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RESEARCH ARTICLE

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

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## Abstract

**Objectives:** Abacavir is a nucleoside analog reverse transcriptase inhibitor (NRTI), antiretroviral drug; it is used in treatment of AIDS. The present study deals with the formulation and evaluation of Abacavir nanoparticles.
**Methods:** 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.
**Results:** Particle size range of nanoparticles was in the range of 121.4-140.6 nm. Zeta potential of formulations was 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.
**Conclusion:** The study concludes that nanoparticles can be a promising drug delivery system for sustained release of Abacavir in terms of increased bioavailability.
**Keywords:** Abacavir, entrapment efficiency, nanoparticles, solvent displacement method, Zeta potential.

## INTRODUCTION

Nanoparticles are promising drug delivery systems of controlled and 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 and 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]. It has *in-vitro* activity against a range of HIV-1 and HIV-2 strains. It has been well tolerated, the main side effect is hypersensitivity, this can be severe, and in rare cases, possibility for fatal. It has short half life and frequent administration of it is necessary to maintain its effective plasma concentration[4]. In anti-retroviral therapy, a sustained release drug delivery system is required for reduction of side effects and improving the bioavailability[5]. So, Abacavir is a suitable candidate to develop and evaluate as nanoparticles formulations[5].

## MATERIALS AND METHODS

Abacavir was a gift sample from Green life Pharmaceuticals Ltd. 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 proportions 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 turned the solution slightly turbid then; acetone was removed by continuous stirring at 35-40°C. The prepared suspension was centrifuged, supernatant was removed and the sediment was freeze dried for further analysis[6].

**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 the formulations was determined by zeta potential probe model DT- 300.

**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 |

**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 0.45 µ membrane filter, diluted suitably and 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:

$$\% \text{ Drug content} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of nanoparticles}} X100$$

**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 sediment nanoparticles were collected, freeze dried and calculated for % yield[9].

$$\% \text{ Yield} = \frac{\text{Weight of recovered particles}}{\text{Weight of drug and polymer used}} X100$$

**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 sample will be assayed by UV-spectrophotometer at 271 nm[10].

$$\% \text{ DEE} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of drug used}} X100$$

***In vitro* release studies**

*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 in the receptor was agitated continuously using a magnetic stirrer a temperature was maintained at 37±1°C. 5ml of sample of receptor compartment were taken at various intervals of time over a period of 24h and each time fresh buffer was replaced. The amount of drug released was determined spectrometrically at 271 nm[11].

**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 equations like zero order, first order and Higuchi'smodel[12].

**Statistical analysis**

Experimental results were expressed as mean±SD. Student's *t*-test and one-way analysis of variance (ANOVA) were applied to check significant differences in drug release from different formulations. Differences were considered to be statistically significant at $p<0.05$.

## RESULTS AND DISCUSSION

Four different Abacavir nanoparticles formulations were prepared by 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 somewhat among the formulation due to variation in the composition of formulations. Particle size 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 depends upon the polymer concentration.

**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 |

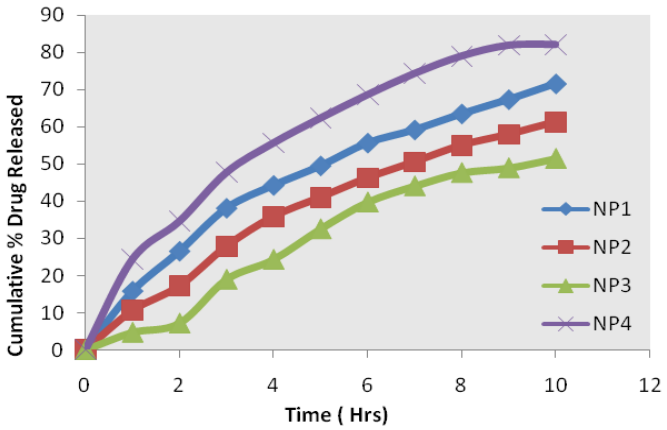


**Figure 1: *In-vitro* drug release profile of Abacavir nanoparticles.**

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.

**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 |

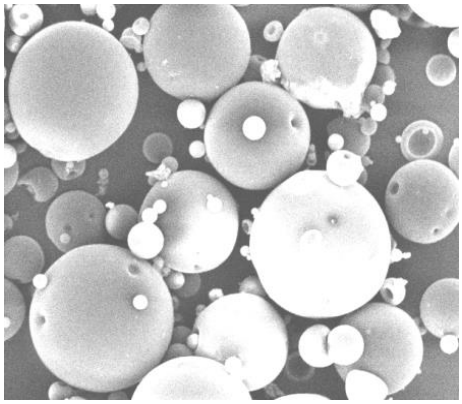

**Figure 2: SEM of Abacavir nanaoparticles of batch NP4.**

The *in vitro* release data was applied to various kinetic models to predict the drug release kinetic mechanism. Nanoparticles were fitted with various kinetic equations like zero order, first order and Higuchi's model. 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.

## CONCLUSIONS

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 of Abacavir 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.

## AUTHOR'S CONTRIBUTION

**Yusuf FS:** writing original draft, conceptualization, methodology, investigation. **Yunus AA:** Writing, review, and editing, supervision, resources. **John DF:** writing, review, and editing. **Chigbo UJ:** writing, review, and editing. Final manuscript was read and approved by all authors.

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## DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

## CONFLICT OF INTEREST

None to declare.

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