DEVELOPMENT TECHNOLOGY AND EVALUATION OF STAVUDINE NANOPARTICLES

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ABSTRACT

In Novel Drug Delivery, Nanoparticles provides a promising drug delivery system of controlled and targeted drug release. Nanoparticles are specially designed to release the drug in the vicinity of targeted tissue. Polymeric nanoparticles have been considered as promising drug delivery systems for variety of drugs like anticancer agents, biological macromolecules and vaccines. Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing the side effects. Nanoparticles mediated targeting plays an important role in inhibiting inflammation, angiogenesis and tumor progression. Especially polymeric nanoparticles have greater deal that provides numerous properties such as simple to synthesize, inexpensive, biocompatible, biodegradable, non-toxic, non-immunogenic and water soluble for an effective drug delivery and drug targeting. The main applications of nanotechnology in medicine are materials and devices for diagnosis and for drug delivery. The aim of this study is to formulate the Stavudine loaded nanoparticles of chitosan, cross linked with Tween 80 for antiretroviral therapy, in order to enhance the bioavailability and to reduce the dose frequency. Formulations of Stavudine loaded nanoparticle were prepared by double emulsion solvent evaporation and solvent diffusion methods. Fourier transmission infrared spectroscopy studies indicated no chemical interaction between drug and polymer. In vitro release studies were performed by the dialysis membrane method. All the drug loaded batches were followed first order and sustained drug release over a period of 20 hrs.

Keywords: *Stavudine, Nanoparticles, Double emulsion solvent evaporation and Solvent diffusion.*

INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with size in rangeof 10-1000 nm in which drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix[1]. Polymeric nanoparticles with a size in the nanometer range protect drugs against *in vitro* and *in vivo* degradation.It releases the drug in a controlled manner andalso offers the possibility of drug targeting[2-3]. The use of polymeric drug nanoparticles is a universal approach to increase the therapeutic performance of poorly soluble drugs in any route of administration. There are many methods were there to prepare nanoparticles includes emulsification-solvent diffusion, solvent diffusion, emulsion evaporation, nanoprecipitation method, salting out method, polymerization method, emulsion polymerization[4-8]. The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of drug at therapeutically optimal rate and dose regimen[9-10].

Stavudine is a synthetic nucleoside analogue which acts as a reverse transcriptase inhibitor. Stavudine is used for the treatment ofChronic Hepatitis and Human immunodeficiency Virus (HIV) infections with a half-life of nearly 5-7 hours. Conventional formulations of Stavudine 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[11-12].

So that there is a need to develop

Stavudine nanoparticles to control the drug release. The objective of the present study was to prepare nanoparticles of Stavudine to overcome some of these problems. Hence, formulated nanoparticles containing Stavudine by emulsion followed by solvent evaporation method and evaluate its physicochemical characteristics such as particle size, shape, zeta potential, drug loading capacity and *in vitro* release characteristics.

MATERIALS AND METHODS

Stavudine was obtained as gift sample from Hetero Labs, Hyderabad. Polymers like Hydroxy propyl methyl Cellulose (HPMC K4M), Glyceryl monostearate and Ethyl cellulose were purchased from AR Chemicals, Hyderabad. Dichloromethane, Methanol, Sodium Lauryl sulphate and other chemicals were purchased from SD. Fine Chemicals Ltd. Mumbai, India. All other chemicals used were of analytical grade.

METHODOLOGY

Formulation of Stavudine nanoparticles[4-8]

Stavudine nanospheres were prepared by using emulsion followed by solvent evaporation technique as an effective technology in preparation of nanodrugs. Polymers dissolved in chloroform then 10 mg of drug of Stavudine was completely dispersed in polymer solution and 1% SLS solution add to this under stirring at 400-500 rpm up to 20 min then beaker placed into probe sonicator for 15 min after sonication kept for continuous stirring by magnetic stirrer and temperature was maintained at 10˚C by using ice bath. Nanoparticles occurred immediately upon mixing.

Table 1: Formulation of Stavudine Nanoparticles

Ingredients	Formulation code								
	LF1	LF2	LF3	LF4	LF5	LF6	LF7	LF8	LF9
Stavudine	30	30	30	30	30	30	30	30	30
(mg)	0	0	0	0	0	0	0	0	0
HPMC K4M (mg)	75	150	225	-	-	-	75	150	225
Glyceryl monostearate(mg)	-	-	-	75	150	225	75	150	225
Ethyl cellulose (mg)	75	150	225	75	150	225	-	-	-
Dichloromethane (ml)	10	10	10	10	10	10	10	10	10
Methanol (ml)	10	10	10	10	10	10	10	10	10
1% SLS (ml)	50	50	50	50	50	50	50	50	50

CHARACTERIZATION OF NANOPARTICLES

Fourier Transform Infra-Red spectroscopy(FT-IR) analysis[13]

The FT-IR spectra of pure Stavudine and nanoparticles loaded with Stavudine were recorded using PERKIN ELMER FT-I Insf. USA. The samples were scanned from 4000 to 400 cm^{-1} in FT-IR spectrophotometer. Similarly, the IR spectra of all the individual drug and prepared nanoparticles were also recorded. Physical appearance of samples & appearance or disappearances of peaks in the spectra were observed to access any possible physical and chemical interaction between the drug and polymers.

Differential Scanning Calorimetry (DSC)measurement[14]

The thermal properties of lyophilized powder samples were investigated with a DSC-41 apparatus (Shimadzu, Japan). The scanning temperature for each lyophilized powder sample was set from 25 to 200˚C with a heating rate of 10˚C/min. 10 mg of each sample was analyzed in an open aluminum pan and magnesia was used as reference. In order to evaluate the internal structure modifications after nanosizing process,thermal analysis was performed on Stavudine & excipients.

Scanning Electron Microscopy (SEM)[15]
Scanning electron microscopy was used to characterize the particle morphology of the

unprocessed drug as well as the fabricated drug nanoparticles. A small fraction of eachdrug powder sample was fixed on a double-sided conductive carbon tape and sputter-coated with 5 nanometers of a Pt–Pd alloy.Micrographs were obtained on a Zeiss DSM 982 Field Emission Gun Scanning ElectronMicroscope (Carl Zeiss AG, Germany).

Particle size, Particle Size distribution[16]

The size of drug nanoparticles was measured immediately after precipitation by dynamic lioaser light scattering (Nanoparticle size analyzer, Malvern). Before analysis, the drug suspension was diluted by purified water to 0.2 mg/ml. Graphic mean size (Mz) & calculated surface area (Cs) were used to interpret the results of particle size analysis.

The morphology of prepared Stavudine nanoparticles was spherical structures as resolute by using scanning electron microscope (SEM). The surfaces of the particles were rough and rounded. It was reported that, when ratio of polymer was increased, the relative sizes of the pores also lean to increase Zeta potential[17]

Zeta potential is an abbreviation for electrokinetic potential in colloidal systems. Zeta potential is electric potential in theinterfacial Double Layer (DL) at the location of the slipping plane versus a point in the bulk fluid away from the interface. The surface charge (Zeta potential) was determined by measuring the electrophoretic mobility of the nanoparticles using a Malvern zeta sizer (Malvern instrument, UK). Samples were prepared by diluting with distilled water.

Assay

Weigh accurately about 0.3 g of Stavudine, (fabricated nano crystals), dissolve in exact 40 ml of methanol, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Stavudine, when dried, contains not less than 99.0% and not more than 101.0% of Stavudine.

Entrapment efficiency[18]

For the determination of encapsulation efficiency accurately weighed NPs (10 mg) were added to 10 ml of distilled water and afterthe equilibrium solubility was attained, clear supernatant after centrifugation was filtered and 1 ml of the filtrate was mixed with 4 ml of methanolic HCl. Resulting sample was analyzed on UV visible spectrophotometer at 275 nm. The encapsulation efficiency wasdetermined by using the following formula Encapsulation efficiency (%) =[1-(Drug in supernatant liquid / Total drugadded)] x 100.

***In Vitro* Dissolution Test[19]**

In vitro release of Stavudine nanoparticles was conducted by a dialysis membrane havingpore size of 2.4 mm with 75 ml of pH 6.8phosphate buffer at 37°C. Briefly in a 100 ml beaker 75 ml of pH 6.8 phosphate buffer was taken. A 2 ml of formulation was taken into a dialysis bag and dipped into the buffer solution. The dialysis membrane was activated prior using by soaking in 1% w/v NaOH overnight. The flask was kept on a magnetic stirrer. Stirring was maintained at 50 rpm and the temperature of the buffer was maintainedat 37°C. Sampling was done by withdrawing 5 ml of aliquots from a beaker. Immediately 5 ml of fresh buffer was added to maintain the sink condition. Samples were analyzed after adequately diluting with methanol by using a UV-Visible Spectrophotometer at a wave length of 275 nm.

Drug release kinetics -model fitting of the release data[20]

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.

EVALUATION OF NANOPARTICLES

Table 2: Different evaluation parameters of nanoparticle formulation

Formulation Code	Particle size (nm)	% Yield	Entrapment efficiency (%)	% Drug content
LF1	200.5	88.5	77.8	85.21
LF2	210.2	60.7	67.5	86.87
LF3	246.7	75.5	77.6	81.56
LF4	198.2	86.2	75.2	79.68
LF5	205.3	67.5	60.2	74.69
LF6	226.7	79.8	71.8	81.23
LF7	197.2	78.8	77.4	84.57
LF8	220.2	84.2	83.4	77.63
LF9	245.3	86.5	85.2	81.69

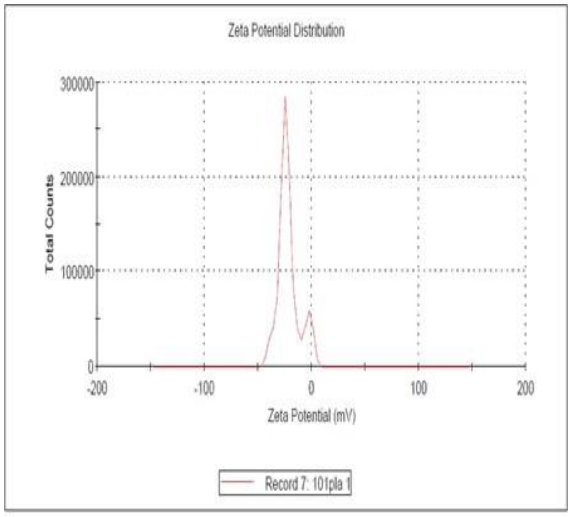

Fig. 1: Zeta potential analysis of formulation LF

Table 3: *In vitro* dissolution study

Time (hr)	% Drug release								
	LF1	LF2	LF3	LF4	LF5	LF6	LF7	LF8	LF9
1	15.2±0.21	12.5±0.31	10.8±0.42	20.8±0.12	17.8±0.33	15.2±0.26	25.4±0.23	22.8±0.16	9.5±0.41
2	38.9±0.53	25.9±0.29	21.6±0.23	28.9±0.54	25.9±0.38	21.8±0.19	38.9±0.61	30.2±0.43	17.8±0.32
4	46.4±0.41	34.5±0.22	30.8±0.22	35.4±0.31	31.8±0.44	29.6±0.43	46.2±0.41	42.7±0.25	28.3±0.79
6	54.8±0.11	46.4±0.64	42.7±0.41	48.9±0.16	43.6±0.79	40.9±0.36	54.8±	51.8±0.38	39.4±0.61
8	68.9±0.78	58.5±0.55	55.8±0.31	56.1±0.13	50.7±0.25	49.4±0.35	61.7±0.18	59.7±0.44	47.5±0.45
10	82.5±0.39	67.5±0.53	63.7±0.13	69.8±0.34	59.8±0.43	56.8±0.42	76.8±0.50	71.5±0.60	54.5±0.53
12	96.7±0.14	85.4±0.26	81.6±0.52	84.7±0.25	76.8±0.15	71.2±0.67	89.5±0.28	85.3±0.54	67.6±0.61
14	-	94.8±0.71	90.8±0.63	96.8±0.11	87.8±0.09	83.5±0.17	97.6±0.17	94.5±0.46	79.7±0.28
16	-	-	96.2±0.71	-	95.5±0.43	93.7±0.24	-	97.8±0.45	87.2±0.36
20	-	-	-	-	-	-	-	-	98.9±0.76

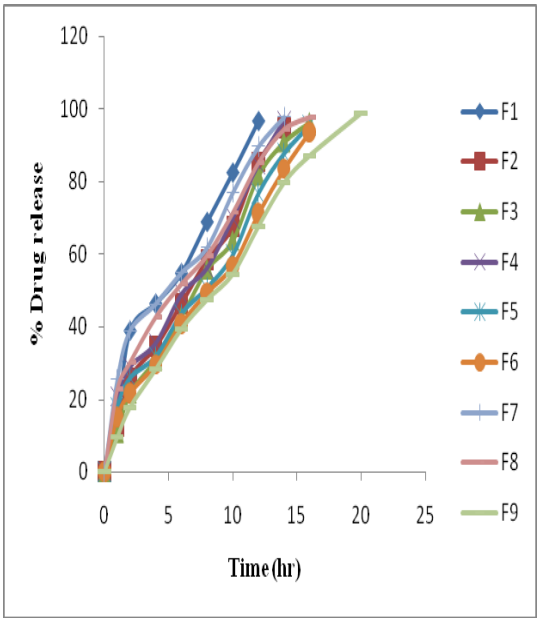

Fig. 2: *In-vitro* drug release studies of Stavudine

FT-IR Spectra for Stavudine Drug–excipient compatibility studies (FT–IR)

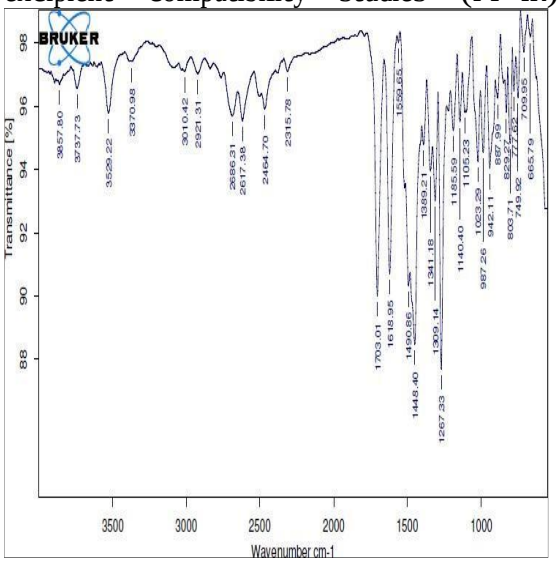

Fig. 3: FT-IR Spectra for Stavudine

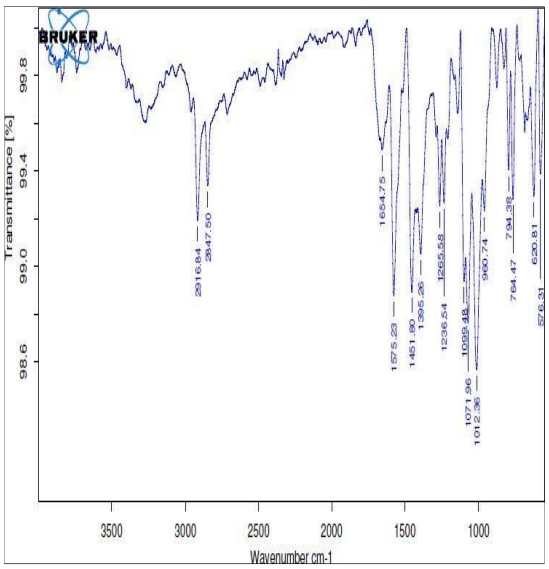

Fig. 4: FT-IR Spectra for Stavudine optimized formulation

Kinetic analysis of dissolution data

To analyze the drug release mechanism the *in-vitro* release data was fitted into various release equations and kinetic models zero order, first order, Higuchi and Korsmeyer-Peppas model. The release kinetics of optimized formulation is shown in Table 4.

Table 4: Kinetic Analysis of Optimized formulation

Formulation code	Zero order	First order	Higuchi	Peppas	
	R^2	R^2	R^2	R^2	n
LF9	0.99	0.8	0.96	0.99	0.8

Stability studies

There was no significant change in physical and chemical properties of the nanoparticles of formulation LF-9 after 3 months.

Table 5: Stability studies of optimized formulation

S. No.	Parameters	Initial	1 month	2 month	3 month	Limits as per specification
1	40^0C/ 75% RH % Release	98.9	98.52	97.79	96.56	Not less than 85 %
2	40^0C/ 75% RH Assay Value	98.9	97.96	96.22	96.00	Not less than 90 % Not more than 110 %

RESULTS AND DISCUSSION

The present investigation was under taken to formulate and evaluate Stavudine nanoparticles. A total of 9 formulations LF1 to LF9, based on the varied concentrations of drug carrier forming polymers namely, HPMC K4M, Glyceryl monostearate and Ethyl cellulose were chosen for the study. Stavudine nanospheres were prepared by using emulsion followed by solvent evaporation technique. It was found to be an effective technology in preparation of nanoparticles.

The FTIR study prior to the formulation of nanoparticles revealed no drug-excipient incompatibilities. The drug excipient compatibility was confirmed further with thehelp of DSC. There was no significant interaction and internal structure modifications were observed after the DSC studies. The average particle size in formulations LF1 toLF9 was found to be in the range of 197.2 to 246.7 nm.

The % yield of nanoparticles obtained by this method of formulation was found to be least in case of LF2 with 60.7% whereas, higher yieldswere found for LF1, LF4, LF9 (i.e., 88.5, 86.2, 86.5%). Entrapment efficiency (%) was found highest for LF9 with 85.2%. The entrapment efficiency was found to be affected by increasing conc. of the polymer. The drug content for all formulations was found to be in the range of 74.69 to 86.87%. Optimized formulation (LF9) has shown -29.6 (Negative Zeta Potential) which indicates that it has excellent stability.

The *in vitro* release profile of all formulation is shown in Fig. 2. The release of Stavudine mainly depended upon the polymer concentration. The burst release of Stavudine from nanoparticles at initial stage resulted from the dissolution of drug crystals on the surface of nanoparticles. On increasing polymer concentration, the release rate of Stavudine from nanoparticles decreased drastically. 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 nanoparticles was best explained by zero order kinetics for best formulation LF9 as the plots shows highest linearity. The correlation coefficient (r^2)was found 0.99 for LF9. There was nosignificant change in physical and chemical properties of the nanoparticles of formulation LF-9 after 3 Months.

CONCLUSION:

Success of the *in vitro* drug release studies recommends product for further *in vivo* studies, which may improve patient compliance. From the results, formulation LF9 containing Stavudine nanoparticles using combination of polymers evolved as the optimized formulation and it releases more than 98.9% drug in 20 hrs. FT-IR spectroscopic studies indicated that there is no drug-excipient interaction in optimized formulation. The optimized formulation LF9 can be considered as a promising sustained drug delivery system of Stavudine nanoparticles providing nearly zero order drug release over a period of 20 hrs.

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