ABACAVIR LOADED NANOPARTICLES: PREPARATION, PHYSICOCHEMICAL CHARACTERIZATION AND IN VITRO EVALUATION

ABSTRACT:

The present study deals with the formulation and evaluation of Abacavir nanoparticles. Abacavir is an antiretroviral drug, it is used in treatment of AIDS. Abacavir nanoparticles were formulated by solvent displacement method using Eudragit RL-100, chitosan and Poloxamer-188. Nanoparticles were characterized by determining its particle size, drug entrapment efficiency, particle morphological character and in-vitro drug release. Particle size range of nanoparticles was in the range of 121.4-140.6 nm. Zetapotential of formulations were determined, and it was found in range of 16.5-20.45 mv. The in-vitro release of nanoparticles were carried out which exhibited a sustained release of Abacavir from nanoparticles up to 10 hrs. The study concludes that nanoparticles can be a promising drug delivery system for sustained release of Abacavir in terms of increased bioavailability.

Keywords: Abacavir, nanoparticles, solvent displacement method, Zeta potential, entrapment efficiency, in-vitro release.

INTRODUCTION:

Nanoparticles are promising drug delivery systems of controlledand targeted drug release. Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. They possess unique properties like small size, high surface area, and ease of suspending in liquids, deep access to cells and organelles, variable optical and magnetic properties are offered by nanoparticles[1]. Their advantages includes increased bioavailability, site specific drug delivery, sustained release of drug over longer period of time, retention of dosage form in entire length of gastrointestinal tract and convenient to patient due to reduction in dosing frequency[2].

Abacavir is a nucleoside analog reverse transcriptase inhibitor (NRTI), antiretroviral drug, it is used in treatment of AIDS. It is used together with other HIV medications, and is not recommended by itself[3,4,5].

MATERIAL AND METHOD:

Eudragit RL-100 was obtained from Neimeth, and chitosan from Emzor Pharmaceuticals. All other chemicals used were of analytical grade.

Preparation of Abacavir nanoparticles-

Abacavir nanoparticles were prepared by the solvent displacement method. Drug and various proportion of polymers i.e. Eudragit RL-100, and chitosan were dissolved in acetone. This solution was poured drop wise into solution of poloxamer 188 with magnetic stirring at room temperature. Nanoparticles were spontaneously formed and turnedthe solution slightly turbid then; acetone was removed by continuous stirring at 35-40°C. Theprepared suspension was centrifuged, supernatant was removed and the sediment was freeze dried for further analysis[6].

Table 1: Composition of different Abacavir nanoparticles

Formulation code	Eudragit RL-100 (mg)	Chitosan (mg)	Water (ml)	Acetone (ml)	Poloxamer-188 (mg)
NP1	100	-	40	10	10
NP2	200	-	40	10	20
NP3	-	100	40	10	30
NP4	-	200	40	10	40

Particle size, surface morphology and zeta potential-

The surface morphology (roundness, smoothness, and formation of aggregates) and particle size were studied by scanning electron microscopy. Zeta potential is an abbreviation for electrokinetic potential in colloidal systems[7]. Zeta potential of theformulations was determined by zeta potential probe model DT- 300.

Drug content-

The drug content in each formulation was determined by weighing nanoparticles equivalent to 30 mg of Abacavir and dissolving in 100 ml of 6.8 pH phosphate buffer, followed by stirring. The solution was filtered through a 0.45µ membrane filter, diluted suitablyand the absorbance of resultant solution was measured spectrophotometrically at 271 nm using 6.8 pH phosphate buffer as blank[8]. The drug content of the prepared nanoparticles was determined by the formula:

% Drug content =(Weight of drug in nanoparticles)/(Weight of nanoparticles) X100

Nanoparticles recovery

The recovery of nanoparticles suspension was analyzed by centrifugation method, where 10 mL suspension was centrifuged at 15000 rpm at 4°C. The sedimentnanoparticles were collected, freeze dried and calculated for % yield[9].

% Yield=(Weight of recovered particles)/(Weight of drug and polymer used) X100

Drug entrapment efficiency

15 mg of freeze dried nanoparticles were taken in a volumetric flask filled with distilled water for extraction of drug and kept for 24 hours. The mixture was sonicated for 20 min. Then filtered by using vacuum filter to obtain complete clear solution and samplewill be assayed by UV-spectrophotometer at 271 nm[10]. The percentage drug entrapment efficiency can be calculated by using following equation

% DEE=(Weight of drug in nanoparticles)/(Weight of drug used) X100

In vitro release studies[11]

In vitro release studies were carried out by using dialysis tubes with an artificial membrane. The prepared Abacavir nanoparticles and 10 ml of phosphate buffer pH 7.4 was added to the dialysis tube and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 250 ml of phosphate buffer pH 6.8. The medium inthe receptor was agitated continuously using a magnetic stirrer a temperature was maintained at 37±1°. 5ml of sample of receptor compartment were taken at various intervals of time over a period of 24 h and each time fresh buffer was replaced. The amount of drug released was determined spectrometrically at 271 nm.

Kinetic modeling

In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of nanoparticles were fitted with various kinetic equation like zero order, first order and Higuchi'smodel[12].

RESULT AND DISCUSSION:

Table 2: Physicochemical characterization of Abacavir nanoparticles.

Formulation code	Particle size (nm)	% Drug content	% Yield	Zeta potential (mv)	% Entrapment efficiency
NP1	121.4±0.37	70.44±0.26	59.46	16.5±0.52	99.22
NP2	125.5±0.25	78.32±0.41	64.38	18.31±0.37	99.45
NP3	130.4±0.71	80.35±0.82	69.57	20.45±0.41	99.92
NP4	140.6±0.43	84.22±0.31	70.65	19.72±0.73	99.85

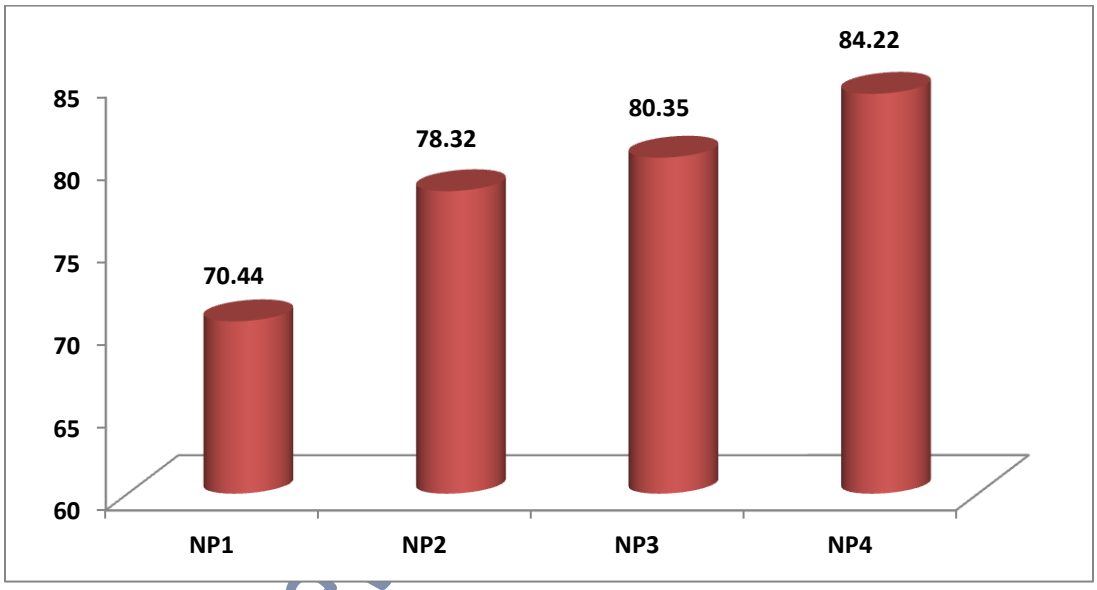

Fig 1: % Drug content of Abacavir nanoparticles

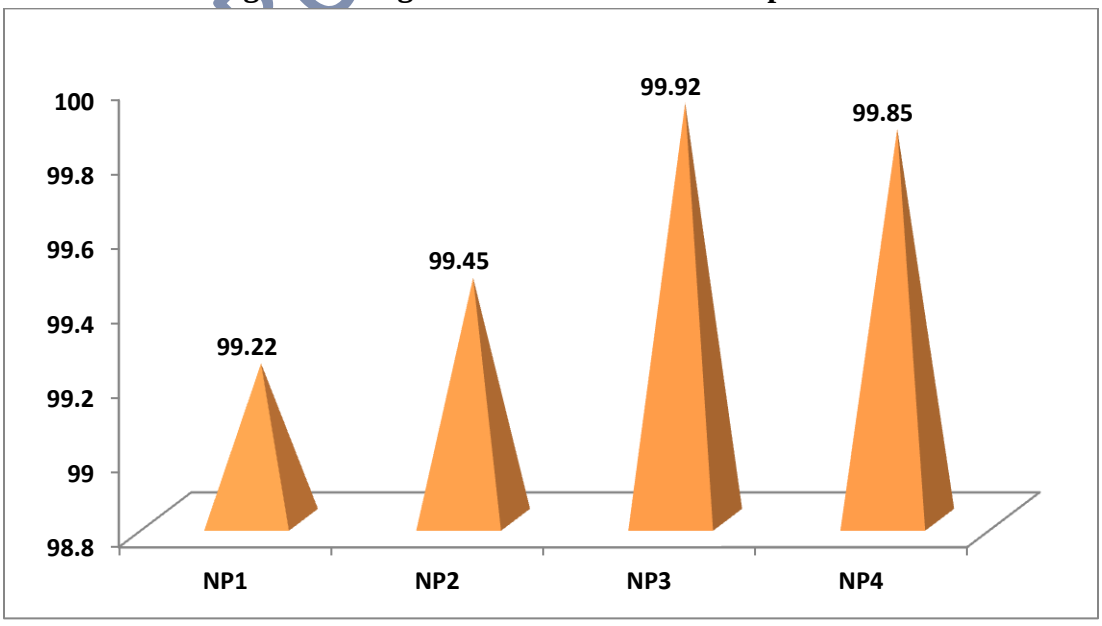

Fig 2: % Drug entrapment efficiency of Abacavir nanoparticles

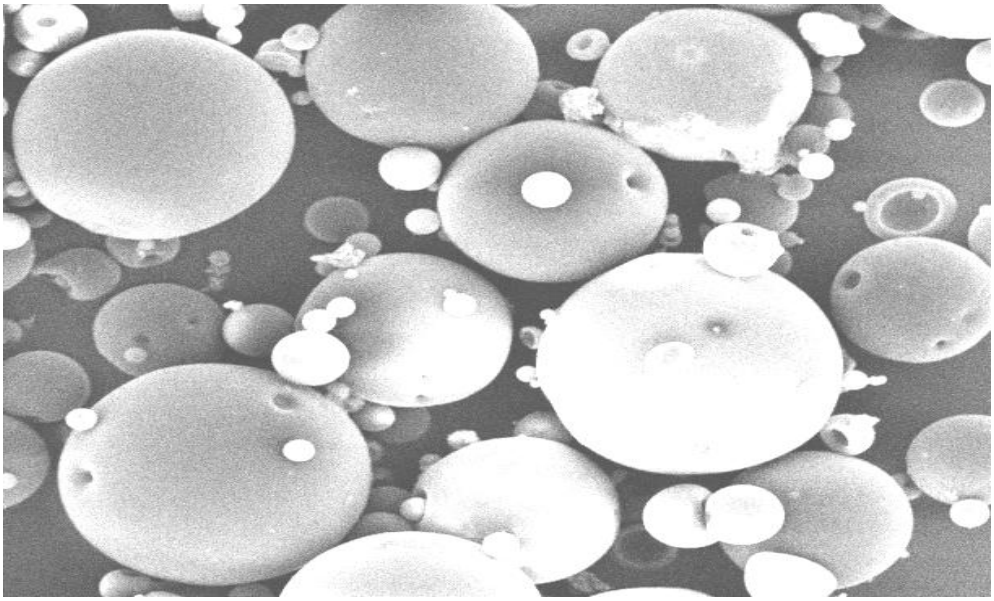

Fig 3: SEM of Abacavir nanaoparticles of batch NP4

Four different Abacavir nanoparticles formulations were preparedby the solvent displacement method with varying proportions of Eudragit RL-100, and chitosan.

The scanning electron microphotograph indicate that Abacavir nanoparticles have a discrete spherical structure without aggregation.

The particle size of nanoparticles varied some what among the formulation due to variation in the composition of formulations. Particle size range of nanoparticles was in the range of 121.4-140.6 nm.

Zeta potential of best formulation was determined and it was found in range of 16.5-20.45 mv. Since there was a decrease of surface potential, it could be concluded that a part of drug was absorbed on the polymeric particles.

The drug content was maximum in formulation NP4. In general nanoparticles exhibited an increase in drug content with an increased in the polymer ratio, up to particular concentration. A decrease in drug content was observed after that point due to the saturation capacity of polymer. The percent entrapment efficiency was found to be more than 99 % in all formulations.

The *in-vitro* release study was conducted for 10 hrs.The release of Abacavir mainly depend upon the polymer concentration. The burst release of Abacavir from nanoparticles at initial stage resulted from the dissolution of drug crystals on the surface of nanoparticles. Nanoparticles of batch NP3 shows maximum release 82.11% in 10 hrs.

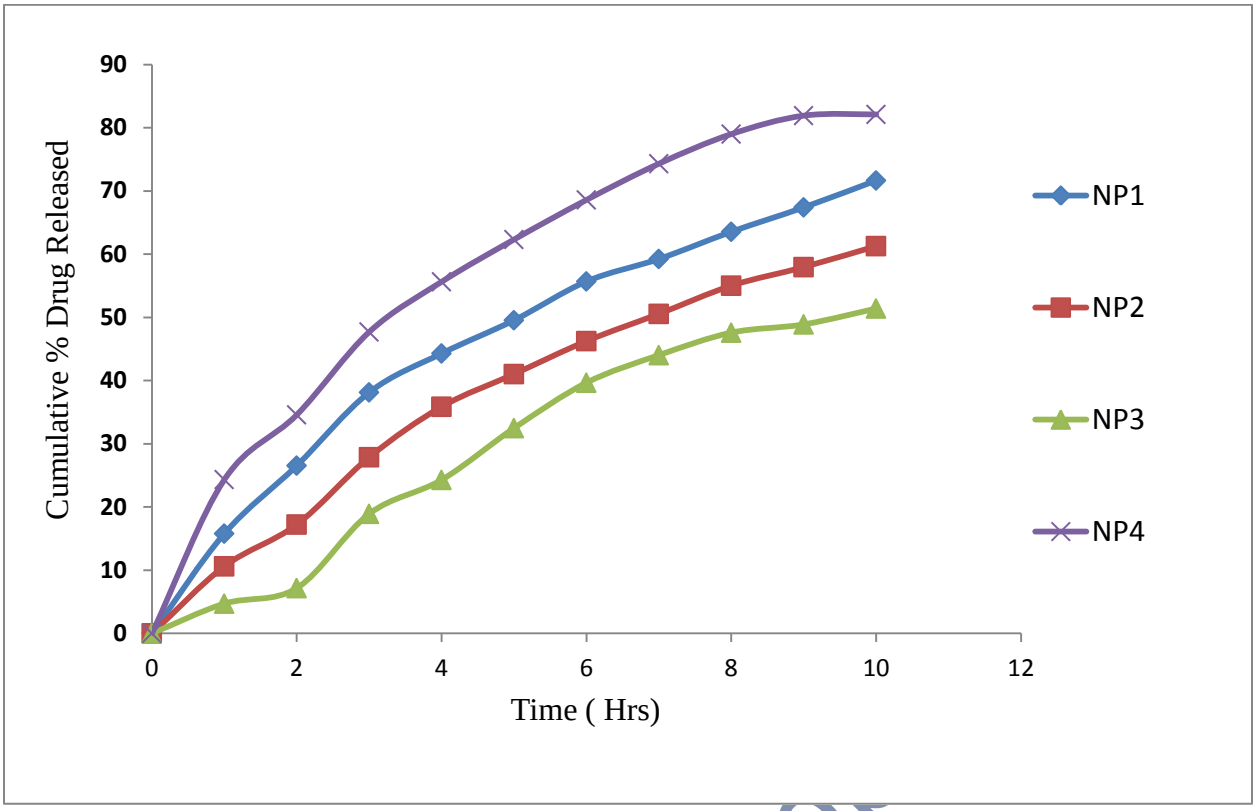

Fig-4: In-vitro drug release profile of Abacavir Nanoparticles

Table 3: Correlation coefficent of different nanoparticles formulations.

Formulation code	Correlation Coefficient (r^2)		
	Higuchi kinetics	First order kinetics	Zero order kinetics
NP1	0.993	0.882	0.956
NP2	0.992	0.884	0.950
NP3	0.982	0.917	0.893
NP4	0.976	0.925	0.900

The in vitro release data was applied to various kinetic models to predict the drug release kinetic mechanism. Nanoparticles were fitted with various kinetic equation like zero order, first order and Higuchi'smodel. The release constant was calculated from the slope of appropriate plots, and the regression coefficient (r^2) was determined by the means of PCP Disso software version 3.0.

CONCLUSION:

The method used for preparation of nanoparticles of Abacavir was found to be simple and reproducible. The slow and constant release of Abacavir from nanoparticles maintain constant drug plasma concentration thereby increasing therapeutic efficacy. The developed formulation overcome and alleviates the drawbacks and limitations ofAbacavir sustained release formulations. The development of effective nano delivery systems capable of carrying a drug specifically and safely to a desired site of action is one of the most challenging tasks of pharmaceutical formulation investigators

On the basis of different parameters i.e. physicochemical and in-vitro release study, nanoparticles of batch NP4 are concluded as optimum formulations.

Further, it can be concluded that the nanoparticulate formulation can be an innovative and promising approach for the delivery of Abacavir.

REFERENCES:

1. Tamizhrasi S, Shukla A, Shivkumar T, Rathi V, Rath JC, Formulation and evaluation of lamivudine loaded polymethacrylic acid nanoparticles, *International Journal of Pharm Tech Research*, 2009,1(3), 411-415.
2. Yaowalak B, Ampol M, Bernd WM. Chitosan drug binding by ionic interaction. *Eur. J. Pharm. Sci*, 2006, 62,267-74.
3. Rauch A, Nolan D, Martin A. Prospective genetic screening decreases the incidence of abacavir hypersensitivity reactions in the Western Australian HIV cohort study. *Clinical Infectious Disease*s, 2006, 43 (1): 99–102.
4. Heatherington. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir, Lancet, 2006, 359 (9312): 1121–1122.
5. Mallal S, Phillips E, Carosi G. HLA-B5701 screening for hypersensitivity to abacavir. New England Journal of Medicine, 2008, 358 (6): 568–579.
6. Kheradmandnia S, Vasheghani-Farahani E, Nosrati M, Atyabi F. Preparation and characterization of ketoprofen-loaded solid lipid nanoparticles made from beeswax and carnauba wax, *Nanomed Nanotechnol Biol Med*; 2010, 6:753-9.
7. Min-Soo Kim, Shun-Ji Jin, Jeong-Soo Kim, Hee Jun Park, Ha-Seung Song, Reinhard HH. Neubert Sung-Joo Hwang. Preparation, characterization and in vivo evaluation of amorphous atorvastatin calcium nanoparticles using supercritical antisolvent (SAS) process. *European Journal of Pharmaceutics and Biopharmaceutics*, 2008, 69,454–465.
8. Corsini A, Bellosta S, Baetta R, Fumagalli R, Paoletti R, Bernini F, New insights into the pharmacodynamic and pharmacokinetic properties of statins, Pharmcol. Ther, 1994, 84413–428.
9. Schwarz C, Mehnert W, Freeze-drying of drug-free and drug-loaded solid lipid nanoparticles (SLN). International journal of pharmaceutics, 1997, 157(2): 171-179.
10. Shegokar R, Singh KK, Müller RH. Production and stability of stavudine solid lipid nanoparticles-from lab to industrial scale. Int J Pharm, 2011, 416: 461-470.
11. Mohanty C, Sahoo SK. The in vitro stability and in vivo pharmacokinetics of curcumin prepared as an aqueous nanoparticulate formulation. Biomaterials, 2010, 31: 6597-6611.
12. Venkateswarlu V, Manjunath K. Preparation, characterization and *in-vitro* release kinetics of clozapine solid lipid nanoparticles. *J. Cont. Rel*., 2004, 95: 627-638.