IJBPAS, December, 2022, 11(12): 6197-6210

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

FORMULATION AND EVALUATION OF AZILSARTAN DRUG LOADED NANOPARTICLES

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Received 26th Dec. 2021; Revised 25th Jan. 2022; Accepted 12th March 2022; Available online 1st Dec. 2022

https://doi.org/10.31032/IJBPAS/2022/11.12.6689

ABSTRACT

Azilsartan, is a selective AT1 receptor blocker **(Figure 1)** that binds tightly and slowly separates **[1]**, thus preventing the binding of angiotensin II leading to vasodilation and minimized aldosterone effects. The present study was aimed to develop a nanoparticulate drug delivery system of antihypertensive drug Azilsartan using polymer (poly vinyl alcohol). The prepared formulation were characterized for loading efficiency, entrapment efficiency, particle size, particle size distribution , zeta potential and drug polymer compatability studies. The entrapment efficiency of the optimized formulation F7 (drug 50mg, polyvinyl alcohol 75mg, β –cyclodextin 10 mg) was 99.38 ±0.08 and invitro drug release was 98.46% after 24 hours. It also obey the zero order, follows diffusion and erosion mechanism of release. Surface morphology of optimized formulation (F7) indicated that lrbesartan nanoparticles were found to be in average nanometer range(358.4nm) and showed ideal surface morphology. The stability test performed revealed that the formulation (F7) showed no change in its characters. The optimized formulation (F7) was also examined for zeta potential determinations.

Keywords: Nanoparticles, Azilsartan, optimization, Drug release

INTRODUCTION

Azilsartan, is a selective AT1 receptor blocker **(Figure 1)** that binds tightly and slowly separates **[1]**, thus preventing the binding of angiotensin II leading to vasodilation and minimized aldosterone effects **[2, 3]**. It was developed by Takeda Global Research Development and approved in Japan in 2012 for the management of hypertension and marketed as a prodrug, azilsartan medoxomil, which is hydrolyzed to its active moiety, azilsartan, at gastrointestinal tract. Absolute bioavailability of azilsartan is 60% due first pass metabolism by CYP2C9 and elimination half-life of 11 hours **[2]**. According to biopharmaceutical classification system (BCS), azilsartan belong to class II with low solubility of 4.28X10-3 mg/L in water at 25°C, (practically insoluble) and high permeability **[4]** which make the drug attractive for solubility enhancement techniques as its dissolution is the rate limiting step for azilsartan absorption. Several methods with different principles have been utilized for increased aqueous solubility of azilsartan such as solid dispersion **[5, 6]**, and liquisolid compact **[7]**. Nanocarriers are the most evolving tools for solubility enhancement, nanocarriers have been used to improve the therapeutic effect of drugs and minimize the side effect. Nanocarriers are defined as particulate dispersion or solid particles with a size in the range of 10–1000 nm in at least one dimension that act as a carrier module for other molecules such as drugs, where the drug is dissolved, entrapped, encapsulated, or attached to a nanocarrier **[8, 9]**. Inorganic nanoparticles, including metal (gold, copper, silver, iron), metal oxide (iron oxide, zinc oxide) and quantum dots (cadmium selenide and cadmium sulfide) are among the nanoparticles of biomedical advantages including their utilization as drug delivery system **[10, 11]**.

EXPERIMENTAL INVESTIGATIONS

CONSTRUCTION OF STANDARD CURVE FOR AZILSARTAN UV Spectroscopy Method

Azilsartan is estimated spectrophotometrically at 220 nm and it obey Beer-Lambert` s Law in the range of 5-50 mcg /ml.

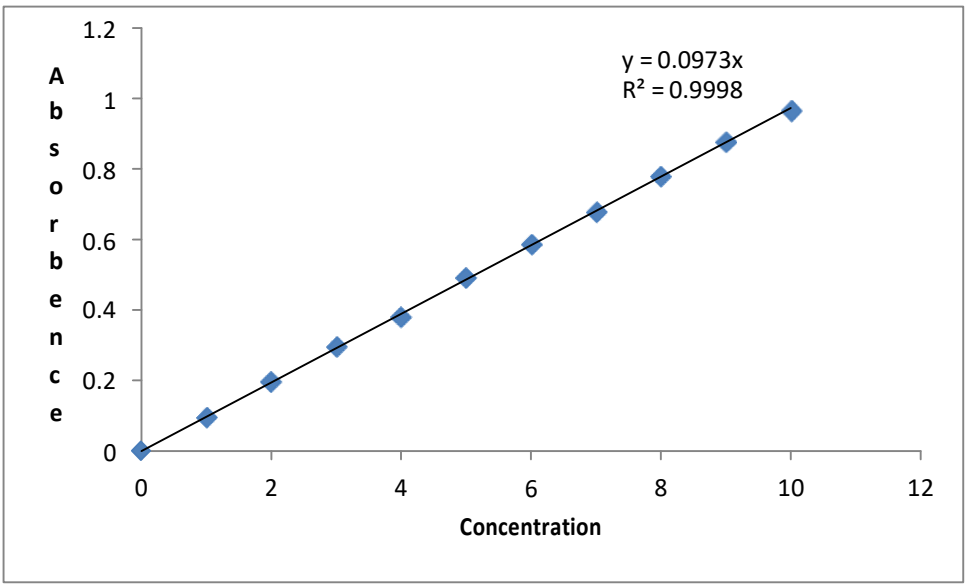

Figure 1: Standard Curve For Azilsartan

PRE FORMULATION STUDY

IR studies:

Identification of the pure drug was performed using IR spectroscopy. IR spectroscopy (using Perkin Elmer) by KBr pellet method carried out on drug. They are compressed under 15 tones pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000-400cm^{-1} in a spectrophometer and peaks obtained were identified.

METHOD OF PREPARATION OF AZILSARTAN NANOPARTICLE NANOPRECIPITATION METHOD:

All batches of nanoparticles were prepared by nanoprecipitation method. The required quantity of polymer dissolved in 3ml ethanol, and drug was dissolved in 3ml of ethanol, added finally both were mixed together and added β-cyclodextrin. The mixer was homogenized in vortex mixture for 1 min and then the Final volume of the preparation was to 10ml. Then this preparation was centrifuged at 15000rpm at 4^0c for half an hour. The supernatant was discarded and precipitate was washed 3times with distilled water. The nanoparticles thus obtained were dried overnight in oven at 60^0c and stored in desiccators. The prepared formulation were characterized for loading efficiency, entrapment efficiency, particle size, particle size distribution , zeta potential and drug polymer compatability studies.

Table 1: Various Composition of Nanoparticles Formulation

FORMULATION CODE	DRUG (Azilsartan) in mg	PAV in mg	β CYCLODEXTRIN
F1	50	25	5
F2	50	50	5
F3	50	75	5
F4	50	100	5
F5	50	25	10
F6	50	50	10
F7	50	75	10
F8	50	100	10
F9	50	25	15
F10	50	50	15

EVALUATIONOF NANOPARTICLES

DRUG ENTRAPMENT STUDY

The entrapment efficiency study was determined by free drug content in the supernatant which is obtained after centrifuging the solid lipid suspension at (15,000rpm for 20 min at zero using ultra centrifuge) The absorbance was measured at 220 nm by UV spectrophotometrically.

INVITRO DRUG RELESE STUDIES

BY UV Spectrophotometric Method:

The *invitro* drug release study was carried out by using the diffusion membrane technique. The nanoparticles preparation was placed in a dialysis membrane and it is dropped in a beaker containing 200ml of diffusion medium (phosphate buffer saline pH 7.4) the medium was maintained at 37^{o} C under magnetic stirred at constant speed. At fixed time interval of 1ml sample was taken from the diffusion medium for every 1 hrs and it was replaced by 1 ml fresh medium. This process was carried out for 24 hrs. The sample was measured UV spectrophotometrically at 220nm. The percentage of drug released at various time intervals was calculated from calibration graph.

SCANNING ELECTRON MICROSCOPY

The optimized formulation was morphologically characterized by scanning electron microscopy (SEM).The sample for SEM analysis was mounted in the specimen by using an adhesive, small sample which was mounted directly in scotsch double adhesive tape. The sample was analyzed in scanning electron microscope operated at 15 kv and image was taken.

SURFACE CHARGE (ZETA POTENTIAL DETERMINATION)

Zeta potential is an important parameter to evaluate and establish an optimum condition for stability of colloidal or dispersed systems .The prepared nanoparticle suspension were characterized with respect to zeta potential by using zeta potential analyser

(Malvern Zeta seizer). Zeta potential is electrical charges on particles surface it create electrical barrier it is very important for drug stability. The effect of polyvinyl alcohol and β-cyclodextrine on the surface characteristics of the nanoparticle was studied.

pH AND PHYSICAL APPERANCE:

The pH of the formulation was measured using pH meter. It plays a vital role in process of stability and formulation activity. The physical appearance of the formulation such as colour and suspended foreign particulate matter were to be examined.

STABILITY STUDIES OF NANOPARTICLES

The Stability studies of nanoparticles involves observing the formulation at 45^{O}C /70% RH which constitutes accelerated condition and (4^{O}C) on refrigerator and room temperature. The formulations were kept in both the temperature for 3 months and sufficient amount of sample were taken at periodic intervals, for performing the following tests.

a) Physical appearance
b) pH of the solution
c) *In vitro* drug release (Dissolution)
d) Percentage of drug entrapment

DRUG RELEASE KINETICS STUDIES

The optimized formulation was subjected to graphical treatment to assess the kinetics of drug release

ZERO ORDER PLOT

The zero order plot obtained by plotting cumulative % drug release versus time.

HIGUCHI PLOT

The Higuchi plot was made by plotting cumulative percentage (%) drug release versus Square root of time.

KORESMEYER PLOT

The graph was obtained by log cumulative percentage (%) drug release versus log time.

FIRST ORDER KINETIC RELEASE STUDY

The first order plots were obtained by plotting log remaining cumulative percentage drug release versus time.

RESULTS AND DISCUSSION

DEVELOPMENT OF AZILSARTAN NANOPARTICLES

All batches of nanoparticles were prepared by nanoprecipitation method. The required quantity of polymer dissolved in 3ml ethanol, and drug was dissolved in 3ml of ethanol, added finally both were mixed together and added β-cyclodextrin. The mixer was homogenized in vortex mixture for 1 min and then the Final volume of the preparation was to 10ml. Then this preparation was centrifuged at 15000rpm at 4^{0}C for half an hour. The supernatant was discarded and precipitate was washed 3 times with distilled

water. The nanoparticles thus obtained were dried overnight in oven at 60^0C and stored in desiccators.

Formulations with different ratios of polymer were prepared, several physiochemical characteristics of nanoparticles such as particle size determination, drug release profile, were investigated and stability of optimized formulation at various temperature was evaluated.

DRUG AND POLYMER COMPATABILITY STUDIES BY FTIR

Identification of the pure drug was performed using IR spectroscopy. IR spectroscopy (using Perkin Elmer) by KBr pellet method carried out on drug. They are compressed under 15 tones pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000-400cm^{-1} in a spectrophotometer and peaks obtained were identified.

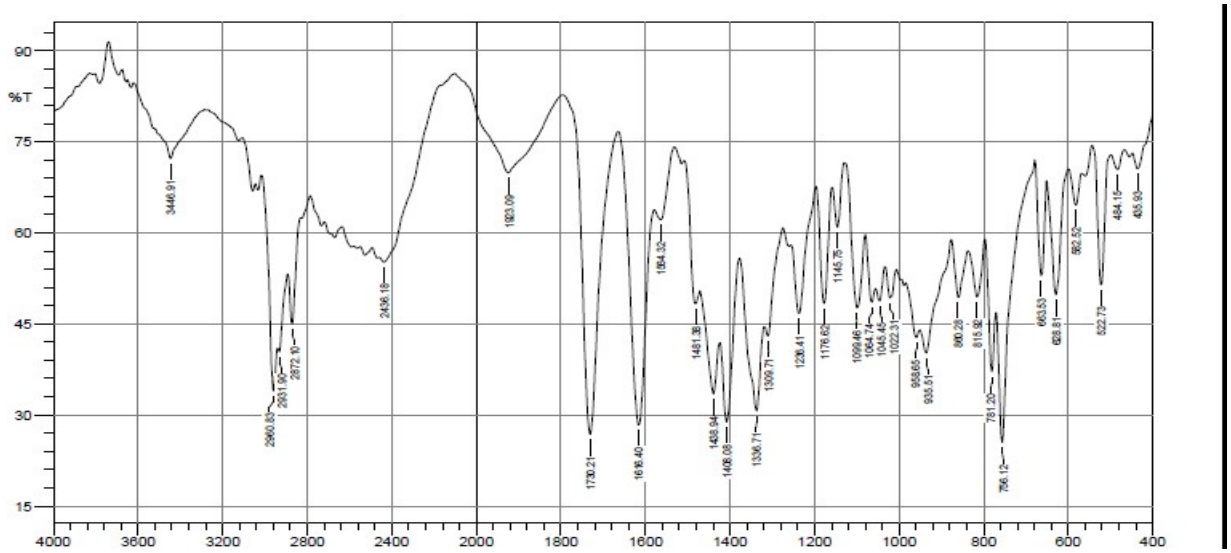

Table 2: I.R Spectra Data For Pure Azilsartan

Wave no. (cm^{-1})	Group		Assigned
1730.21	C=O	–	Stretching
1408.06	C=C	–	Stretching
1099.46	C-N	-	Stretching

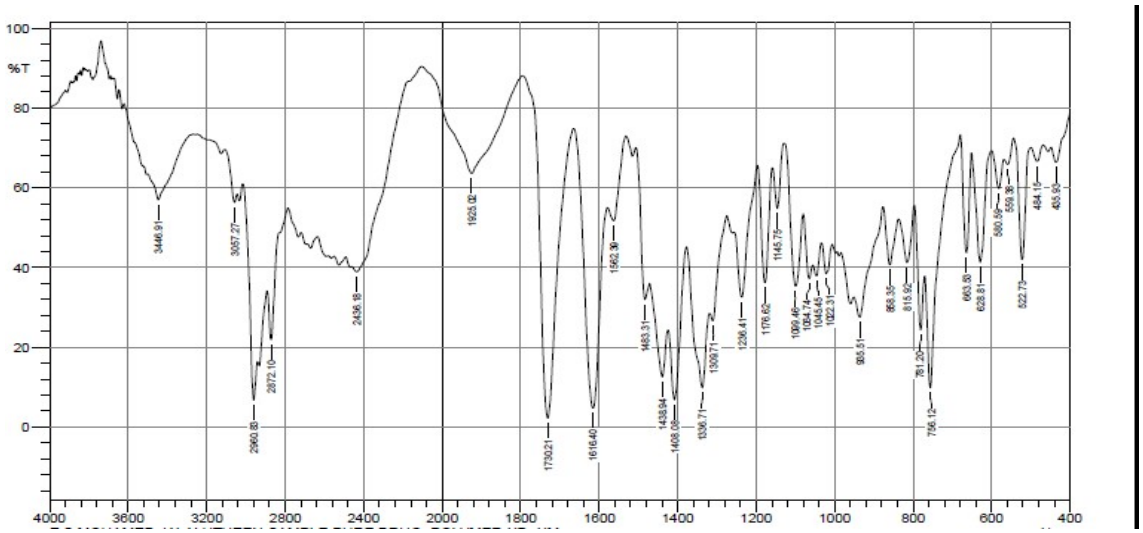

Table 3: I.R Spctera Data For Physical Mixture

Wave no.(cm^{-1})	Group Assigned
1734.01	C=O – Stretching
1409.06	C=C – Stretching
1099.43	C-N - Stretching

REPORT:

In FTIR spectra the peaks of physical mixture was compared with the original spectra. Same peaks were observed, indicates no possible molecular interaction between the drug and the polymer.

ENTRAPMENT EFFICIENCY OF NANOPARTICLES

The entrapment efficiency of Azilsartan nanoparticles was prepared by nano precipitation method. The formulation F1(Azilsartan 50 mg with 25 mg of Polyvinyl alcohol and β -cyclodextrin) shows less entrapment value of 60.16% this may be the due to repulsive force between drug and the polymer.

Table 4: Entrapment efficiency of Azilsartan nanoparticles

Formulation code	Drug (mg)	Poly vinyl alohol (mg)	β cyclodextrin (mg)	Ethanol	Entrapment Efficiency (%)
F1	50	25	5	2%	60.16±0.14
F2	50	50	5	2%	64.15±0.17
F3	50	75	5	2%	68.28±0.15
F4	50	100	5	2%	71.12±0.09
F5	50	25	10	2%	88.23±0.12
F6	50	50	10	2%	94.26±0.18
F7	50	75	10	2%	99.38±0.08
F8	50	100	10	2%	87.42±0.09
F9	50	25	15	2%	85.35±0.06
F10	50	50	15	2%	82.25±0.04

In formulation F2 Polymer concentration was increased (Azilsartan 50 mg with 50mg of Polyvinyl alcohol and 5 mg β -cyclodextrin) the entrapment efficiency was to 64.15%. Further increase in polymer concentration in formulation F3 (Azilsartan 50 mg with 75 mg of Polyvinyl alcohol and 5mg β -cyclodextrin)entrapment efficiency was 68.28%. Further increase in polymer concentration in formulation F4 (Azilsartan 50 mg with 100 mg of Polyvinyl alcohol and5 mg β -cyclodextrin) entrapment efficiency was 71.12%. Formulation F5, F6, was carried out by same process as like previous formulation but changes in polymer concentration 25mg, 50mg, 50 mg of Azilsartan and10 mg β -cyclodextrin was taken. The entrapment efficiency was found to be F5, 88.23% for F6,94 .26% .

Formulation F7 was carried out by increasing the polymer concentration same (Azilsartan 50 mg with 75 mg of Polyvinyl alcohol and 10 mg β -cyclodextrin) the entrapment efficiency was increased to 99.38%.

Formulation F8 was carried out by increasing the concentration (Azilsartan 50 mg with 100 mg of Polyvinyl alcohol and 10 mg β -cyclodextrin) which give the percentage of entrapment efficiency was 87.42% but In F8 the *invitro* release of drug shows less than F7 formulation. So F7 formulation is optimized and further study was carried out.

Further formulation F9 and F10 was carried out in same process, drug and polymer concentration (Azilsartan 50 mg with 25 and 50mg of Polyvinyl alcohol and 15 mg β - cyclodextrin) the Entrapment efficiency is F9 85.35%, F10 82.25% From the above result formulation F7 shows highest percentage of entrapment efficiency of 99.38%. So hence this formulation was optimized and further study was carried out.

In F1,F2,F3, F4 formulations, when increasing the polymer concentration the entrapment efficiency is not satisfactory limit. Nanoparticle using 5 mg β -cyclodextrin showed lower entrapment.

So further increasing the concentration of β –cyclodextrin in F5, F6 and F7 formulations. (Azilsartan 50 mg with 25 mg 50mg and 75 mg of Polyvinyl alcohol and 10 mg β -cyclodextrin). In this formulations the entrapment efficiency was F5 for 88.23%, F6 for 94.26% and F7 for 99.38%.In this the optimum entrapment efficiency obtained in F7.

Further increase the concentration of β –cyclodextrin in formulation F8, F9 and F10.

The entrapment efficiency also decreased.

IN VITRO DRUG RELEASE PROFILE OF NANOPARTICLES

- The *in-vitro* drug release of

Azilsartan nanoparticles can be carried out by membrane diffusion method for 24 hrs.

- The *in-vitro* drug release of Azilsartan nanoparticles with Polyvinyl alcohol and β – cyclodextrin.
- The *in-vitro* drug release of formulation F1 (Irbesartan 50 mg with polyvinyyl alcohol and 5 mg β -cyclodextrin) The percentage of *in vitro* drug release was 97% in 9 hrs.
- The formulation F2 was carried out by the increasing the polymer concentration (Azilsartan 50 mg with 50mg of Polyvinyl alcohol and 5 mg β -cyclodextrin) The percentage of *in vitro* drug release was found to be 96.40% in 11 hrs.
- The formulation F3 was carried out by further increasing in polymer concentration (Azilsartan 50 mg with 75 mg of Polyvinyl alcohol and 5 mg β -cyclodextrin) The percentage of drug release was found to be 98.44% in 13 hrs.
- The formulation F4 was carried out by further increasing in polymer concentration (Azilsartan 50 mg with 100mg of Polyvinyl alcohol and 5 mg β -cyclodextrin). The percentage drug release found to be 96.2% in 16 hrs.
- The formulation F5 was carried out by further increasing in polymer concentration (Azilsartan 50 mg with 25 mgof Polyvinyl alcohol and 10 mg β -cyclodextrin). The percentage drug release was found to be 98.0% in 19 hrs.
- The formulation F6 was carried out by further increased in polymer concentration (Azilsartan 50 mg with 50mg of Polyvinyl alcohol and 10 mg β -cyclodextrin). The percentage drug release was found to be 94.42% in 24 hrs.
- The formulation F7 was carried out by combination of (Azilsartan 50 mg with 75mg of Polyvinyl alcohol and 10 mg β -cyclodextrin). The percentage of drug release was found to be 98.46% in 24 hrs.
- The formulation F8 was carried out by the combination of increasing the polymer concentration of (Azilsartan 50 mg with 100mg of Polyvinyl alcohol and 5 mg β -cyclodextrin) percentage drug release was found to be 88% in 24 hrs.
- The formulation F9 was carried out by the combination of increased polymer concentration (Azilsartan 50 mg with 25 gmofPolyvinyl alcohol

and 15 mg β - cyclodextrin) percentage of drug release was found to be 95% 14 hrs.

- The formulation F10 was carried out by the combination of increased polymer concentration (Azilsartan 50 mg with 50gmof Polyvinyl alcohol and 15 mg β - cyclodextrin) percentage of drug release was found to be 96.4% 17 hrs.
- From the above formulation (F1-F10) confirms that the percentage of drug release was satisfactory in formulation F7 and it shows higher percentage of drug release of 98% for 24 hrs. So it was selected as a optimized formulation.

When increasing the polymer concentration the *in vitro* drug release also increased to a certain extent in the drug and polymer ratio up to 1:1.5

Further the polymer concentration is increased in F8 formulation the *in vitro* drug release increased but not extend upto 24hrs. So F7 was selected as a optimized formulation.

COMPARITIVE INVITRO RELEASE STUDY OF AZILSARTAN NANOPARTICLE FORMULATIONS F1 TO F10

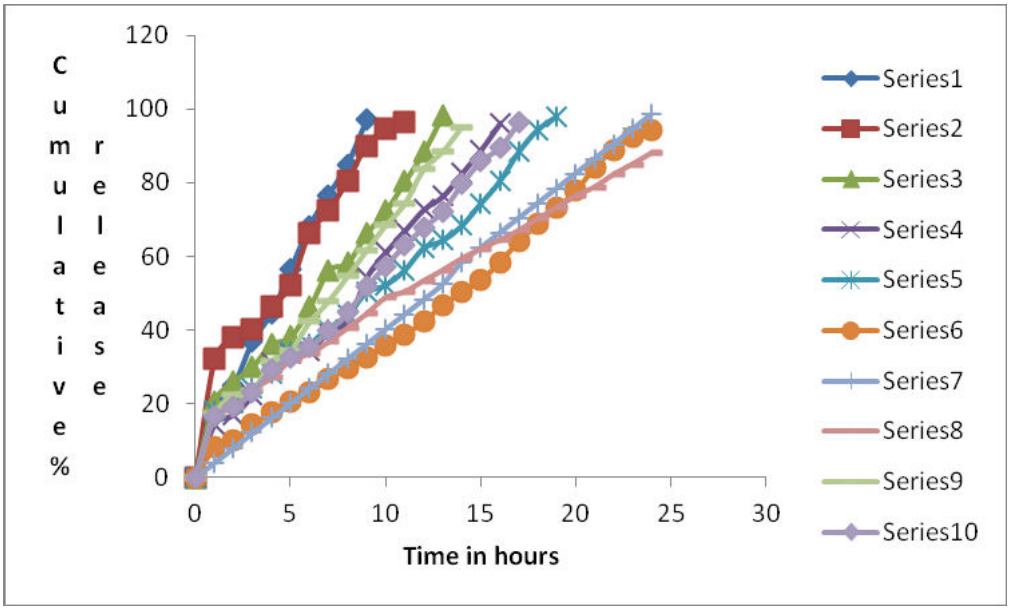

Figure: 3

SCANNING ELECTRON MICROSCOPY

The surface characteristics of optimized formulation (F7) particle size were studied by scanning electron microscope.SEM image of prepared nanoparticle formulation shows the coating of polymer mixture on drug particles. The appearance of nanoparticles in scanning electron microscope is in granule form, which indicates a thin and uniform coating over the drug. SEM image revealed that the Azilsartan nanoparticles were in nano size range,and smooth spherical in shape in this F7 Formulation.

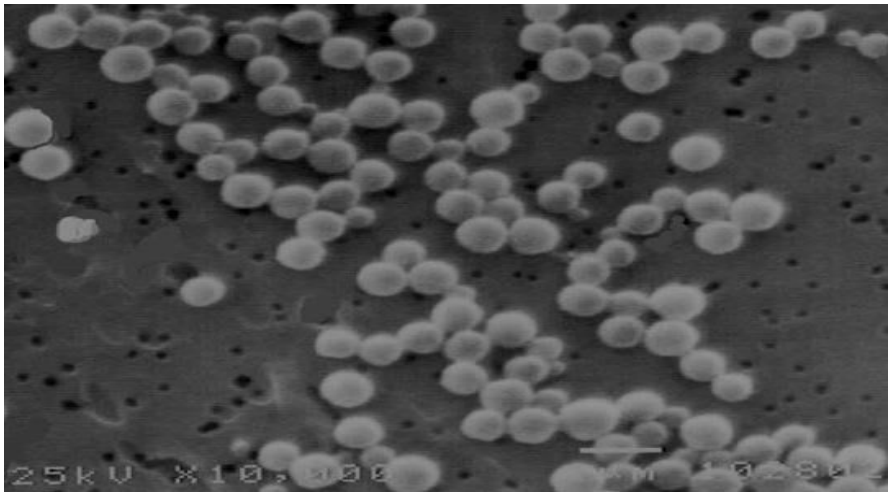

Figure 4: SEM Image of F7

SURFACE CHARGE (ZETA POTENTIAL

The zeta potential of a nanoparticles is commonly used to characterize the surface charges property of nanoparticles. It reflects the electrical potential of particles influenced by the composition of the particles band the medium in which it is dispersed. When nanoparticles formulations are administered through intravenous route they are easily identified and detected by the phagocytes. The particle size and the hydrophobicity surface of the nanoparticles determine the adsorption of blood components (proteins) called as opsonins. The opsonin in turn decides the fate of the nanoparticles. Binding of these opsonins on to the surface is known as Opsoniazation. Non modified nanoparticles were rapidly opsoniazed and gets easily eliminated from the body. Hence, to increased minimize the opsoniazation and to prolong the circulation of nanoparticles *invivo*. The zeta potential of the nanoparticle formulation with poly vinyl alcohol (formulation F7) particles which present in the formulation are de-aggregated and remain same and more stable in the substance and zeta potential (mV) is 38 mv and zeta Deviation (mV) is 9.16 and

conductivity (mS/cm) is 0.899.So this polymer is more suitable for nanoparticles preparation and the result shows smooth surface character and efficient repelled action and it decreases the opsoniazation.

Kinetic of drug release of first order for optimized formulation F7

The optimized formulation F7 was introduced into graphical treatment for kinetics of drug release.

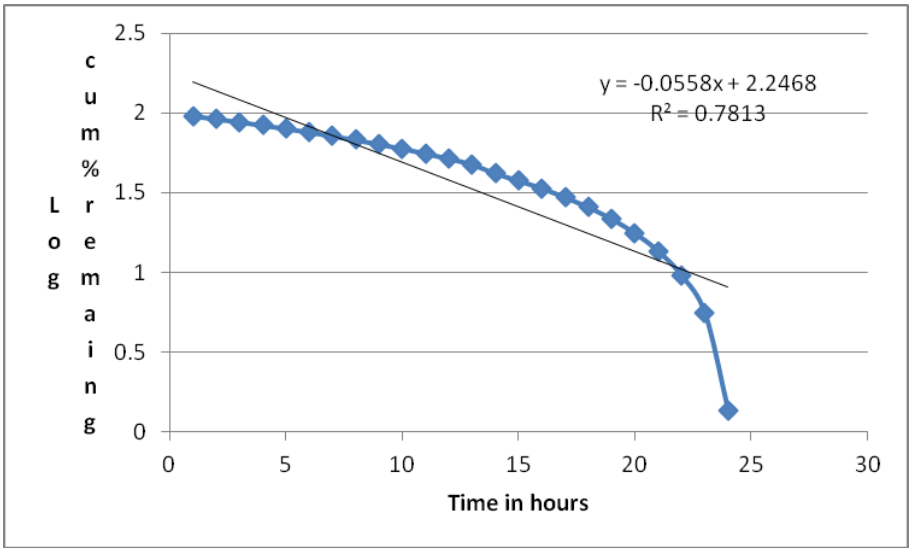

Figure 5: First order plot for formulation F7

Regression = 0.7813

The optimized formulation F7 of nanoparticles is more suitable for parentral administration it shows the *invitro* release kinetic study. The first order plots were obtained by plotting log remaining cumulative percentage drug release versus time. The regression value is 0.7813.

CONCLUSION

The present study was aimed to develop a nanoparticulate drug delivery system of antihypertensive drug Azilsartan using polymer (poly vinyl alcohol).The polymer enhances the binding of Azilsartan nanoparticles in specific or targeted site with sustained release of drug increasing therapeutic efficacy. These nanoparticles may also reduce the dose frequency with desired therapeutic response.

All batches of nanoparticles (F1-F10) were prepared by nano precipitation method.

The entrapment efficiency of the optimized formulation F7 (drug 50mg, polyvinyl alcohol 75mg, β –cyclodextin 10 mg) was 99.38 ±0.08 and *invitro* drug release was 98.46% after 24 hours. It also

obey the zero order, follows diffusion and erosion mechanism of release.

Surface morphology of optimized formulation (F7) indicated that lrbesartan nanoparticles were found to be in average nanometer range(358.4nm) and showed ideal surface morphology.

The stability test performed revealed that the formulation (F7) showed no change in its characters. The optimized formulation (F7) was also examined for zeta potential determinations.

The formulation(F7) showed maximum deviation of 9.16 mV which demonstrated that the particles are separate and highly repelling property found to be more useful in decreasing opsonization and favors target specificity. The developed Azilsartan nanoparticle formulation increases water solubility, reduces the dose frequency and improves the bioavailability of drug.

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