metabolite. These nucleoside analogues get incorporated into viral DNA by inhibiting HIV reverse transcriptase, and results in DNA chain termination[1]. Conventional drug delivery approaches including high active anti-retroviral therapy have increased the quality of life HIV patients but 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[2].

Nanoparticles are colloidal polymeric particles of size below 1µm with a therapeutic agent either dispersed in polymeric matrix or encapsulated in polymer. Nanodrug delivery systems can alter the pharmacokinetics and pharmacodynamics of drug substance in order to improve the therapeutic efficacy. Nanoparticle formulations were prepared for lamivudine by double emulsion evaporation method and nanoprecipitation Method[3-5] and stavudine nanoparticles by Nanoprecipitation Method[6]. The main objective is to prepare the nanoparticles of Lamivudine with stavudine by a new method by Nanoprecipitation Method by different polymers Eudragit RL 100 and Poloxamer 407 to achieve a higher entrapment efficiency and to prolong the drug release.

MATERIAL AND METHODS

Reagents and chemicals

Lamivudineis obtained as a gift sample from Cipla Pharmaceuticals Ltd, Mumbai and stavudine from Hetero drugs Ltd. Glacial acetic acid is procured from Qualigens Fine Chemicals. Eudragit RL 100 is purchased from Evonik Pharma polymers. Poloxamer 407 from Sigma Aldrich. Distilled water is used throughout the study. Remi Magnetic stirrer is used.

Method

Preparation of Lamivudine and stavudine loaded Eudragit RL 100 nanoparticles

The Eudragit RL 100 nanoparticles containing Lamivudine (150mg), Stavudine (30mg) were prepared through a nanoprecipitation method[7-11] using different drug to polymer ratios (1:1 to 1:5). Lamivudine (150mg) and Stavudine (30mg) was dissolved in 5mL of water. Separately, different amounts of Eudragit RL 100 (180, 360, 540, 720 and 900 mg) were dissolved in 5mL of acetone. The mixture was formed by injecting the drug aqueous solution drop wise into the Eudragit RL 100 organic solution and was magnetically stirred at 500 rpm. This mixture was then added into 10mL of an external aqueous solution under agitation containing 2 % (w/v) of poloxamer 407 as a suspension stabilizer. Thereafter, the mixture was magnetically stirred at room temperature for 1h at a speed of 1200rpm to evaporate the organic solvent.

The nanoparticles were recovered by centrifugation for 45min at 6000rpm and washed by resuspending the nanoparticles in 5mL of deionized water.

Optimization

Nanoparticles were prepared by varying parameters like Drug to Polymer ratio, centrifugation time to optimize their concentrations.

Drug to Polymer ratio

Drug to Polymer ratio was varied from 1:1 to 1:5 by keeping Stirring time period (60 min) and centrifugation time (45 min at 6000rpm) as shown in Table No.1.

Effect of Centrifugation time

Of all the formulations NP1 was considered as optimized based on the Entrapment efficiency, the effect of Centrifugation time was studied i.e., 30, 45and 60 min to get maximum yield as shown in Table No.2.

Preformulation studies

Compatibility of drug with excipients was determined by carrying out FTIR studies and DSC studies.

Fourier Transform Infra-Red Spectroscopy

Compatibility of drug with excipients was determined by carrying out FTIR studies using Fourier Transform Infrared spectrophotometer BRUKER, using KBr Pellet method. KBr pellet obtained was scanned in the range of 400-4000cm^{-1}. The characteristic peaks were recorded for lamivudine and stavudine pure drug and drugs with excipients.

Differential Scanning Calorimetry

The thermal behaviour of lamivudine and nanoparticles containing drug were examined by using DSC 60, Shimadzu. Sample of about 5mg was placed in aluminium pan and analysed at a scanning temperature range from 40 to 240^{0}C at the heating rate of 10^{0}C/min under constant purging of Nitrogen at 20ml/min.

Evaluation of nanoparticles

Nanoparticle formulations prepared were evaluated for Entrapment efficiency and Percentage yield.

Entrapment efficiency

Take 10ml of suspension of nanoparticles and subject it to ultracentrifugation at 10000 rpm for 30min and the amount of free lamivudine in the supernatant was measured by UV spectrophotometer at 270nm by diluted with pH 7.4 Phosphate buffer and the amount of drug entrapped was found by following equation.

$$\% EE = \frac{\text{Total amount of drug - amount of drug in the supernatant}}{\text{Total amount of drug}} \times 100$$

Percentage Yield

It was determined by calculating initial weight of raw materials and final weight of the product. The initial weight is the theoretical mass and dry weight of the obtained nanoparticles is taken as practical mass. The percentage yield is calculated by the given formula.

$$\textbf{Percentage Yield} = \frac{\text{Practical mass of Nanoparticles}}{\text{Theoretical mass (Polymer + Drug)}} \times 100$$

Nanoparticles characterization

The optimised NP1 Formulation was analysed for the Particle Size, zeta potential and surface morphology. Average particle size of the formulated nanoparticles was determined by using Malvern Mastersizer by placing the wet sample in the measurement cell. Particle size was found by the light scattering pattern shown by particles in the sample.

The Zeta potential of the nanoparticles was determined by using a Malvern Zetasizer. The zeta potential is an indirect measure of the surface charge and predicts about the storage stability of colloidal dispersion in which the samples were placed in a disposable cuvettes to find the potential. Surface morphology of lamivudine and stavudine loaded nanoparticles was studied using high resolution Scanning Electron microscopy (SEM).

Compatibility studies

Compatibility of drug with excipients was determined by carrying out FTIR studies and the thermal behaviour of lamivudine and lamivudine with excipients were examined by using DSC 60, Shimadzu.

***In vitro* drug release studies**

In vitro drug release studies were carried out by using dialysis bag method. 10 ml of Lamivudine and stavudine loaded nanoparticles suspension containing 150mg of Lamivudine and 30 mg of stavudine was taken in dialysis bag and placed in 500ml of Phosphate buffer pH 7.4 at 37^0C under continuous magnetic stirring in a dissolution bowl at 50 RPM. At specified time intervals of 2, 4, 8, 12, 16, 20 and 24 hours, 5ml of aliquots were withdrawn from the medium and replaced with equal volume of 7.4 phosphate buffer. The concentration of drug was assayed by UV spectrophotometer at 260nm.

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 and Stavudine nanoparticles

Effect of the variables like Drug to Polymer ratio, centrifugation time on the entrapment efficiency was studied. The results of nanoparticles prepared using various factors are as follows:

Drug to Polymer ratio

Drug to Polymer ratio was varied from 1:1 to 1:5 and the entrapment efficiency of NP1 to NP5 formulations were shown in Table No.3 and depicted in Figure No.1. As the ratio increased, entrapment efficiency decreased.

Centrifugation time

NP1 formulation was considered as optimized based on the Entrapment efficiency, the effect of Centrifugation time was studied on that formulation i.e., 30 45 and 60 min to get maximum yield as shown in Table No.4. As the Centrifugation time increased entrapment efficiency increased up to 45 min and then decreased were shown in Table No.4 and depicted in Figure No.2.

Preformulation Studies

FTIR Analysis

The IR spectrum and Characteristic peaks of lamivudine, Stavudine and Drugs with nanoparticle excipients were shown in Figure No.3. The peaks identified for the pure drug was same when observed with the Nanoparticles as shown in Table No.5 and 6. This indicates that no interaction occurred between the drug and the excipients.

Differential Scanning calorimetry

DSC thermogram of lamivudine, Stavudine and nanoparticles containing drug were shown in Figure No.4. The thermogram of lamivudine and Stavudine pure drug showed a sharp melting peak at 161.2^{o}C and 159.7^{o}C respectively. Lamivudine and stavudine with nanoparticle excipients showed sharp melting peak at 160.5^{o}C and 159.5^{o}C. It indicates that there was no effect of polymers on the thermal behaviour of the drug.

Characterization of drug loaded Nanoparticles

Evaluation of nanoparticles

SEM Analysis

SEM Analysis shows that the Lamivudine-Stavudine loaded nanoparticles prepared has spherical shape as shown in Figure No.5.

Particle Size determination

The Particle Size of optimized Lamivudine Stavudine loaded nanoparticle formulation was shown in Figure No.6. The mean particle size of the optimized formulation was found to be in the range of 80-90nm.

Zeta potential

The zeta potential of -15.8mv shown in Figure No.7 indicates good stability of formulation.

Drug release and Release Kinetics for lamivudine and stavudine loaded nanoparticles

The % cumulative drug release and *in vitro* release pattern of lamivudine and stavudine loaded nanoparticles prepared by nanoprecipitation method for NP1-NP7 were shown in Table No.7 and Figure No.8. It reveals that the rate of drug release from NP1 Formulation was 96.88% after 24 hours and it was considered as the optimized formulation.

Drug Release Kinetics of optimized formulation

The *in vitro* dissolution data for best formulation NP1 were fitted in different kinetic models i.e, zero order, first order, Higuchi and korsemeyer-peppas equation and results were plotted and shown in Figure No.11. The 'n' value was 1.354 for the optimised formulation (NP1) i.e., n value was n > 0.89 this indicates Super case II transport. The release kinetics for the optimized formula was shown in Table No.8.

Stability Studies of NP1 formulation

The optimised formulation of Lamivudine stavudine nanoparticles was subjected to accelerated stability studies for 90 days at temperature and humidity of 40^{o}C±2^{o}C and 75%±5 RH (as per ICH guidelines). The nanoparticles prepared was filled into an amber glass bottle and flushed with nitrogen gas prior to air- tight closure with a plastic cap. The nanoparticles were evaluated for drug release at the end of 30th, 60th, 90th days. The percentage of drug release assessed by UV spectrophotometer was found to be 96.34% after 24 hrs as shown in Table No.9 and Figure No.12. After 90 days, the nanoparticles were evaluated for Particle Size and Zeta Potential shown in Figure No.13 and 14 was found to be in the range of 100-110nm and -19.2mv respectively. It indicates that there was no variation in Particle size and Zeta Potential states the stability of nanoparticles.

Table No.1: Formulation of nanoparticles with different Drug to Polymer ratio

S.No	Code	Drug A(150) B(30mg)	Polymer Eudragit RL 100 (mg)	Drug: Polymer ratio	Polaxomer (ml)
1	NP1	180	180	1:1	10
2	NP2	180	360	1:2	10
3	NP3	180	540	1:3	10
4	NP4	180	720	1:4	10
5	NP5	180	900	1:5	10

Note: A=Lamivudine, B= Stavudine

Table No.2: Effect of Centrifugation time on NP1 Formulation

S.No	Drug A(150) B(30mg)	Polymer Eudragit RL 100 (mg)	Drug: Polymer ratio	Polaxomer (ml)	Stirring time (min)	Centrifugation (RPM)6000
1	180	180	1:1	10	60	30
2	180	180	1:1	10	60	45
3	180	180	1:1	10	60	60

Table No.3: Effect of Drug to Polymer ratio on Entrapment efficiency

S.No	Formulation	Drug to Polymer ratio	Entrapment efficiency
1	NP1	1:1	**81.02**
2	NP2	1:2	71.5
3	NP3	1:3	62.8
4	NP4	1:4	80.6
5	NP5	1:5	81.4

Table No.4: Effect of centrifugation time on Entrapment efficiency

S.No	Centrifugation time	Entrapment efficiency
1	30	80.49
2	45	81.02
3	60	78.22

Table No.5: Interpretation of IR spectrum

S.No	Region(cm^{-1}) for lamivudine	Region(cm^{-1}) For Stavudine	Type of vibration
1	3798.87	3693.51	-OH- Stretching
2	2950.29	2815.80	-C-H Stretching
4	3589.33	3422.43	-NH-Stretching
5	1740.94	1681.76	-C=O Stretching
6	1055.81	1070.03	C-O-C–Stretching

Table No.6: Interpretation of IR spectrum of Lamivudine and Stavudine with nanoparticle excipients

S.No	Region in cm^{-1}	Type of vibration
1	3685.04	-OH- stretching
2	2884.07	-C-H stretching
3	3352.20	-NH-stretching
4	1767.53	-C=O Stretching
5	1008.60	C-O-C–Stretching

Table No.7: Percentage cumulative drug release for lamivudine-stavudine loaded nanoparticles

S.No	Time (hrs)	Cumulative % drug release ± 3						
		NP1	NP2	NP3	NP4	NP5	NP6	NP7
1	0	0	0	0	0	0	0	0
2	2	5.69±0.88	2.05±0.22	1.68±0.31	1.22±0.29	3.65±0.36	3.12±0.26	3.58±0.41
3	4	16.34±0.16	15.69±0.83	10.08±0.65	9.84±0.77	12.69±0.55	8.84±0.63	18.26±0.85
4	8	26.84±0.54	23.24±1.44	16.85±1.02	15.62±1.16	24.58±0.91	19.22±0.85	38.62±1.25
5	12	42.68±0.95	35.08±1.58	35.78±1.24	28.64±1.43	35.64±1.06	38.10±0.90	51.89±1.16
6	16	78.35±0.84	69.16±1.78	62.94±1.82	38.04±1.51	46.18±1.44	66.35±1.06	84.62±1.9
7	20	86.22±0.24	78.30±1.24	71.84±1.96	53.98±1.81	52.68±1.62	82.64±1.12	98.92±0.48
8	24	96.88±0.32	89.13±1.26	80.64±1.02	69.37±1.73	73.86±1.19	90.16±1.64	98.93±0.36

Table No.8: Model dependent kinetics for optimized formulation

S.No	Formulation	Zero order R^2	First order R^2	Higuchi R^2	Korsmeyer – Peppas R^2	Korsmeyer-Peppas (n)
1	NP1	0.976	0.876	0.887	0.966	1.354

Table No.9: Percentage cumulative drug release data of NP1 formulation during Stability studies

S.No	Time (hrs)	0	1 Month	2 Month	3 Month
1	0	0	0	0	0
2	2	5.69±0.88	5.69±0.10	5.62±0.14	5.60±0.62
3	4	16.34±0.16	16.32±0.22	16.28±0.25	16.22±0.85
4	8	26.84±0.54	26.83±0.64	26.81±0.36	26.80±0.61
5	12	42.68±0.95	45.12±0.18	42.18±0.14	42.5±0.05
6	16	78.35±0.84	78.32±0.55	78.22±0.22	77.96±0.24
7	20	86.22±0.24	88.22±0.65	86.08±0.48	85.86±0.35
8	24	96.88±0.32	96.86±0.69	96.8±0.94	96.34±0.41

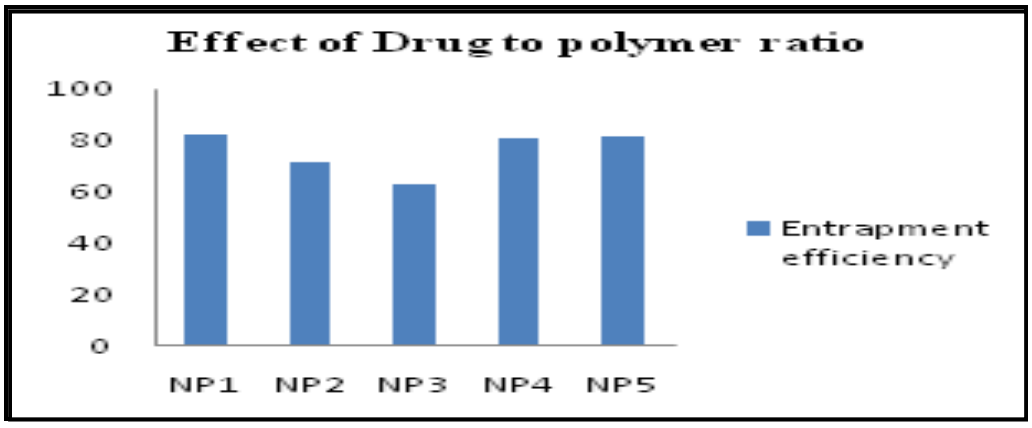


Figure No.1: Effect of Drug to Polymer ratio on Entrapment efficiency

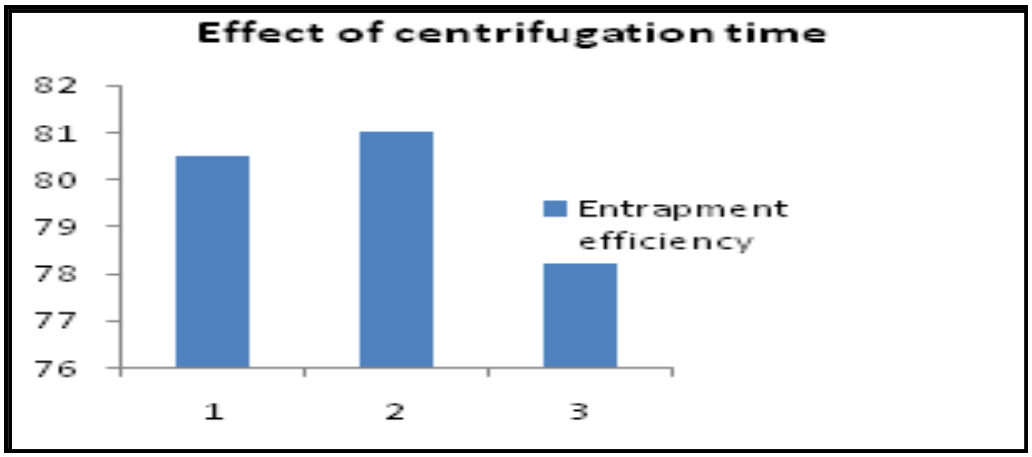
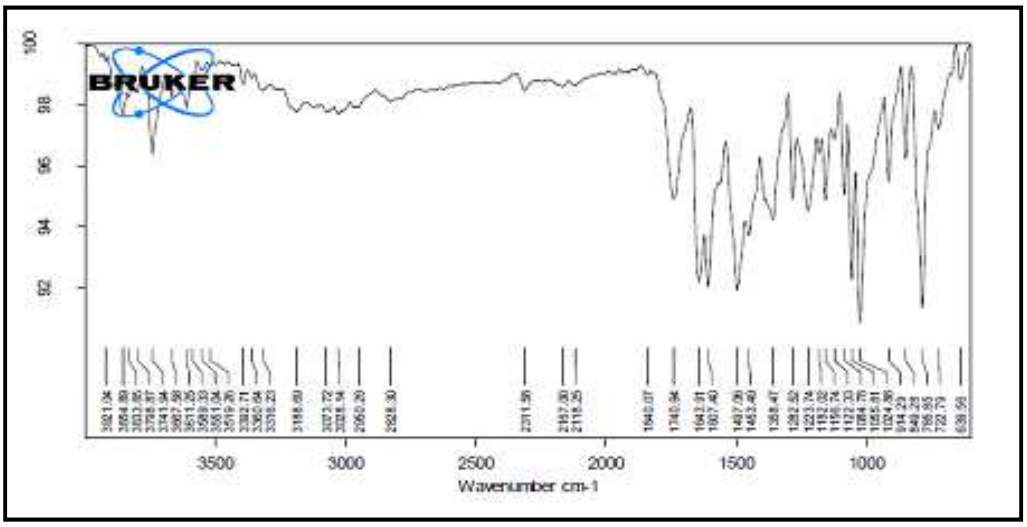
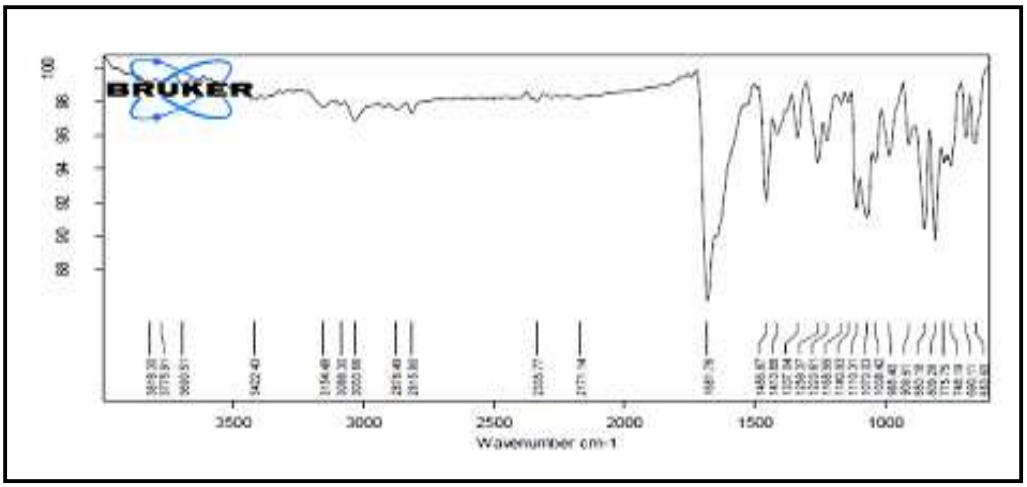


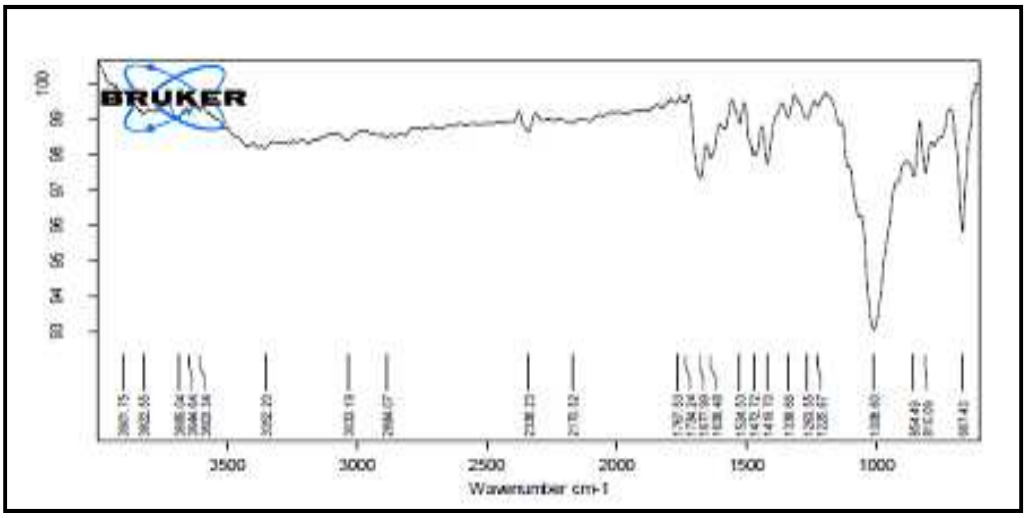
Figure No.2: Effect of centrifugation time on Entrapment efficiency



a)

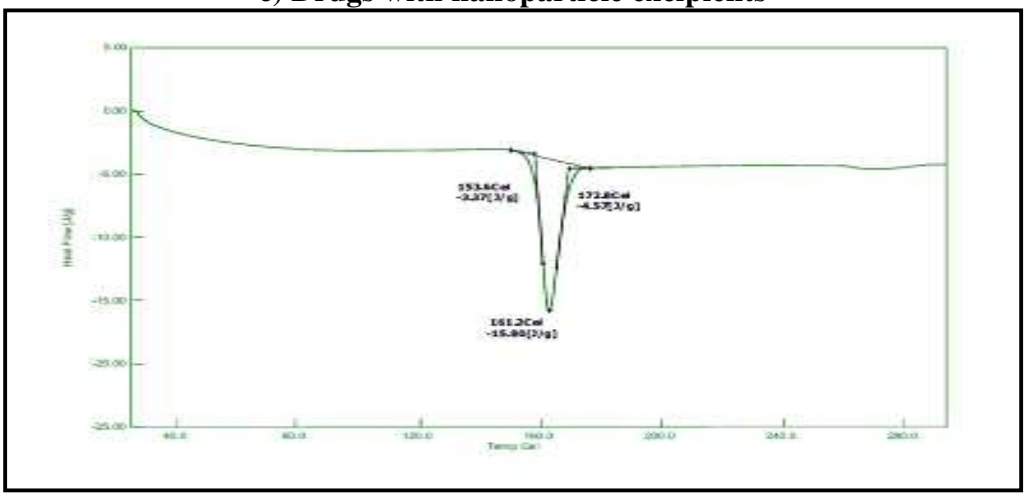


b)

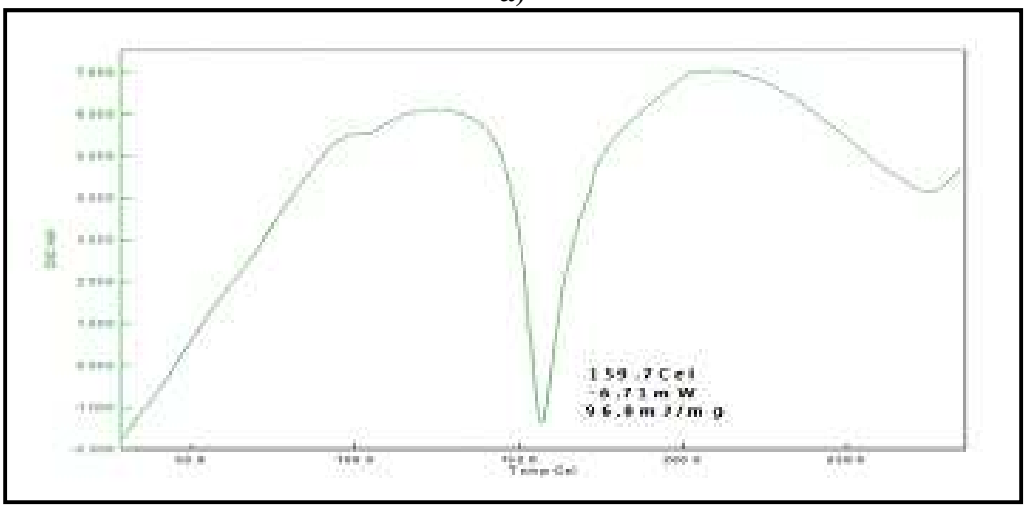


c)

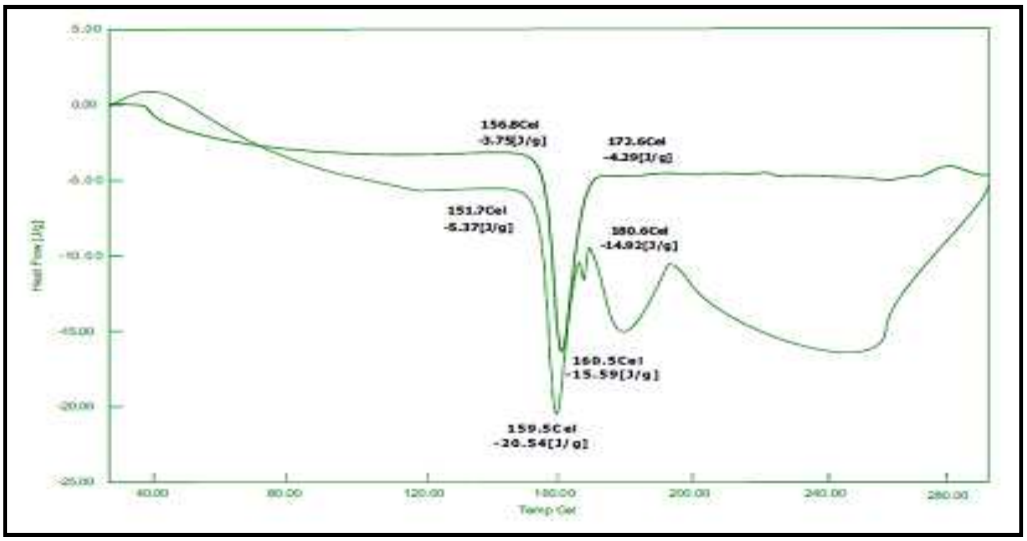
Figure No.3: FTIR spectra of a) pure Lamivudine b) pure Stavudine c) Drugs with nanoparticle excipients



a)



b)



c)

Figure No.4: DSC of a) Lamivudine b) Stavudine c) Drugs with nanoparticle excipients

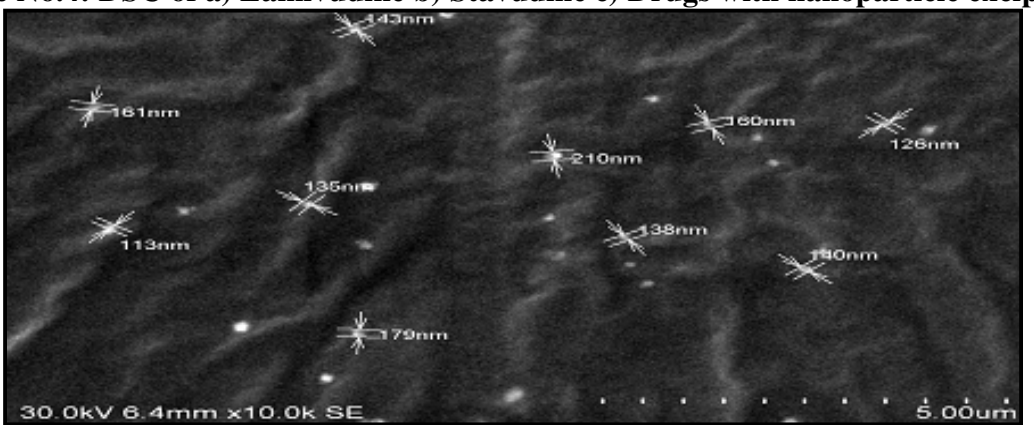


Figure No.5: SEM analysis of NP1 formulation

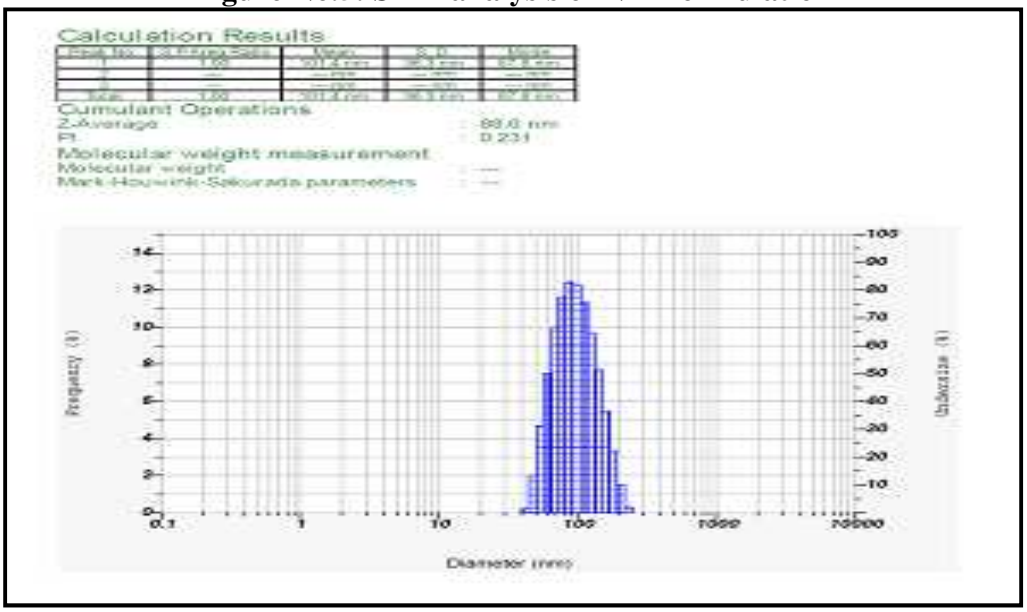


Figure No.6: Particle size of NP1 formulation

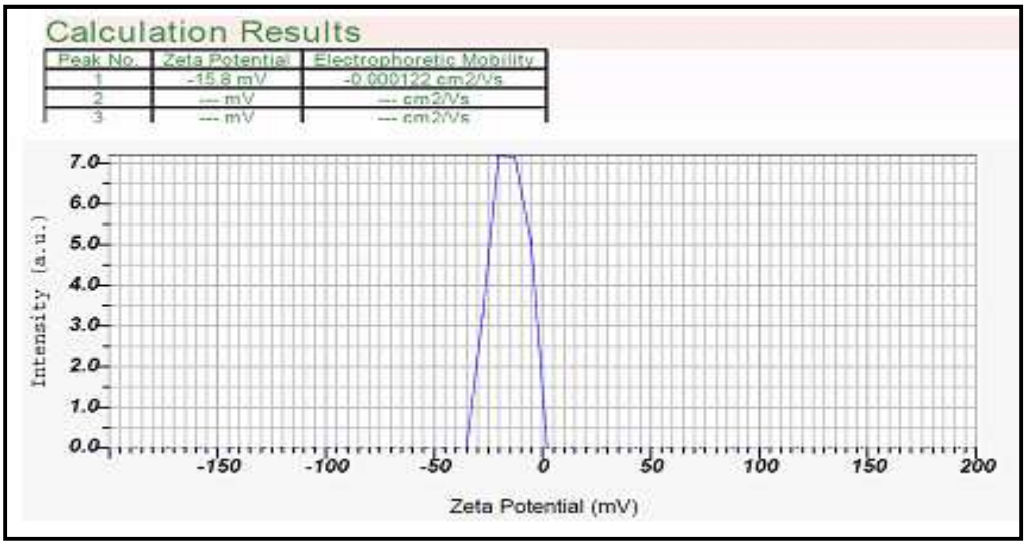


Figure No.7: Zeta potential of NP1 formulation

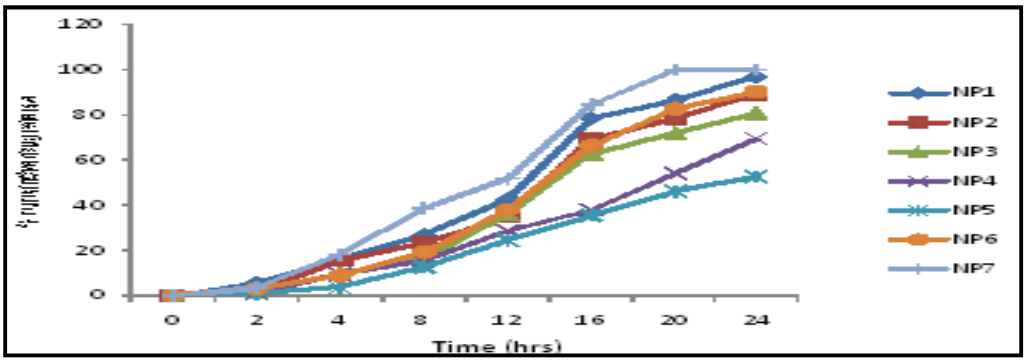


Figure No.9: *In vitro* dissolution studies of Nanoparticles NP1 to NP5

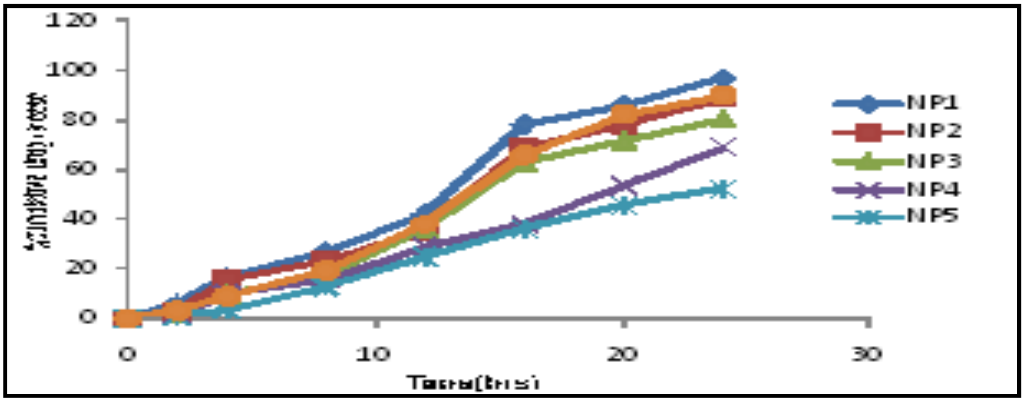


Figure No.9: *In vitro* dissolution studies of Nanoparticles NP1 to NP5

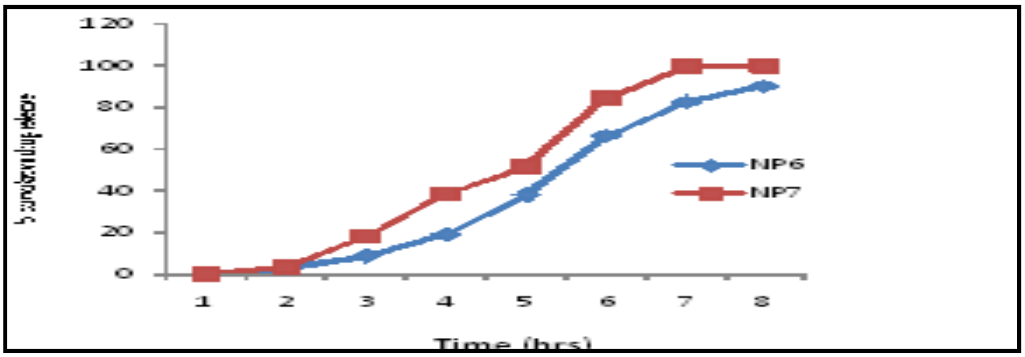
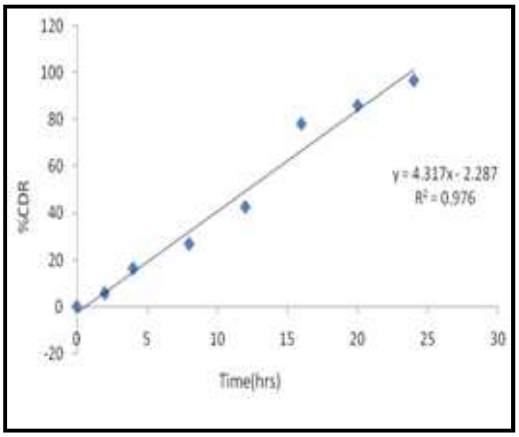
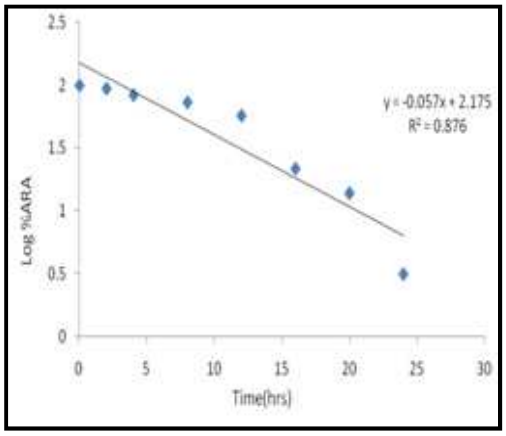


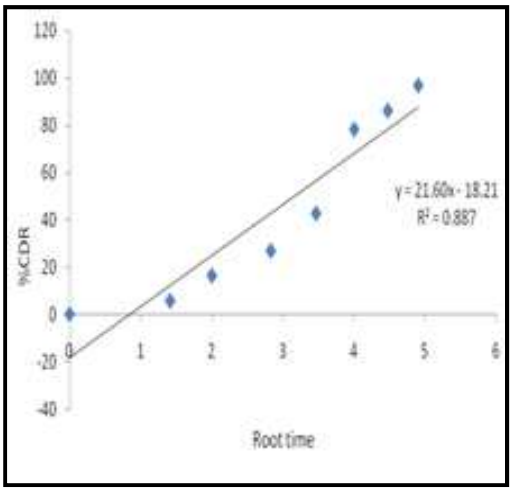
Figure No.10: *In vitro* dissolution studies of Nanoparticles NP6 and NP7



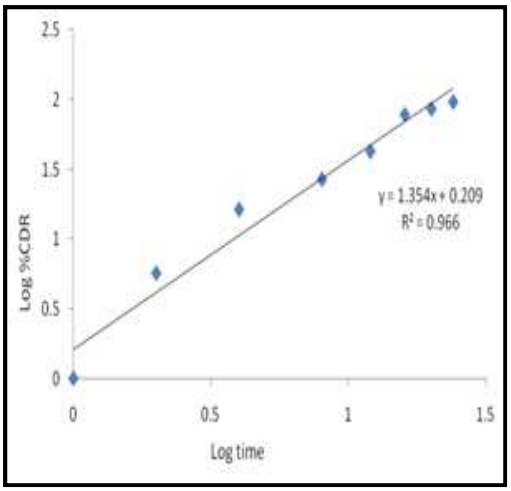
Zero order



First order



Higuchi plot



Peppas plot

Figure No.11: Drug release kinetics for the Nanoparticles optimized formulation NP1

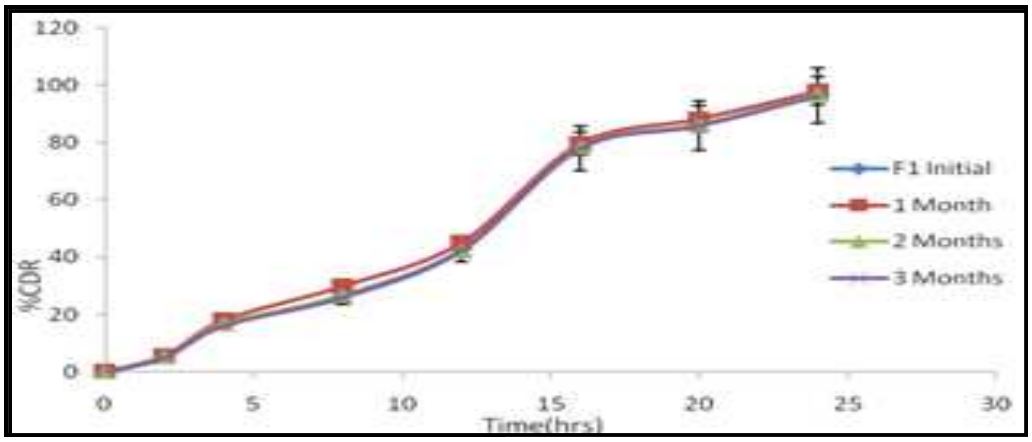


Figure No.12: Drug release profile of F1 formulation during Stability studies

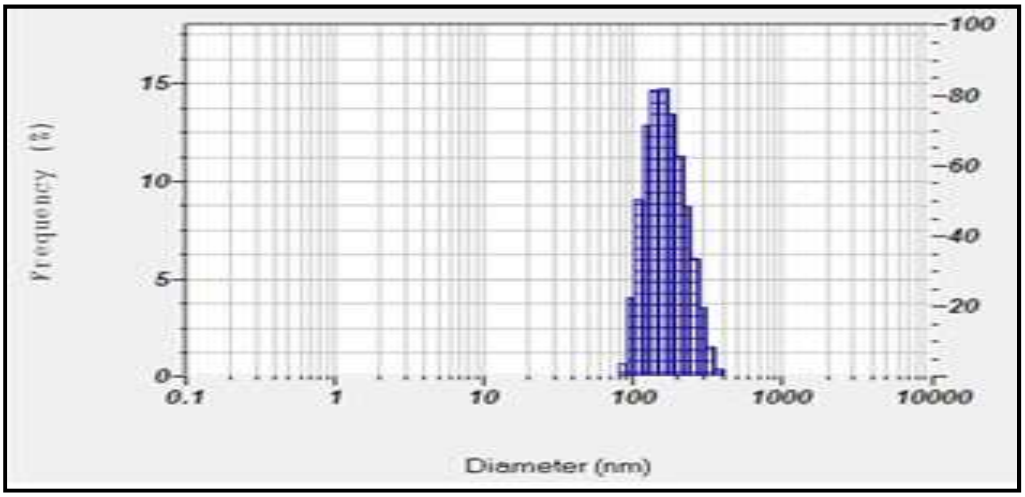


Figure No.13: Particle size of NP1 after Stability studies

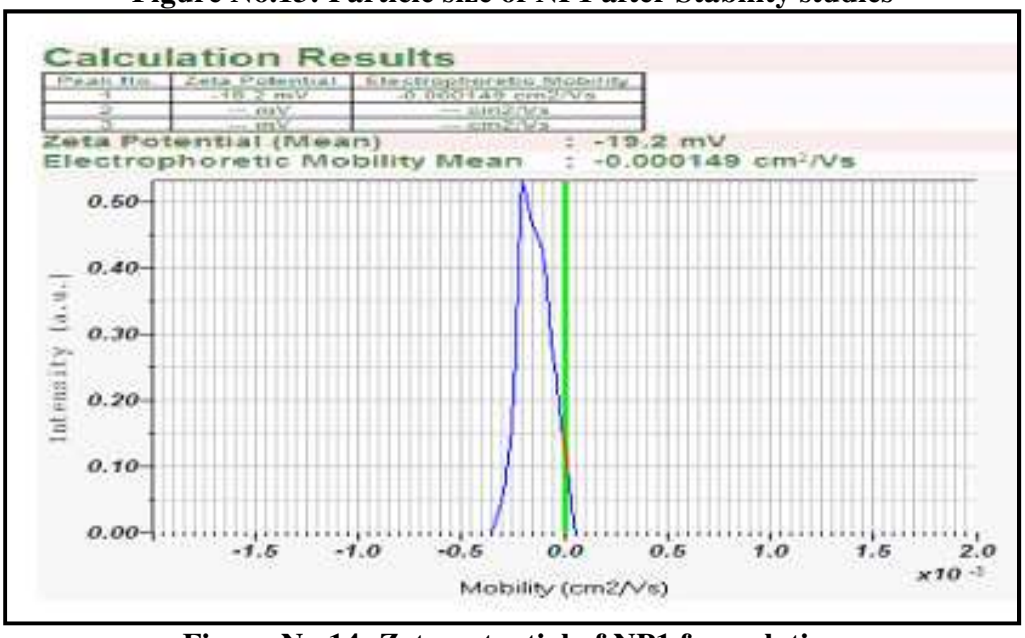


Figure No.14: Zeta potential of NP1 formulation

CONCLUSION

The method of preparation of lamivudine stavudine loaded nanoparticles was found to produce spherical, free flowing and uniform sized particles. Among all the formulations, NP1 optimised formulation was considered as optimum because of 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. The optimised formulation was able to sustain the drug release for a period of 24 hours. The drug release pattern followed zero order for best formulation NP1 and the 'n' value was 1.354 for the optimised formulation (NP1) i.e., value was n > 1.354, this indicates Super case II transport.

ACKNOWLEDGEMENT

I am very thankful to my guide and coguide for supervising me regarding all the matters of work. I would also like to thank the Management, for providing the necessary facilities to carry out this work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Shanmugam S, Kamaraj K, Vetrichelvan T. Formulation and evaluation of lamivudine sustained release matrix tablets using synthetic polymers, *Journal of Pharmacy Research*, 5(2), 2012, 1063-1066.
2. Bhagwat R R, Vaidhya I S. Novel Drug Delivery Systems: An Overview, *International Journal of Pharmaceutical Sciences and Research*, 4(3), 2013, 970-982.
3. Ramadan E. Transdermal microneedle-mediated delivery of polymeric lamivudine-loaded nanoparticles, *Journal of Pharmaceutical Technology and Drug Research*, 5, 2016, 1-8.
4. Bing Wang, Quan Chen. Preparation and Cellular uptake of PLGA particles loaded with lamivudine, *Chinese science bulletin*, 57(31), 2012, 3985-3993.
5. Tamizhrasi S, Shukla A. Formulation and Evaluation of lamivudine loaded polymethacrylic acid nanoparticles, *International Journal of Pharm Tech Research*, 1(3), 2009, 411-415.
6. Kartikeyan D, Srinivas M. Formulation and Evaluation of stavudine Nanoparticles, *International Journal of Novel trends in Pharmaceutical Sciences*, 3(1), 2013, 1-6.
7. Fessi H, Puisieux F. Nanocapsule formation by interfacial polymer deposition following solvent displacement, *International Journal of Pharmaceutics*, 55(1), 1989, R1-R4.
8. Sara Salatin, Jaleh Barar. Development of a nanoprecipitation method for the entrapment of a very water soluble drug into Eudragit RL nanoparticles, *Research in Pharmaceutical Sciences*, 12(1), 2017, 1-14.
9. Das S, Suresh P K. Design of Eudragit RL 100 nanoparticles by nanoprecipitation method for ocular drug delivery, *Nanomedicine,* 6(2), 2010, 318-23.
10. Gabriella Torres, Flores. Preparation of Fenofibrate loaded Eudragit L100 nanoparticles by nanoprecipitation method, *Science Direct*, 13(Part 2), 2019, 428-435.
11. Kefilwe Matlhola, Lebogang Katata Seru. Formulation and Optimization of Eudragit RS PO-Tenofovir Nanocarriers Using Box-Behnken Experimental Design, *Journal of Nanomater,* Article ID 630690, 2015, 1-11.

Please cite this article in press as: Pravalika Reddy P *et al.* Preparation and characterization of lamivudine and stavudine loaded nanoparticles by nanoprecipitation method, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 7(4), 2019, 986-998.