Original Article

Formulation Development And Evaluation Of Atazanavir Nanoparticles

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Abstract

Atazanavir is one of the effective antiviral drugs used in the treatment of HIV. It is a lipophilic drug encountering various problems such as poor solubility, rapid first-pass metabolism and pH based dissolution. To overcome these issues, atazanavir nanoparticles (AZNP1 to AZNP5) were formulated. The formulations prepared were evaluated for drug content, entrapment efficiency, particle size, surface morphology and drug release. AZNP3 nanoparticles of particle size (227.9nm), surface charge (-20.7mV) was found to be optimum. The percentage of drug release was 99.85 up to 24h. The results of evaluation studies support the potential and futuristic applications of atazanavir nanoparticles in the treatment of HIV. In conclusion, the nanoparticles can be a promising drug delivery of atazanavir at a controlled rate.

Keywords: Atazanavir, nanoparticles, chitosan, in vitro drug release, controlled release

INTRODUCTION

Nanoscience is a term used for materials and technology involving nano size. It is a science that is involved in various fields including pharmacy [1]. Nanotechnology is coined for the techniques and methods, whereas nanoparticles refer to the materials and drug delivery system [2]. Nanoparticles in pharmaceutical drug delivery systems may be defined as a carrier system for drugs with the size of less than 100nm in diameter [3]. The carrier used may be a polymer and it may or may not be biodegradable.

Different methods are used to prepare the nanoparticles. The choice of a suitable method depends on the characteristics of the drug and polymer [4]. Normally solvent evaporation, emulsion diffusion and precipitation methods are used for the preparation of nanoparticles [5]. Solvent evaporation involves the emulsification of polymer solution into an aqueous phase and then followed by the evaporation of solvent to obtain nanospheres [6]. The emulsion diffusion method involves encapsulating the drug in the polymer which is dissolved in the partially miscible solvent, then the polymer solution is emulsified in an aqueous solution containing surfactant for the formation of nanoparticles [7]. This method involves the polymer precipitation from an organic solution, which is then poured into an aqueous

solution containing surfactant. For stirring magnetic stirrer can be used.
The characterization is important for the nanoparticles. The size, surface morphology, zeta potential, drug content, entrapment efficiency must be determined to analyze the product [8].
Nanotechnology and nanoparticles are the technology and drug delivery systems widely used in the field of pharmaceutical sciences. The nanoparticles can be entered into the human body via lungs, intestinal tract or skin [9]. The nanoparticles are utilized in drug delivery due to its enhanced bioavailability and reduced toxicity [10]. The advantages of nanoparticles include targeted drug delivery, sustained drug delivery and even faster drug delivery also. AIDS is a condition in which human immunity has dropped down [11]. This is primarily transmitted through blood transfusion and unsafe sexual relationships. It can also be transmitted to kids through pregnant mothers [12]. The virus responsible for the AIDS is human immunodeficiency virus (HIV) which consists of two species of Lentivirus (a subgroup of retrovirus) [13]. HIV infects the significant cells of the immune system like helper T cells, macrophages and dendritic cells. It enters the $CD4^+$ T cells and macrophages by adsorption of the glycoproteins receptor. The pyroptosis mechanism lowers the $CD4^+$ T cells creating a loss in cell-mediated immunity and further susceptible to infections [14].
Atazanavir is a drug used along with other medications in the management of HIV [15]. The bioavailability of the drug is 60-68%. The drug targets the HIV-1 protease and interferes with the maturation of HIV. The drug is having a Cmax of 2 to 2.5 hrs. Protein binding is 86% and metabolized by hepatic cytochrome P450 enzymes. The half-life of the drug is 7 hr [16]. Atazanavir has severe adverse effects like liver problems, neurological problems and muscle pain [17]. The present research study focused on the preparation and evaluation of nanoparticles of atazanavir.

MATERIALS

Atazanavir, tween 80 and chitosan were purchased from Sigma Aldrich Pvt. Ltd. The other chemicals and reagents used were of analytical grade.

METHODS

Preformulation Studies

Preparation of calibration curve for Atazanavir

100mg of atazanavir was taken and dissolved in a buffer solution of pH 1.2, 7.4 and 6.8. From this solution, dilutions were made to obtain a concentration of 10 to 50µg/ml. The absorbance was measured at 278nm [18].

Drug–Excipients Compatibility Studies

Differential Scanning Calorimetry

The interaction studies of the drug alone and in combination with the excipients were done using DSC [19]. The spectra for atazanavir, excipients and blend of atazanavir with excipients were obtained by DSC. Samples were placed in aluminum crucible pans (5-10mg) and scanned in the temperature range of 50-300˚C with a heating rate of 10˚C/min using Shimadzu DSC – 60 equipment.

Physical Compatibility Studies

Physical incompatibility studies were done by placing the drug alone and with additives. The colour, odour and other changes were observed.

Organoleptic properties

The drug characters like colour, odour and appearance play a significant role in sample identification. These were recorded.

FORMULATION OF ATAZANAVIR NANOPARTICLES

The atazanavir nanoparticles were prepared by the emulsion droplet coalescence method [20]. 100mg atazanavir was dissolved in phosphate buffered saline. The polymer chitosan was dissolved in 1% acetic acid. The solution was added to 10ml of liquid paraffin having 2% v/v tween 20. This mixture was stirred in a homogenizer for 3 min to form w/o emulsion. The prepared nanoparticles were subjected to centrifugation at 3000rpm for 1 hr and washed using ethanol and water. This step is done to remove the tween 20 and liquid paraffin. Then it is dried at 50˚C for 4 hours in a hot

air oven. Then it is stored in a desiccator. The chitosan concentration is changed for every trial for optimization. The formula for the formulation was given in table 1.

Table 1 Formula for Atazanavir Nanoparticles

S.No.	Ingredients	Formulations				
		AZNP1	AZNP2	AZNP3	AZNP4	AZNP5
1.	Drug (mg)	100	100	100	100	100
2.	Chitosan (% W/V)	0.25	0.5	0.75	1	1.25
3.	Tween 20 (V/V)	2	2	2	2	2

EVALUATION OF ATAZANAVIR NANOPARTICLES

The atazanavir nanoparticles were characterized for various physicochemical properties.

Drug Content

To analyze the content of the drug, 1gm of atazanavir nanoparticles were accurately weighed and transferred into a volumetric flask. The sample was dissolved in pH 6.8 phosphate buffer. Required dilution was done with the buffer solution. The standard was prepared by using the phosphate buffer. The absorbance of the standard and sample was measured at 278nm using UV-visible spectrophotometer.

Entrapment efficiency

The atazanavir formulations in buffer solutions were exposed to centrifugation at 15000 rpm for 30 min [21]. The clear supernatant liquid was separated. From this 1 ml of the solution was diluted with pH 6.8 phosphate buffer solution and absorbance was measured at 278nm by UV spectrophotometer. The entrapped and unentrapped drug amount were calculated.

Particle Size and Surface Charge

The two parameters particle size and surface charge need to be considered to achieve a stable preparation. The surface charge determines the interaction of drugs with proteins [22]. These two directly related to the stability of the preparations and were analyzed using Malvern Zeta sizer. The zeta potential of above 30mV either positive or negative can be stable [23]. The sample was injected slowly and analyzed with six replicates. The surface charge can be given as zeta potential.

Surface Morphology

This study was determined by scanning electron microscopy. The surface of the nanoparticles is responsible for the drug release pattern. The aggregation or agglomeration also would happen due to the surface properties [24]. So, the surface morphology needs to be studied properly. The sample was kept in sample holder, followed by coating with gold using sputter coater. Then the sample was scanned with a fine beam of electrons. The images of the nanoparticles were obtained.

In vitro release

Drug release studies directly represent the absorption and bioavailability [25]. The in vitro studies were performed for 24 h by Franz diffusion cell using a dialysis membrane. The prepared atazanavir formulations were placed in the dialysis membrane and immersed in pH 6.8 phosphate buffer. The samples were collected at regular intervals and analyzed at 278nm by UV spectrophotometer. The cumulative drug release percent was calculated from the absorbance values.

RESULTS AND DISCUSSION

Preformulation Studies

Calibration Curve of Atazanavir

Atazanavir standard calibration curve was carried out in three different media; pH 1.2, pH 7.4 and pH 6.8 buffers at 278nm. The r^2 value gives nearly 1 which shows linearity. The standard plot of Atazanavir was given in Table 2 and the standard graph is in Figure 1.

Table 2 Calibration curve of Atazanavir

Concentration (µg/ml)	Absorbance value		
0	0	0	0
10	0.025	0.036	0.040
20	0.052	0.073	0.081
30	0.076	0.108	0.122
40	0.101	0.146	0.163
50	0.125	0.182	0.204

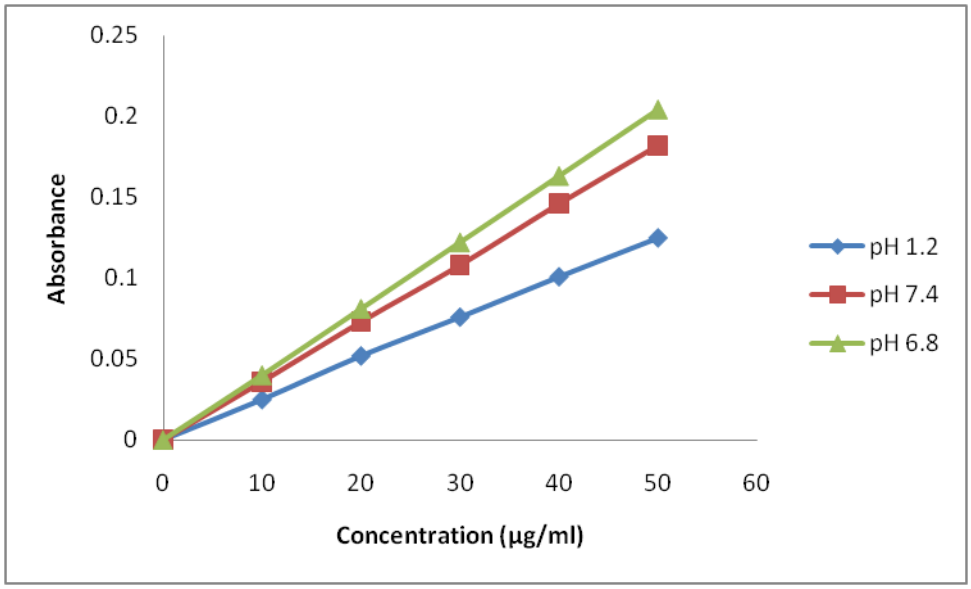

Fig. 1. Standard Calibration Curve in pH 1.2, 7.2 and 6.8 Phosphate buffer

Drug – Excipient compatibility Study

Differential Scanning Calorimetry

DSC of Atazanavir expressed a sharp endothermic peak at about 211ºC. The physical mixture of atazanavir with other additives showed a peak at 207ºC. The values were almost the same and the difference is negligible. This indicated that the drug is compatible with excipients. This study indicated that there was no interaction between the drug and the excipients used in the formulation. The DSC curves were given in Figure 2 and Figure 3.

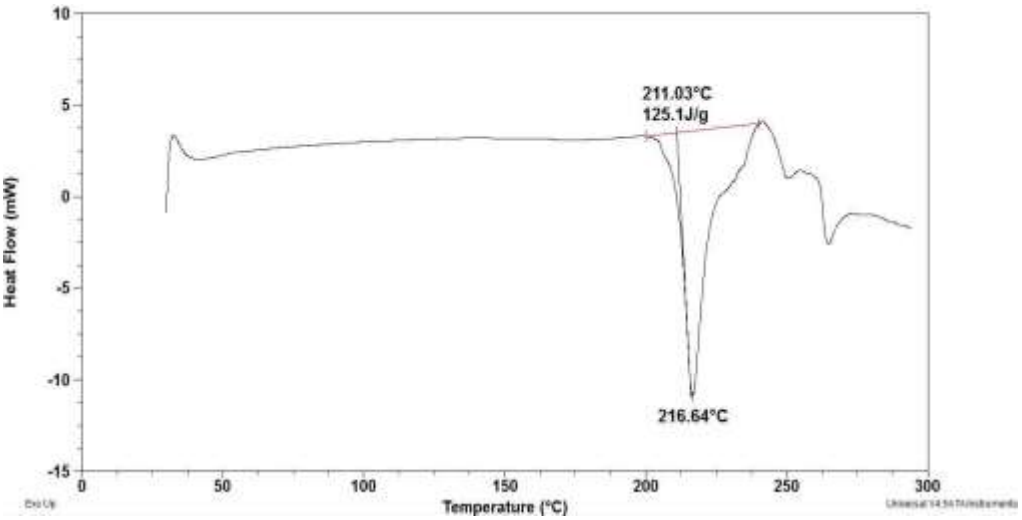

Fig. 2. DSC curve of Atazanavir

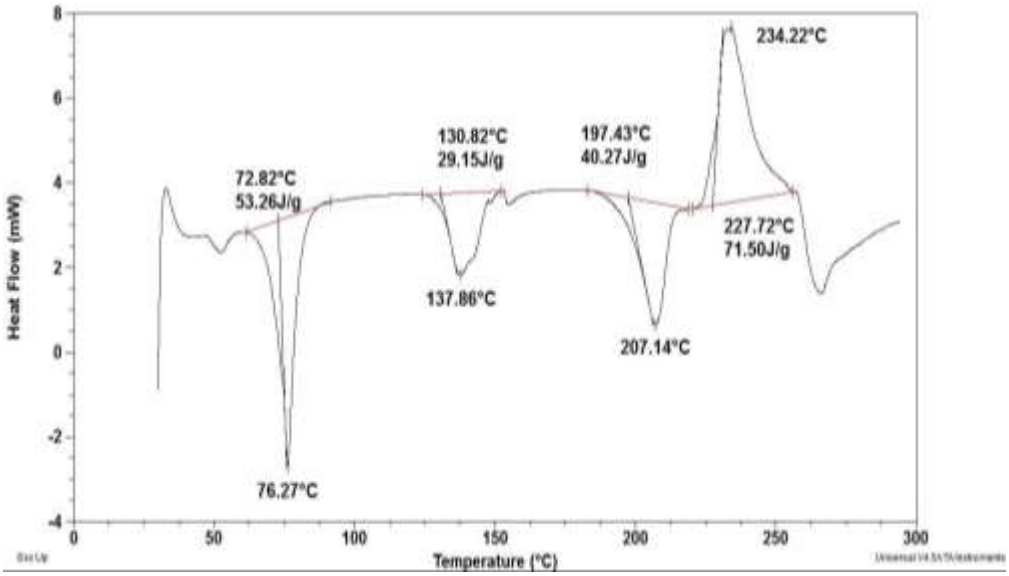

Fig. 3. DSC curve of Atazanavir nanoparticles

Physical Compatibility Studies

The physical compatibility studies report showed that there was no interaction between the drug and additives. The results were given in table 3.

Table 3 Physical Compatibility Studies

S.No	Drug / Additives	Description (Initial)	Description (Final)
1.	Atazanavir	White crystalline powder	No change
2.	Chitosan	Off white powder	No change
3.	Atazanavir + Chitosan	Off White powder	No change

Organoleptic properties

The drug characters like colour, odour and appearance were observed. From the results, it was found that there were no changes in the properties. This indicated that there was no interaction of drugs with excipients.

Drug Content

The drug content for all the atazanavir nanoparticle formulations (AZNP1 - AZNP5) was almost the same. This result suggested that there was no drug loss in the manufacturing of atazanavir nanoparticles. The results were given in table 4.

Entrapment efficiency

The entrapment efficiency of atazanavir nanoparticles increased with an increase in chitosan concentration. This may be due to the availability of chitosan to encapsulate the atazanavir upon increasing the chitosan concentration, the number of layers of chitosan that coat the drug decreases which increases the percentage of entrapment efficiency. The minimum and maximum percentage entrapment efficiencies for atazanavir nanoparticles were found to be 55.76 (AZNP1) and 86.2 (AZNP5) respectively. The results were given in table 4.

Table 4. Drug content (%) and Entrapment Efficiency (%)

Formulations	Drug Content (%)	Entrapment Efficiency (%%)
AZNP1	99.85	55.76
AZNP2	99.79	62.19
AZNP3	99.86	85.65
AZNP4	99.82	85.71
AZNP5	99.84	86.2

Particle Size and Surface Charge

The particle size of the atazanavir nanoparticles ranged from 211.6 to 276.8nm. The chitosan polymer concentration influences the particle size. This is due to increased coating in the number of layers on the drug by chitosan.

The zeta potential values of atazanavir nanoparticles were negative and increased from -17.7 to -22.7mV. The negativity is due to the chitosan. The particle size and zeta potential results were given in Fig. 4 and 5 and Table 5.

The results were given in Fig. 4 & 5 and Table 5.

Table 5. Particle Size and Surface Charge

Formulations	Particle size (nm)	Surface charge (mV)
AZNP1	211.6	-17.7
AZNP2	220.3	-18.6
AZNP3	227.9	-20.7
AZNP4	250.8	-21.4
AZNP5	276.8	-22.7

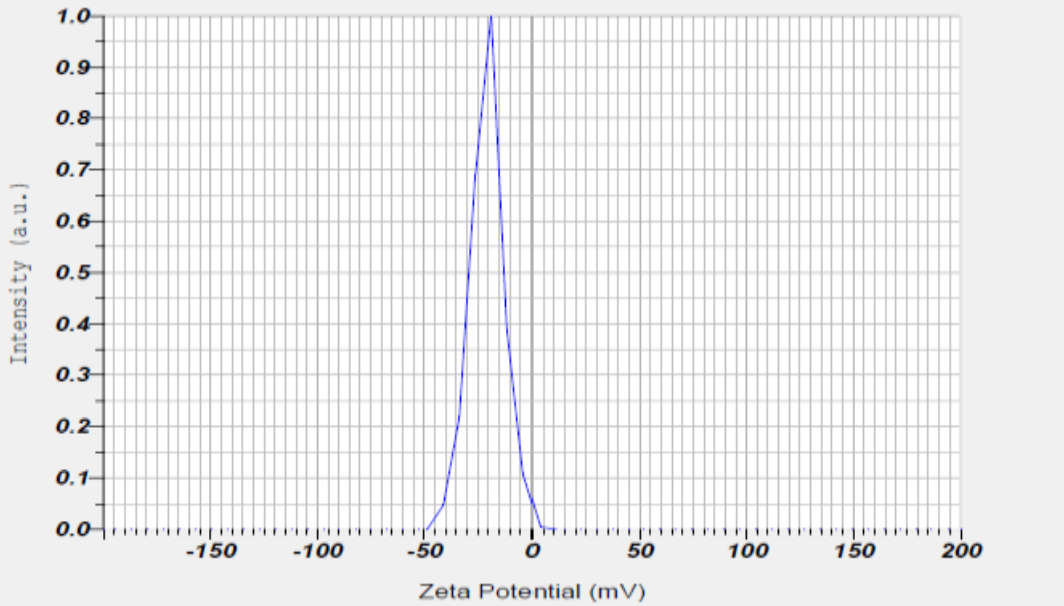

Fig. 4. Zeta potential of Atazanavir Nanoparticles (AZNP3)

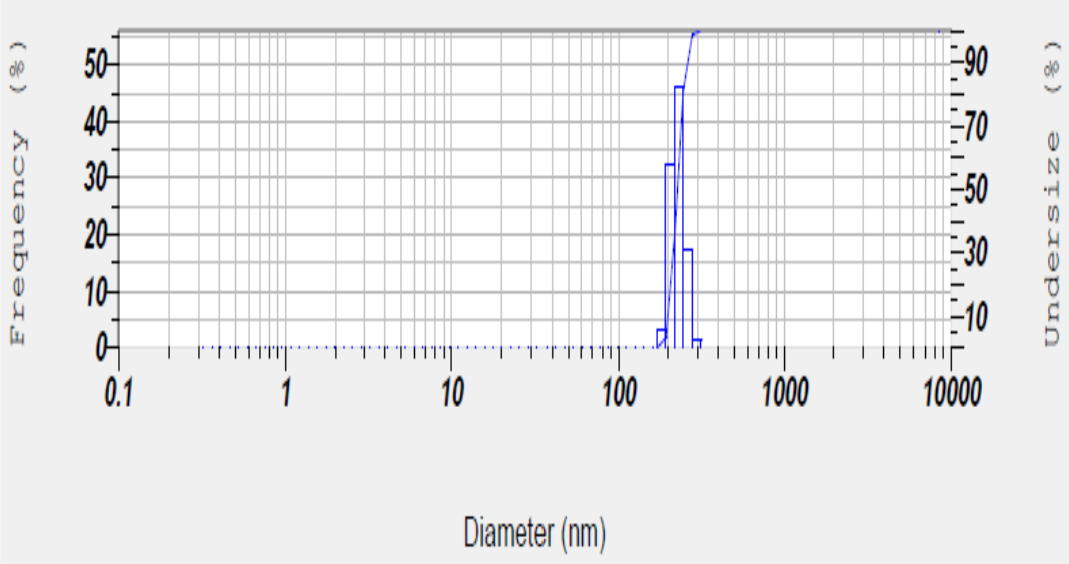

Fig. 5. Particle Size of Atazanavir Nanoparticles (AZNP3)

Surface Morphology

The surface studies of atazanavir nanoparticles were done by SEM. The particle's shapes were spherical. The SEM image of the particles was given in Fig 6.

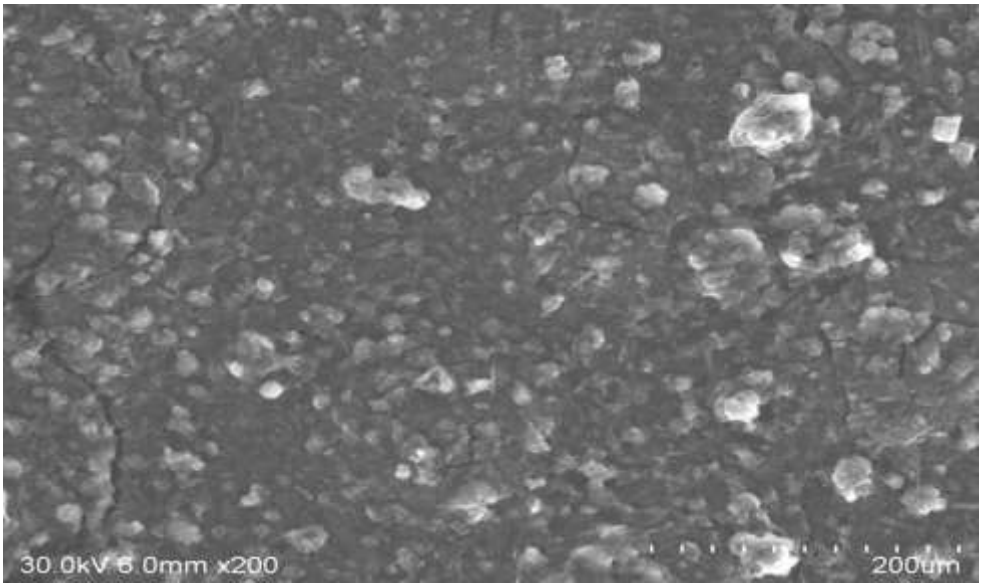

Fig. 6. SEM Image of Atazanavir Nanoparticles

In vitro release

The drug release for all the nanoparticles was a maximum of 99.85 % for ASNP3 which contained 100mg of atazanavir and 0.75 % w/v of chitosan polymer. Below 0.75 % w/v concentration of chitosan, the maximum percentage drug release was 99.81% and 99.82% were observed at the end of 16 h and 20 h respectively since the drug release was achieved soon the formulations AZNP1 and AZNP2 were not desirable. Above 0.75% w/v concentration of chitosan, a reduction in drug release was observed for the trials AZNP3, AZNP4 and AZNP5. The maximum percentage of drug release for AZNP3 was found to be 99.85% at 24 h.

From the overall results of all trials, AZNP3 was chosen as the optimized trial due to its ideal particle size (227.9nm), zeta potential (-20.7mV), entrapment efficiency (85.65%) and drug release was 99.85 % at the end of 24hr. The results suggested that the formulation of atazanavir nanoparticles was successful in delivering the drug. The results of drug release were given in table 6 and figure 7.

Table 6 Cumulative Drug Release

S.No.	Time (hr)	Percentage Cumulative Drug Release				
		AZNP1	**AZNP2**	**AZNP3**	**AZNP4**	**AZNP5**
1	0	0	0	0	0	0
2	0.5	25.57	20.13	15.82	10.16	8.53
3	1	32.68	26.83	21.69	15.87	12.72
4	6	67.84	62.27	57.26	25.89	20.77
5	12	85.81	79.45	71.49	38.48	33.85
6	16	99.81	90.55	85.28	55.29	47.91
7	20	99.80	99.82	94.33	68.26	59.42
8	24	99.79	99.81	99.85	82.67	73.39

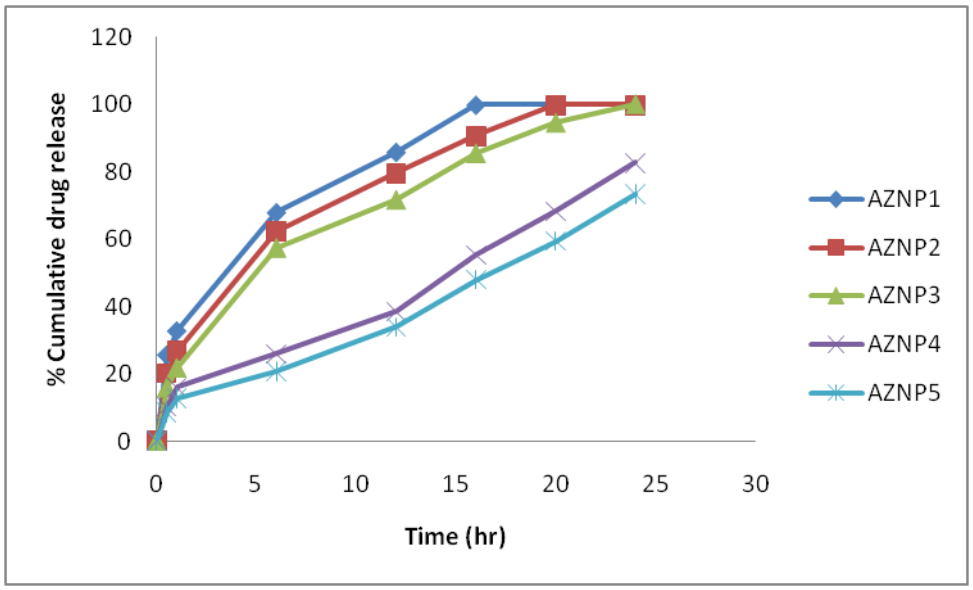

Fig.7. Percentage Cumulative Drug Release of Atazanavir Nanoparticles (AZNP1 – AZNP3)

CONCLUSION

Nanoparticles are formulated to achieve controlled / targeted / sustained drug delivery with reduced toxicity. In some cases, it can be formulated for immediate release also. To improve the bioavailability and to reduce the dosing frequency of the drug atazanavir, nanoparticles are one of the good options. In this present study, atazanavir nanoparticles were formulated and evaluated. Atazanavir nanoparticles formulated in the present study provided a controlled release. Hence it can be concluded that the formulated atazanavir nanoparticles may provide increased bioavailability.

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