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RESEARCH ARTICLE

FORMULATION AND EVALUATION OF AZILSARTAN NANOSUSPENSION BY SOLVENT EVAPORATION METHOD

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ABSTRACT

The current research deals with formulation and evaluation of Azilsartan nano suspension. Nanosuspension containing drug was prepared by solvent evaporation method by using combinations of SLS, Polaxomer, PVP-K25, and methanol and quantity sufficient water). The Nanosuspension were evaluated for parameters such as Entrapment efficiency, scanning electron microscopy, particle size analysis, zeta potential, in-vitro release, drug excipient interactions (FTIR). The entrapment efficacy of the formulated Nanosuspension was found to be in the range of 76.02%- 96.05% respectively. Zeta potential value for the optimized formulation (F6) was found to be within the acceptable limits. Average particle size of nanosuspension of optimized formulations (F6) was found to be 489.7nm. From the invitro studies we can say that formulation F6 shows best drug release of 98.22% within 30 minutes where as all the other formulations takes about 45-60 minutes to release the drug. The drug release from the Nanosuspension was explained by the using mathematical model equations such as zero order, first order. Based on the regression values it was concluded that the optimized formulation F6 follows First order kinetics.

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INTRODUCTION

Azilsartan medoxomil is an angiotensin II receptor antagonist indicated for the treatment of mild to moderate essential hypertension. Efficacy of Azilsartan can be enhanced by improving its dissolution. Reduction in particle size to nanoscale can help in dissolution improvement by increase in surface area. Due to its high half life it can be formulated as a Nanosuspension. Nanosuspension is a novel technique used to increase the solubility of the drug prepared by solvent evaporation method. The Nano suspensions are novel promising target and controlled released dosage form which is gaining importance because of ease of manufacturing and diversified applications. The present trend of pharmaceutical research lies in the usage of biodegradable polymer because of its availability and low toxicity. Nanosuspension containing drug was prepared by solvent evaporation method by using SLS, Polaxomer, PVP K25 and methanol as organic solvent.

MATERIALS AND METHODS

Materials

Azilsartan is obtained as gift sample from Spectrum labs, Hyderabad, Sodium lauryl sulphate, polyoxamer, PVP K25, methanol were used. All the reagents used are of LR grade.

Method of preparation

Solvent evaporation method: Nanosuspensions was prepared by the solvent evaporation technique. Azilsartan was dissolved in methanol at room temperature (organic phase). This was poured into water containing different stabilizers of PVP K25, pluronic F127 and SLS maintained at room temperature and subsequently stirred on magnetic stirrer which is stirred at rpm 800-1000 for 30 min to allow the volatile solvent to evaporate. Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer containing water. Organic solvents were left to evaporate off under a slow magnetic stirring of the Nanosuspensions at room temperature for 1 hour followed by sonication for 1 hour.

Evaluation Parameters

Pre Formulation Studies

Melting Point: The temperature at which the first particle of the substance completely melts is regarded as melting point of the substance. The temperature at which the first particle starts to melt and last particle completely melts is regarded as the range of melting point.

Solubility studies: Solubility of Azilsartan was carried out in different solvents –like 0.1N HCL, 6.8pH buffer, 7.4pH buffer,

ethanol, and methanol . Saturated solutions were prepared by adding excess drug to the vehicles and shaking on the shaker for 48 hr. at 25°C under constant vibration. Filtered samples (1ml) were determined spectrophotometrically at 231 nm.

Drug-Excipient Interactions Studies: There is always possibility of drug- excipient interaction in any formulation due to their intimate contact. The technique employed in this study is IR spectroscopy.IR spectroscopy is one of the most powerful analytical technique, which offers possibility of chemical identification. The IR spectra was obtained by KBr pellet method. (Perkin-Elmer series 1615 FTIR Spectrometer).

Determination of absorption maximum (λmax): Determination of Azilsartan λ-max was done in pH 6.8 buffer medium for accurate quantitative assessment of drug dissolution rate.

Preparation of calibration curve of azilsartan

Procedure for standard curve in pH 6.8: 10 mg of Azilsartan was dissolved in 10 ml of pH 6.8 by slight shaking (1000 µg/ml). 1 ml of this solution was taken and made up to 10 ml with pH 6.8, which gives 100 µg/ml concentration (stock solution). From the stock solution, concentrations of 2, 4, 6, 8, 10 and 12 µg/ml in pH 6.8 were prepared. The absorbance of diluted solutions was measured at 231nm and a standard plot was drawn using the data obtained.

EVALUATION PARAMETERS

Entrapment efficacy: The freshly prepared nanosuspension was centrifuged at 20,000 rpm for 20 min at 5°C temperature using cool ultracentrifuge. The amount of un incorporated drug was measured by taking the absorbance of the appropriately diluted 5 ml of supernatant solution at 231 nm using UV spectrophotometer against blank/control nanosuspensions. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken.

The entrapment efficiency (EE %) could be achieved by the following equation:

%Entrapment efficiency= Drug content *100/Drug added in each formulation

Scanning electron microscopy: The morphological features of Azilsartan nanosuspension are observed by scanning electron microscopy at different magnifications.

Particle size and shape: Average particle size and shape of the formulated nanosuspensions was determined by using Malvern Zetasizer ZS using water as dispersions medium. The sample was scanned 100 times for determination of particle size.

Zeta potential: There are three ways by which a solid particle (colloid) dispersed in a liquid media can acquire a surface charge. First, by the adsorption of ions present in the solution. Second, by the ionization of functional groups on the particle's surface. Third, due to the difference in dielectric constant between the particle and the medium. Attention should be paid to the formation of electric double layer at the solid-liquid interface. The zeta Potential is defined as the difference in potential between the surface of the tightly bound layer (shear plane) and the electro-neutral region of the solution. The potential gradually decreases as the distance from the surface increases. As the concentration of electrolyte increases in the medium, the zeta potential falls off rapidly due to the screening effect of the counter ions (Figure). The zeta potential cannot be measured directly; however, it can be calculated using theoretical models and from experimentally determined electrophoretic mobility data. The theory is based on electrophoresis and can be expressed as:

$\mu = \zeta\varepsilon/\eta$

Where (µ) is the electrophoretic mobility, (ε) is the electric permittivity of the liquid, (η). Is the viscosity and (ζ) us the zeta potential.

***In vitro* drug release study:**[61] In vitro dissolution study was performed by USP dissolution apparatus-type II using 900 ml of 6.8pH buffer as a dissolution medium maintained at 37 ± 0.5°C and stirring speed (50 rpm). The freshly prepared nanosuspensions of drug: stabilizer ratios were added to the dissolution medium, five-milliliter samples were withdrawn at specific intervals of time, then filtered through a 0.45 µm filter paper and analyzed for their drug concentrations by measuring at 231nm wavelength.

The results of in vitro release profiles obtained for the NDDS formulations were fitted into

Four models of data treatment as follows:

- Cumulative percent drug released versus time (zero order kinetic model).
- Log cumulative percent drug remaining versus time (first- order kinetic model).

Table 1.1. Wavelength of maximum absorption of Azilsartan in 6.8pH buffer

Sl. No.	Solvent	λ_{max}
1	6.8pH buffer	231nm

Table 1.2. Standard calibration data of Azilsartan in 6.8pH buffer

Concentration (µg/ml)	Absorbance
0	0
2	0.109
4	0.207
6	0.298
8	0.402
10	0.493
12	0.604

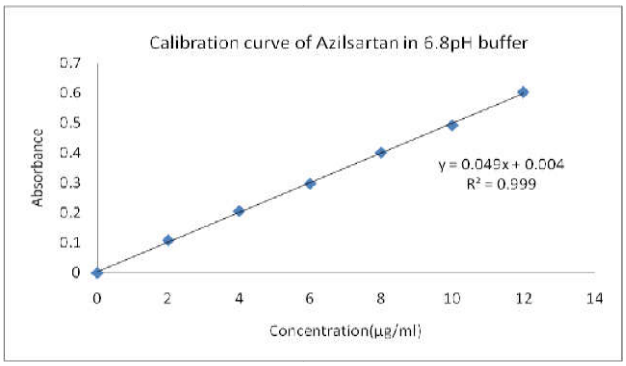


Fig 1.1. Standard calibration curve of Azilsartan in pH 6.8

Where, ft = fraction of dose released at time't'
KH, Ko, and Ks = release rate constants characteristic to respective models

Qo = the drug amount remaining to be released at zero hour
Qt = the drug amount remaining to be released at time't'
Wo = initial amount of drug present in the matrix
Wt = amount of drug released at time't'

Zero Order Kinetics: A zero-order release would be predicted by the following equation.

$A_t = A_0 - K_0 t$

Where:

A_t = Drug release at time 't'
A_0 = Initial drug concentration.
K_0 = Zero-order rate constant (hr^{-1}).

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to K_0.

First Order Kinetics: A first-order release would be predicted by the following equation

$$\textbf{Log C = Log C0-} \frac{Kt}{2.303}$$

Where:

C = Amount of drug remained at time't'
C_0 = Initial amount of drug
K = First-order rate constant (hr^{-1}).

When the data is plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follows First-order kinetics. The constant 'K' can be obtained by multiplying 2.303 with slope values.

RESULTS AND DISCUSSION

Determination of melting point: The melting point of Azilsartan was found to be in range of 212°C which was determined by capillary method.

Solubility studies: Saturation solubility was carried out at 25^0C using 0.1N HCL, 6.8 phosphate buffer, and other solvents. Solubility studies in various buffers says that pH 6.8 phosphate buffer has more solubility when compared to other buffer solutions. So pH 6.8 buffer is used as dissolution medium , based upon the solubility studies on organic solvents methanol has more solubility than others so methanol was used in the nanosuspension formulation.

Determination of absorption maximum (λmax): The absorption maxima of Azilsartan is obtained at 231nm depicted in Table 1.1 and Fig 1.4

Calibration curve of Azilsartan at λmax of 262nm: The linearity was found to be in the range of 2-12 µg/ml in acetone, pH 6.8 buffer. The regression value was closer to 1 indicating the method obeyed Beer-lamberts' law given in table 1.2 and Fig:1.1.

Drug polymer interaction (FTIR) study: Drug and excipient compatibility was confirmed by comparing spectra of FT-IR analysis of pure drug with that of various excipients used in the formulation. From the drug excipient compatibility studies we observe that there are no interactions between the pure drug (Azilsartan) and optimized formulation (Azilsartan+ excipients) which indicates there are no physical changes as shown in Fig 1.2 and Fig 1.3.

Entrapment efficiency: The entrapment efficacy of formulation F1-F9 was found to be 76.02%- 96.05% from Table 1.4

Surface morphology (SEM): The surface morphology of the Azilsartan was studied by SEM. SEM photographs of the optimized formulation was shown in the Fig.1.5. The surface of particles was found to be smooth from SEM analysis.

Zeta Potential: The measurement itself is a particle electrophoresis, the particle velocity is determined via the doppler shift of the laser light scattered by the moving particles. The field strength applied was 20 V/cm. The electrophoretic mobility was converted to the zeta potential in mV using the Helmholtz-Smoluchowski equation.

At standard measuring conditions (room temperature of 25 °C, water) this equation can be simplified to the multiplication of the measured electrophoretic mobility (µm/cm per V/cm) by a factor of 12.8, yielding the ZP in mV. zeta potential value for the optimized formulation (F6) was found to be within the acceptable limits was shown in Fig: 1.6.

Particle size analysis: Average particle size of nanosuspension of optimized formulations (F6) was found to be having maximum particles at a range of 489.7 nm was shown in Table 1.7.

***Invitro* dissolution studies:** The *invitro* performance of Azilsartan nanosuspension was as shown in table . Among F1-F9 formulations, F1-F3 formualtions were formulated by using SLS in three different ratios (i.e., 20mg, 30mg, 40mg). From the above invitro studies we can say that 40mg of SLS shows maximum drug release at the end of 60mins. So further trails were formulated to decrease the drug release time. F4-F6 formualtions were formulated by using PVPK-25 in three different ratios (i.e., 20mg, 30mg, 40mg). From the above invitro studies we can say that 40mg of PVPK-25 shows maximum drug release at the end of 30mins, where as remaining F4 & F5 formulatoions 45-60mints. So further trails were formulated to decrease the drug release time. F7-F9 formualtions were formulated by using poloxamer in three different ratios (i.e., 20mg, 30mg, 40mg). From the above invitro studies we can say that 40mg of poloxamer shows maximum drug release at the end of 45mins, where as remaining F7 & F8 formulations at 60mints.From the above invitro studies we can say that increase in the polymer concentration decrease in the dissolution time of all the formulations. So F6 is considered as optimized formulation as it shows drug release with in 30mins.

Release kinetics of Azilsartan Nano suspension: In vitro drug release data of all the Nanosuspension formulations of Azilsartan was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetics and according to equations of drug release.

Table 1.3. Formulation design of Azilsartan Nano suspensions using different ratios of drug and polymers

Ingredient(mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Azilsartan	40	40	40	40	40	40	40	40	40
SLS (mg)	20	30	40	--	--	--	--	--	--
PVP K25(mg)	--	--	--	20	30	40	--	--	--
Pluronic F127	--	--	--	--	--	--	20	30	40
Methanol (ml)	3	3	3	3	3	3	3	35	3
Water (ml)	40	40	40	40	40	40	40	40	40

Drug polymerinteraction (FTIR)study

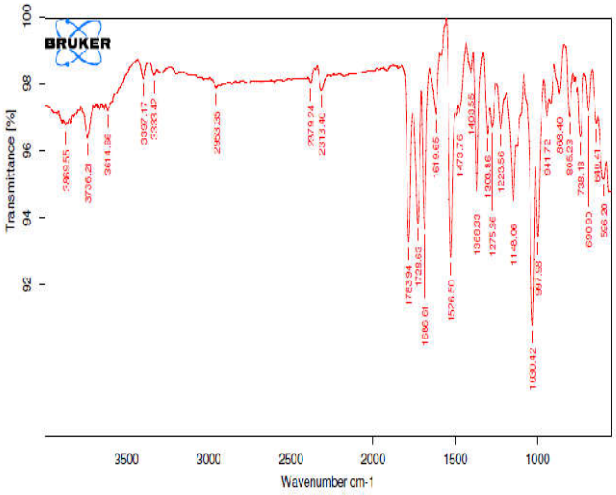


Fig 1.2. IR spectra of Azilsartan

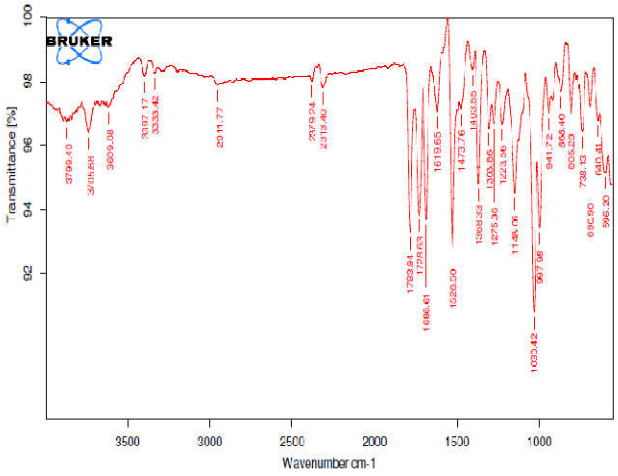
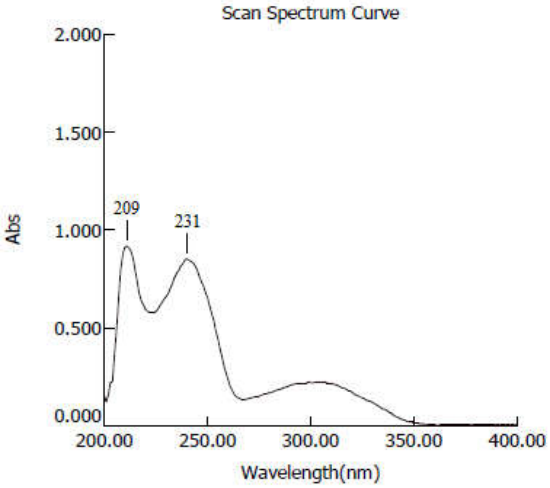


Fig 1.4. λmax of Azilsartan in 6.8 PH Phosphate buffer



Surface morphology-Scanning Electron Microscopy (SEM)

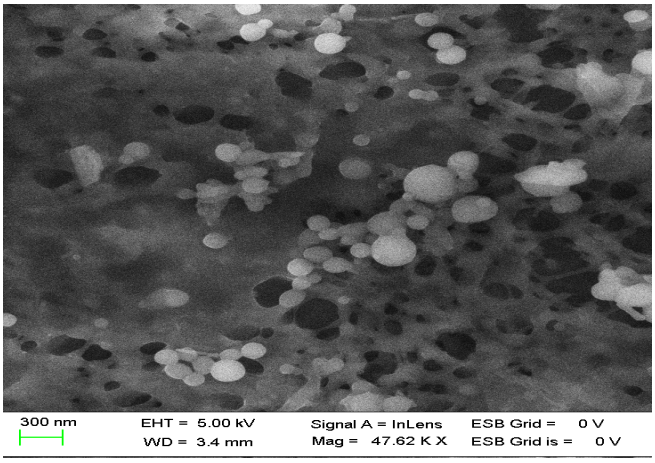
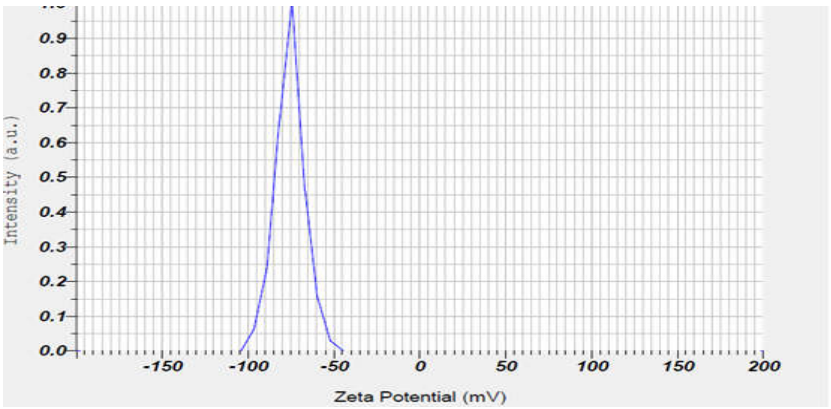


Fig 1.5. SEM photographs of Azilsartan optimized formulation



Measurement Results

Zinc-1.nzt

Measurement Results

Measurement Type	: Zeta Potential
Sample Name	:
Temperature of the Holder	: 25.2 °C
Dispersion Medium Viscosity	: 0.892 mPa·s
Conductivity	: 0.183 mS/cm
Electrode Voltage	: 3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-75.6 mV	-0.000587 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	: -75.6 mV
Electrophoretic Mobility Mean	: -0.000587 cm²/Vs

Fig: 1.6. Zeta potential of optimized formulation

Particle size analysis

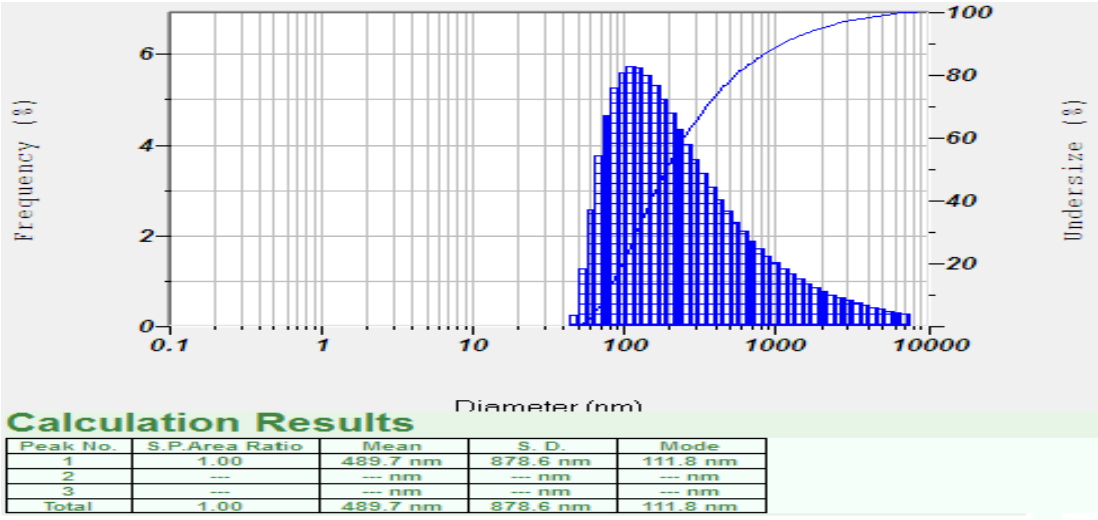


Fig. 1.7 Particle size analysis

Table 1.4. Entrapment efficiency of formulated Nanosuspensions

Formulation code	Mean % entrapment efficiency
F1	76.52±0.12
F2	82.23±0.62
F3	86.04±0.34
F4	83.14±0.85
F5	90.63±0.96
F6	95.42±0.01
F7	90.61±0.34
F8	91.23±0.61
F9	96.05±0.42

Table: 1.5 *In-vitro* drug release data of Azilsartan Nanosuspensions from formulations F1to F9

TIME (Min)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
5	23.62±0.52	36.84±0.02	42.75±0.23	43.56±0.23	49.63±0.12	50.63±0.56	32.52±0.53	39.84±0.2	47.85±0.96
10	29.52±0.41	43.51±0.34	49.52±0.45	52.41±0.40	56.95±0.36	64.29±0.42	39.84±0.26	49.61±0.13	59.61±0.32
15	35.08±0.96	57.84±0.65	60.52±0.16	63.41±0.85	69.85±0.63	74.63±0.18	46.82±0.09	57.06±0.56	66.75±0.63
20	49.12±0.32	64.85±0.98	68.52±0.33	69.85±0.54	78.51±0.59	86.41±0.64	53.74±0.14	70.33±0.91	74.08±0.44
30	60.85±0.05	76.84±0.86	82.63±0.69	76.95±0.91	89.63±0.45	98.22±0.35	69.85±0.32	79.82±0.38	89.63±0.01
45	72.63±0.54	85.63±0.43	89.63±0.93	85.41±0.62	97.42±0.21	-	77.05±0.60	89.63±0.62	98.4±0.20
60	80.96±0.23	92.63±0.24	98.4±0.51	92.63±0.32	-	-	86.63±0.22	99.02±0.23	-

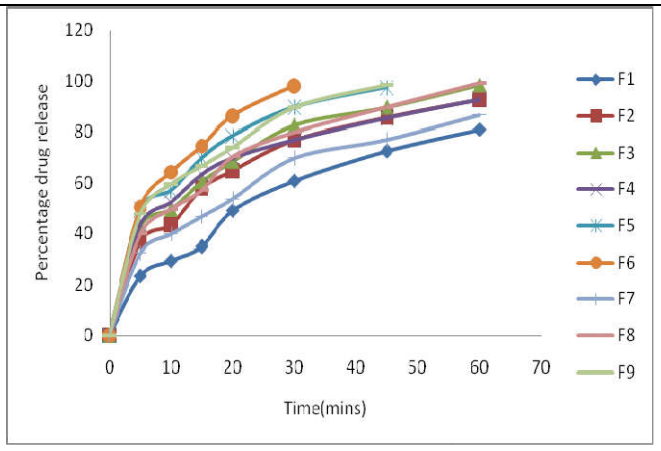


Fig: 1.8 Dissolution parameters for the formulations F1-F9

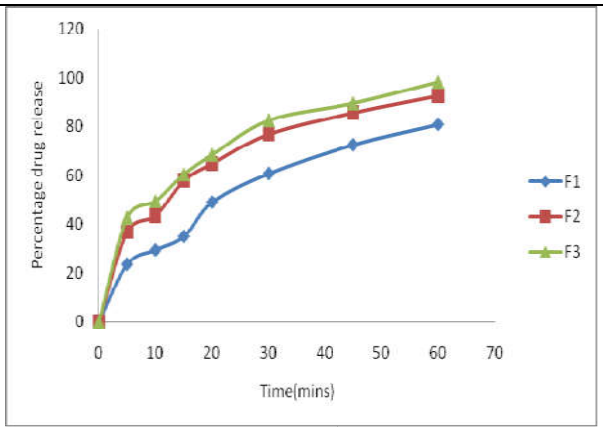


Fig : 1.9 Dissolution parameters for the formulations F1-F3

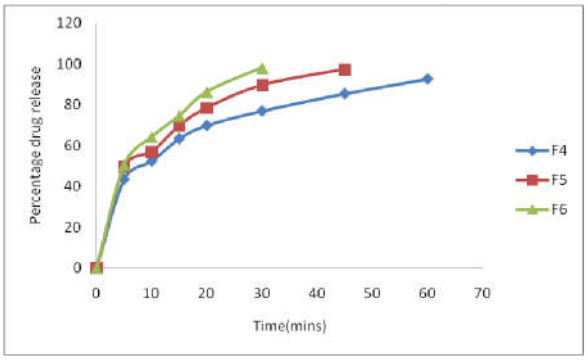


Fig: 1.10 Dissolution parameters for the formulations F4-F6

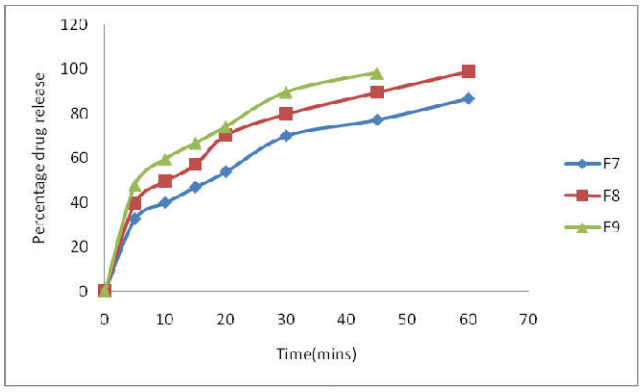


Fig:1.11 Dissolution parameters for the formulations F7-F9

ZERO ORDER RELEASE KINETICS:

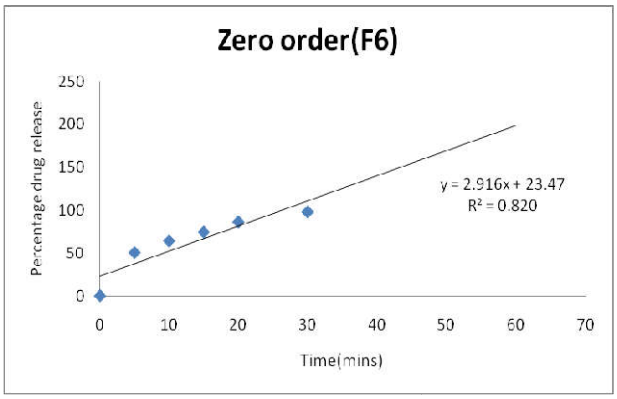


Fig. 1.12. Zero order release profile of formulation F6

FIRST ORDER RELEASE KINETICS:

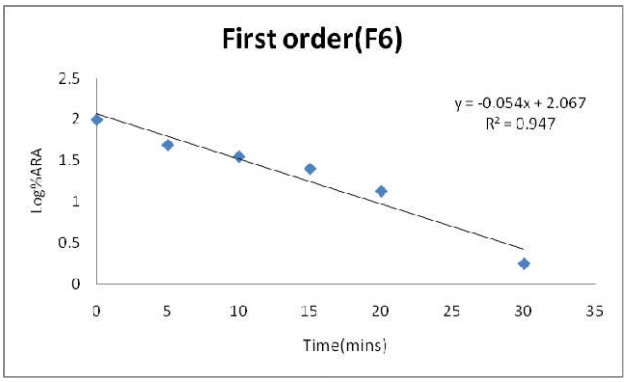


Fig. 1.13. First order release profile of formulation F6

Table1.6. Kinetic data of the formulation F6 (optimized formulation)

ORDE OF KINETICS	ZERO ORDER	FIRST ORDER
REGRESSION	0.820	0.947

The results of linear regression analysis including regression coefficients from the above data it is evident that the optimized formulation (F6) follows zero-order release kinetics. The drug release from the Nanosuspension was explained by using mathematical model equations such as zero order, first order. Based on the regression values it was concluded that the optimized formulation F6 follows First order kinetics.

Conclusion

From the above experimental results it can be concluded that: Oral Nanosuspension of Azilsartan by solvent evaporation method using various polymers such as SLS, Polaxomer, PVP-K25 and Methanol. The entrapment efficacy of the formulated Nanosuspension was found to be in the range of 76.02%-96.05% respectively. As the polymer concentration increases, the drug release time decreases, whereas Nanosuspension strength increases. Optimized formulations of Nanosuspension displayed first order release kinetics and drug release. IR spectroscopic studies indicated that there are no drug-excepient interactions. When compared to other all the formulations F6 is the best formulation which showed 98.22% of drug released respectively with in 30 min and follows first order release kinetics. Hence from the study it was concluded that the solubility of Azilsartan drug was successfully enhanced by using Nanosuspension prepared by solvent evaporation method using PVP K-25(40mg).

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