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Development and Evaluation of Multiparticulate Pulsatile Drug Delivery System Containing Azelnidipine

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ABSTRACT:

The objective of this work was to develop and evaluate multiparticulate pulsatile drug delivery for the chronotherapeutic delivery of Azelnidipine. The drug loaded pellets was prepared by solution layering technique using PVP K-30 (2%) as binder. The drug loaded nonpareil beads were coated with polymer solution mixture of (Eudragit RS 100 and RL 100) at different composition using triethyl citrate (TEC) as a plasticizer and talc antisticking agent in a mixture of IPA: DCM (7:3). The prepared pellets were then evaluated for FTIR, Flow properties, friability, drug content, SEM and in vitro dissolution study. FTIR study reveals no interaction between drug and polymer. SEM study confirmed the smooth appearance of coating over the surface of pellets. Pellets coated with 10% polymer coating weight gain showed promising lagtime and delay drug release. Batch F6 prepared with 60:40 ratio of eudragit RS and RL 100 polymer gives total lag time of 4hrs and drug release of 96.23±5.41% in 8 hr. Further optimized coated pellets formulation was evaluated for pharmacokinetics study. In vivo study also confirmed the delay appearance of drug in plasma after considerably long lag time. This study concludes that solution layering technique followed by coating with combination of eudragit polymer can be used for the development of multiparticulate drug delivery system for the chronotherapeutics management of hypertension.

Keywords: Azelnidipine, Eudragit RS 100, Eudragit RL 100, Multiparticulate Pellets, etc.

1. Introduction

The oral controlled-release system shows a typical pattern of drug release in which the drug concentration is maintained in the therapeutic window for a prolonged period of time, thereby ensuring sustained therapeutic action. There are certain conditions for which such a release pattern is not suitable that demand release of drug after a lag time. i.e. pulsatile drug delivery system (PDDS) [1,2]. A pulsatile drug delivery system is a specialized type of drug delivery system designed to release a drug in a pulsatile or pulsating manner, rather than the continuous release observed in conventional drug delivery systems. This system is particularly useful for drugs that require a specific pattern of release to achieve optimal therapeutic effects or when the drug's release needs to be synchronized with the body's natural circadian rhythms or biological events.

Hypertension is a serious medical condition that can lead to severe complications and escalate the risk of heart diseases, stroke [3]. Hypertension exhibits circadian variation and this contribute to the increase of acute cardiovascular events that occur in early morning hours. The occurrence of these events can be prevented by reducing the morning rise in blood pressure.

It was evidenced that hypertensive patients have higher heart rate and blood pressure during morning hours, this is basically due to the decrease in sympathetic output occurring at bed time. In morning when the individual wakes up have a rapid rise in both systolic blood and diastolic blood pressure. The capillary resistance and the vascular reactivity are higher in early morning and then declines slowly during the day time. This shows the risk of myocardial ischemia, acute myocardial infarction, angina pectoris, cardiac failure, and sudden cardiac arrest with higher expectancy during first hours of morning or in the late afternoon. Expert studies reported that the risk of heart attack and cardiac death are greater between 6.00 am and 12.00 noon. At the same time angina attack and congestive heart failure symptoms occur mostly during sleeping hours [4,5]. Many antihypertensive drugs can't control the early morning rise in blood pressure with once daily morning dose. Thus; there is the need of chronotherapeutics in hypertensive management that could reduce blood pressure and modify the distorted circadian profile more effectively. Azelnidipine is a calcium channel blocker, widely utilized for the treatment of hypertension. Hence the aim of this research was to developed the multiparticulate pulsatile drug delivery utilizing Azelnidipine for the effective chronotherapy and management of hypertension.

2. Materials and Methods

Azelnidipine was supplied as gift sample by Ipca laboratories, Mumbai India. Eudrgit RS 100 and RL 100 were purchased from Loba Chem, India. All other materials and solvents used were of analytical grade.

Preparation of Drug Loaded Pellets of Azelnidipine:

Drug loaded pellets of azelnidipine was prepared by solution layering technique. Solution mixture of drug azelnidipine was prepared along with PVP K-30 (2%) as binder in solvent mixture of IPA and water in the ratio of (80:20). The solution was then sprayed onto the rotating nonpareil seeds in laboratory coating pan. The flow rate of solution was maintained constant at 1 ml/min, so as to prevent the agglomeration of pellets during coating process. The inlet air temperature was maintained at 45°C. The drying time after each application of solution mixture was kept as 2 min. The speed of coating pan was maintained 25 rpm throughout the process. The layering process was continued till all the drug solution was

applied on surface. The drug-loaded pellets were finally dried in oven at 70°C for 30 min and were used for further study [6].

Formulations of coated Pellets

The drug loaded nonpareil beads were coated with polymer using solution mixture of two grade of polymer Eudragit RS 100 and RL 100 at different composition. 5% fixed polymeric solution concentration of Eudragit RL and RS 100 polymer was prepared by dissolving it in solvent mixture of IPA: DCM (7:3). Triethyl citrate (TEC) 15 %, w/w based on polymer weight was added as plasticizer and talc was added as antisticking agent (5% w/w). Required quantities of drug loaded pellets were taken and added into the coating pan. Pellets were then coated with polymeric solution. During the coating process the flow rate coating solution was maintained at 1 ml/min. The inlet drying temperature was maintained at 45°C. Drying time after each coating cycle was 3 min. Coating pan speed was used as 25 rpm. Coating of drug loaded pellets by polymeric solution was continued until attainment of weight gain of 5% and 10% w/w respectively. Finally, all coated pellets were store in amber color bottle at room temperature for further studies. The composition for coating of pellets was shown in table 1 [7,8].

Table 1: Formulation of Coated Pellets

Formulation Code	Weight Gain (%)	Drug loaded Pellets (g)	Eudragit RS 100 : RL 100 (Ratio)	IPA (ml)	DCM (ml)	TEC (mg)	Talc (mg)
F1	5	50	80:20	70	30	0.75	0.25
F2		50	60:40	70	30	0.75	0.25
F3		50	40:60	70	30	0.75	0.25
F4		50	20:80	70	30	0.75	0.25
F5	10	50	80:20	70	30	0.75	0.25
F6		50	60:40	70	30	0.75	0.25
F7		50	40:60	70	30	0.75	0.25
F8		50	20:80	70	30	0.75	0.25

Evaluation of Pellets

Fourier transforms infrared spectroscopy

The infrared (IR) spectrum of the coated pellets was compared with that of pure drug to confirm the chemical integrity of the drug in the formulations. The samples were powdered and mixed with dry powdered potassium bromide. The powdered mixture was taken in sampler and scanned in FTIR spectrophotometer (Jasco, FT/IR 4100).

Friability

Friability of pellets was determined by subjecting 10 g of pellets in (Roche friabilator) at 4 min at 25 rpm. The abraded samples were sieved and the pellets retained on the sieve were weighed and percent friability was calculated from the difference in the weight of the pellets before and after friability.

Flow properties

The flow properties of polymer coated pellets were studied by determining its Carr compressibility index and Hausner ratio. The data of the tapped density and bulk densities were utilized for the determination of flow properties of the coated pellets.

Drug content

Required weight of drug loaded pellets containing equivalent weight of 8 mg of azelnidipine were taken and crush to powdered. The powder was then transferred in to 100 ml volumetric flask and dissolved in 100 ml of methanol. The solution was shaken and then filtered. After appropriate dilution the drug content was measured using a UV spectrophotometer by taking absorbance of sample at 255 nm. [9]

Scanning electron microscopy (SEM)

Scanning electron microscopy was performed to examine the surface morphology of the formed pellets. SEM images of the pellets were taken before and after coating using (JEOL, Japan) Scanning microscopy. The sample was manually spread onto a sample stub with help of double adhesive carbon coated tape that was glued to an aluminum stub. Fine gold coating was applied with help of sputter coater under a pressure of 0.1 torr, that gives thin coating (30Å) of gold. The samples were examined at various magnification to examine surface morphology.

In vitro drug release

In vitro drug release study was performed in dissolution test apparatus (Electrolab). The study was conducted in 900 ml of dissolution medium maintained at 37±0.5°C with a basket. The rotation speed of basket was at 50 rpm. Polymer coated pellets was added in to the 900 ml of 0.1N hydrochloric acid (pH 1.2) as a dissolution medium for first 2 hrs followed by phosphate buffer pH 6.8 for rest of the time. Samples 5 ml were withdrawn at predetermined times interval using pipette. The amount of sample withdraw was replenished immediately with the same volume of dissolution medium in order to keep the total volume constant. Up on suitable dilution the sample were analyzed by UV Spectrophotometer (Shimadzu 1700) at 255 nm. [10,11]

In Vivo Pharmacokinetic Study

Study Design

In vivo pharmacokinetic studies was conducted on rabbits as per the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), after approval of study. The study was performed on healthy albino rabbits. Six male rabbits were randomly chosen having body weight of 1.5 - 2 kg. Experimental animals were kept under standard laboratory conditions for 1 week. Before drug administration, animals were allowed free access to water and kept fasted overnight and fasting will have continued until 4 hrs post dose. The 6 rabbits were divided into two groups each group contain 3 rabbits. Optimized formulation F6 was given to Group I rabbits, while group II rabbits received uncoated immediate release pellets as reference. Formulation was given to rabbit in capsule with feeding tube so as to avoid the biting of the formulation by rabbit. 1 ml of blood samples were collected though the marginal ear vein at time interval of 0.5, 1, 1.5, 2,3,4,6,8,10 and 12 hrs after dose administration. The collected blood sample were transfer in to heparin tubes. Blood samples were then centrifuged at 4,000 rpm for 10 min to obtained plasma. The obtained plasma was then stored at 4°C till further use. To analyzed the plasma sample, 100 µl of plasma, was mixed with 200 µl of acetonitrile so as to precipitate protein. The mixture was then vortexed for 2 min and centrifuged at 10,000 rpm for 10 min. The

supernatant was separated by filtration. The 20 µl sample was then injected into a HPLC (Agilent 1260, Agilent Technologies, USA), C18 column (Cosmosil, 5m, 4.6 x 250 mm), at a column temperature of 35°C, at a flow rate of 1 ml/min and examined at a wavelength of 255 nm.

Pharmacokinetic Data Analysis

The maximum plasma concentration (Cmax), the time taken to reach the maximum plasma concentration (tmax), and the area under the plasma concentration-time curve (AUC0→t and AUC0→∞), mean residence time (MRT) and t ½ were calculated using the PK solver software. Statistical analysis was performed using oneway ANOVA and $p < 0.05$ was considered statistically significant. [12]

3. Results and Discussion

Azelnidipine loaded pellets were prepared by using two grades of eudragit polymer (RS 100 and RL 100) at different composition. Eight different batches were prepared with 5% and 10% weight gain of pellets. Friability study of all batches of the coated pellets showed friability value less than 1% indicating optimum ability to withstand mechanical shock. The flow properties of all batches of coated pellets were determined. Tapped density and bulk densities for all the batches were falls within acceptable limit indicating good flow properties. Percent compressibility index and hausner ratio also indicates excellent flow properties for all batches of pellets. Drug content for all batches coated pellets were determined after dissolving the pellets in methanol and after specific dilution. The sample were analyzed spectrophotometrically the results for all the batches were falls in the ideal range of pharmacopoeial standard indicates effective distribution and deposition of drug among the pellets. The results for drug content were shown in table 2.

Table 2: Characterization of formulations F1 to F8

Batch Code	Bulk density (g/cc)	Tapped density (g/cc)	Compressibility Index (%)	Hausner's Ratio	Angle of Repose(Ɵ)	Friability (%)	Drug Content (%)
F1	0.288 ± 0.024	0.323 ± 0.014	10.83 ± 0.032	1.12 ± 0.024	20.32 ± 0.540	0.46 ± 0.24	96.18 ± 2.18
F2	0.288 ± 0.014	0.328 ± 0.011	12.19 ± 0.026	1.13 ± 0.021	21.26 ± 0.602	0.42 ± 0.36	98.60 ± 0.54
F3	0.284 ± 0.032	0.32 ± 0.017	11.25 ± 0.025	1.12 ± 0.031	20.5 ± 0.472	0.48 ±0.12	95.47 ± 1.22
F4	0.287 ± 0.016	0.336 ± 0.016	14.58 ± 0.031	1.17 ±0.024	22.56 ± 0.543	0.51 ±0.24	96.48 ± 0.58
F5	0.28 ± 0.027	0.328 ±0.024	14.63 ± 0.025	1.17 ± 0.026	24.64 ± 0.614	0.54 ±0.18	96.30 ± 1.23
F6	0.287 ± 0.018	0.321 ± 0.018	10.59 ± 0.027	1.11 ± 0.027	20.14 ± 0.862	0.42 ± 0.22	98.18 ± 1.51
F7	0.278 ± 0.026	0.32 ± 0.015	13.12 ± 0.034	1.15 ± 0.032	21.34 ± 0.844	0.56 ± 0.31	96.16 ± 0.62
F8	0.276± 0.012	0.324 ± 0.020	14.81 ± 0.030	1.17 ± 0.017	22.35 ± 0.723	0.44 ± 0.43	95.14 ± 1.65

All values represent mean ± standard deviation (n=3)

Scanning electron microscopy was performed to characterize the surface of the formed pellets. SEM photographs of the polymer coated nonpareil pellets were taken before and after coating. Optimized formulation F6 was examined for its surface morphology. Surface morphology study showed smooth surface of coated pellets as compare to its uncoated pellets counterpart which showed rough surface. It was also observed that polymer coating on the pellets was uniform and free from cracks which can able to hold the drug for considerable time. The SEM image of uncoated and coated pellets were shown in figure 1 and 2 respectively.

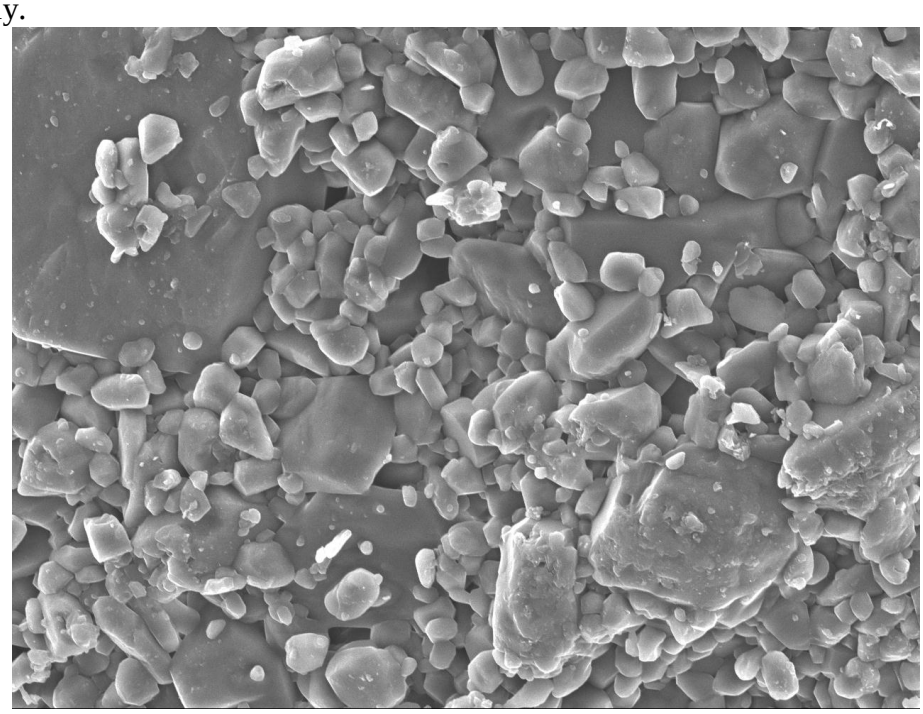

Figure 1: SEM of Uncoated Pellets at 100x

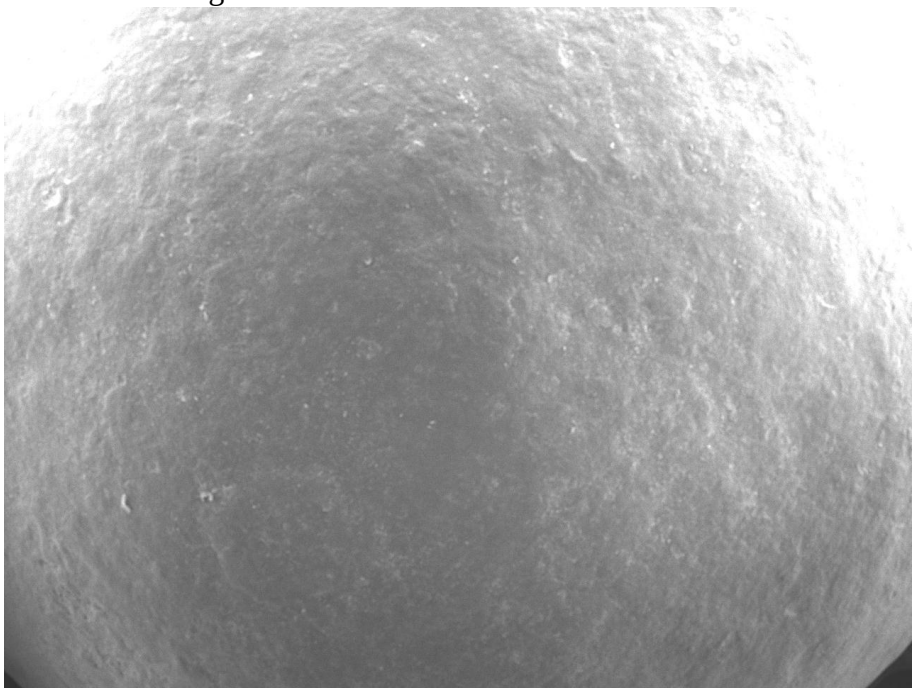

Figure 2: SEM of Azelnidipine Coated Pellets at 100x

In Vitro drug release

In vitro drug release study of prepared coated pellets was determined by performing dissolution study in 900 ml of dissolution medium. Basket type apparatus was utilized at rotation speed of 50 rpm. Two different type of dissolution medium was utilized for the study. 0.1N hydrochloric acid (pH 1.2) was used for the first 2 h and phosphate buffer pH 6.8 was used for remaining period of time. Formulation F1, F2, F3 and F4 was prepared with different ratio of Eudragit RS and RL 100 polymer, with weight gain of 5% showed 98.23%, 94.54%, 97.30% and 94.36% of azelnidipine release respectively, with lag time of 3 hrs, 2hrs, 1hrs and 0hrs respectively. It was observed that differences in the permeability and porosity of Eudragit RS100 and RL 100 polymer majorly affect the lag time and drug release. It was observed that as the concentration of more permeable Eudragit RL 100 increases, the drug release rate also increased and lag time decreased, due to availability of more pore for diffusion of drug. From the study it was found that formulation batch F1 to F4 was not optimum in the maintaining lag time and drug release up to 8 hrs, which confirmed that 5% coating weight gain is not enough to hold the drug for for time.

Formulation F5, F6, F7, and F8 prepared with similar composition of Eudragit RS and RL polymer with 10% of coating level weight gain, showed optimum drug release of 80.16%, 94.32%, 95.52% and 96.51% at the end of 8 hrs. Batch F5 and F6 showed lag time of 4 hrs, while batch F7 and F8 showed lag time of 3 hrs. This extended lag time in batch F5 and F6 might be due to higher concentration of less porous RS100, which provide minimum pathway for the drug release. Rate of drug release was slow up to the 6 hrs after that burst drug release was observed. As the concentration of more porous RL 100 increased in batch F7 and F8, the fall in lag time and increased in drug release observed. From the above observation, it was concluded that coated pellets with 5% polymer load (F1 to F4) was not able to maintain sufficient lag time and, release the drug in faster rate and didn't meet the requirement for this type of formulation. Coated pellets prepared with 10% polymeric weight gain (F5 to F8) was considered to be effective in term of sufficient lag time and drug release in given time period. Thus this study clearly indicates that, 10% coating level with this polymeric ratio is more suitable in the formulation and development of multiparticulate pulsatile drug delivery of azelnidipine. Among the all formulation, batch F6 prepared with eudragit RS100:Rl100 (60:40) ratio with 10% of polymeric level, was considered optimized on the basis of sufficient lag time and drug release for 8 hrs. Dissolution profile of batch formulation F1 to F6 is shown in figure 3.

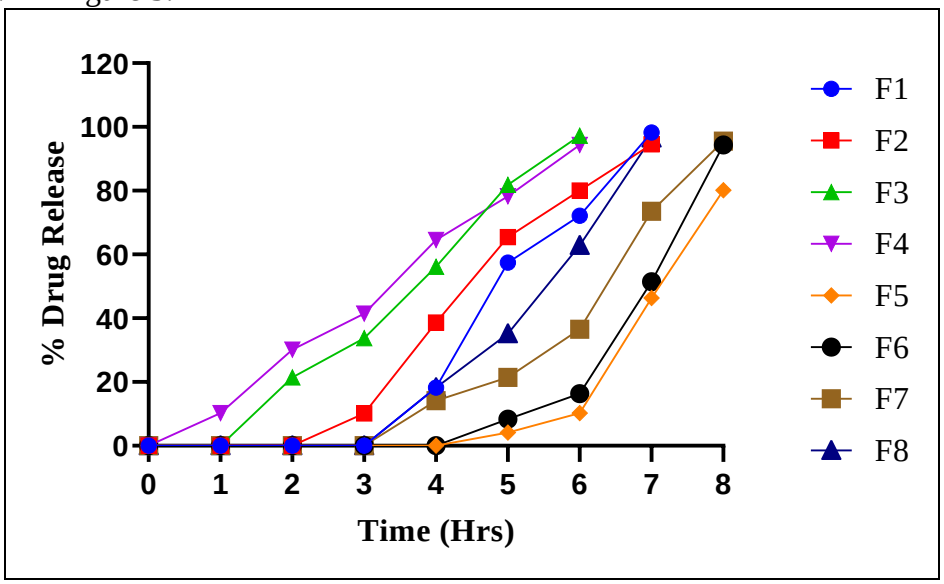

Figure 3: Dissolution profile of Azelnidipine from batch F1 to F8

In order to determine the possible mechanism of drug release form coated pellets the dissolution data of all batches were determined and calculated for various kinetic models like zero order, first order, higuchi model and Korsmeyer-Peppas model for both drug. Fitting of the Azelnidipine release rate data to the various models revealed that formulations F1, F2 F3, F4 and F8 follows the zero order model, while formulation F5, F6, and F7 follows Korsmeyer-Peppas model. Optimized formulation F6 followed Korsmeyer-Peppas model. The 'n' value of Korsmeyer-Peppas model for formulation F6 was 0.565, which indicates that the drug release mechanism from coated pellets was non-Fickian diffusion. [13,14] The details of Kinetic data analysis of formulations F1 to F8 for is shown in table 3

Table 3: Kinetic Data Analysis of Formulations F1 to F8 for Azelnidipine

Formulation Code	**Zero Order**	**First Order**	**Higuchi**	**Korsmeyer-Peppas**	
	R2	**R2**	**R2**	**R2**	**'n'**
F1	0.836*	0.724	0.526	0.715	0.465
F2	0.912*	0.808	0.638	0.882	0.304
F3	0.968*	0.765	0.796	0.917	0.251
F4	0.994*	0.852	0.882	0.989	1.04
F5	0.602	0.517	0.393	0.633*	0.542
F6	0.631	0.463	0.417	0.670*	0.565
F7	0.805	0.598	0.576	0.819*	0.478
F8	0.804*	0.7	0.522	0.718	0.435

*Indicates best fitted model

In-Vivo Pharmacokinetic study

In-vivo pharmacokinetic study was conducted on chosen optimized formulation F6. Non compartmental pharmacokinetic study was performed on azelnidipine after dosing the drug through oral route to the healthy rabbit. Optimized formulation F6 was given to Group 1 rabbits as a test, while group 2 rabbits received uncoated immediate release pellets as reference. The peak plasma concentration (C_{max}) of azelnidipine in uncoated immediate release pellets was found as 214.72± 10.86ng/ml in 4hr, while C_{max} of azelnidipine in coated pulsatile pellets was found as 204.70 ± 11.47ng/ml in 8 hr. The plasma drug concentration data suggests the delay in absorption and appearance of drug in plasma as compare to uncoated pellets, which suggest delay in drug absorption from pulsatile formulation. The AUC_0-t of azelnidipine in uncoated immediate release pellets and coated pellets (F6) was found to be 1237.31 ± 27.85 and 869.14 ± 38.71ng/ml*h. While the AUC_0-∞ value 1401.50 ± 102.41 and 1236.48 ± 98.16ng/ml*hr respectively. The MRT for azelnidipine in uncoated and coated formulation was found to be 6.48 ± 0.72 and 11.03 ± 0.27hr respectively.

Pharmacokinetic study clearly suggested the delay appearance of drug in plasma from coated optimized pellets formulation after oral administration when compared with their immediate release counterpart. This suggest that, drug will be in hold for first few hours in formulation and will release in burst after lag time, suggesting pulsatile form of drug delivery. The resultant pharmacokinetic parameters were tested for significance using one-way ANOVA. P value lesser than 0.05 was considered as significant. The in vivo pharmacokinetic parameters of uncoated and optimized coated pellets formulation (F6) is shown in table 4

Table 4: Pharmacokinetic Parameters of Azelnidipine in uncoated immediate release pellets and Optimized coated pellets (F6)

Pharmacokinetic Parameter	**Azelnidipine in Uncoated Pellets (Mean ± SD)**	**Azelnidipine in Optimized Coated Pellets (F6) (Mean ± SD)**
T_{max} (h)	4 ± 0.00	8 ± 0.00
C_{max} (ng/ml)	214.72± 10.86	204.70 ± 11.47
$t_{1/2}$ (h)	3.35 ± 0.74	3.06 ± 0.24
AUC $_{0-t}$ (ng/ml*h)	1237.31 ± 27.85	869.14 ± 38.71
AUC $_{0-inf.}$ (ng/ml*h)	1401.50 ± 102.41	1236.48 ± 98.16
MRT (hr)	6.48 ± 0.72	11.03 ± 0.27

Mean ± SD (n=3)

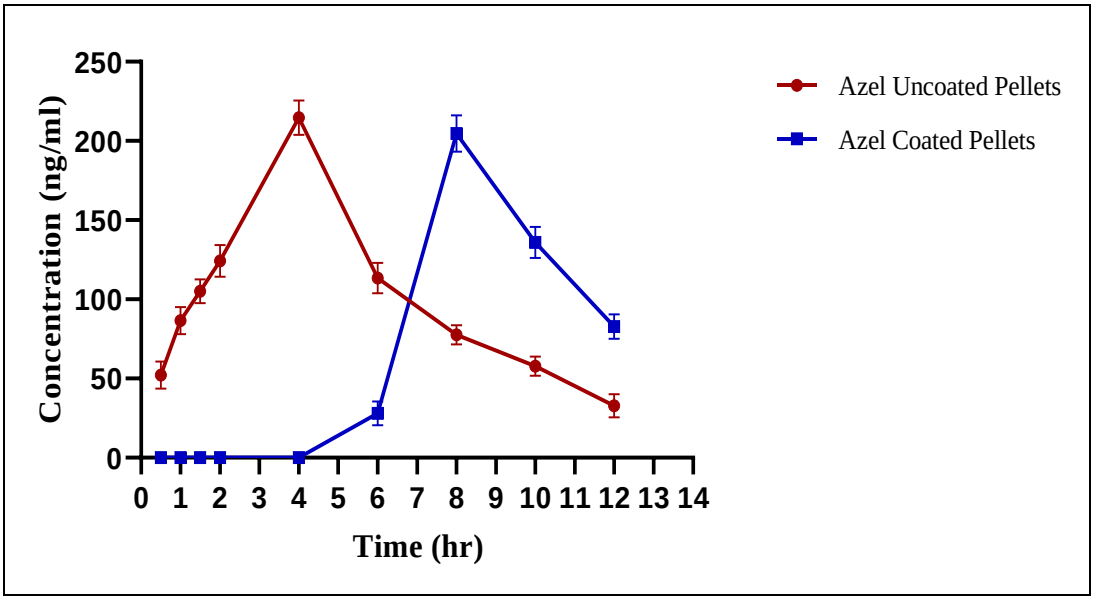

Figure 4: Plasma drug concentration profile of Azelnidipine in uncoated immediate release pellets Vs Optimized Coated Pellets (F6)

4. Conclusion

The present study was carried out to develop the multiparticulate pulsatile drug delivery system for azelnidipine. Drug loading on pellets by solution layring technique followed by coating drug loaded pellets using different composition and different weight gain by using coating pan given desired multiparticulate pulsatile pellets formulation which effectively delaying the drug release for 8 hrs. SEM study confirmed the effective and smooth coating of polymer over pellets surface. In vitro dissolution study confirmed that, 10 % polymer coating weight gain gives better results in term of sufficient lag time and delay drug release. Use of combination of polymer Eudragit RS 100 and RL 100, would be a better choice for the development of multiparticulate pulsatile drug delivery system.

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5. References

1. Jain D., Raturi R, Jain V., Bansal P., Singh. Recent technologies in pulsatile drug delivery. Biomatter.2011;1:1, 1-9.
2. Raghavendrarao NG., Soumya P , Revathi A., Nayak SB . A review on pulsatile drug delivery system. International Research Journal of Pharmacy. 2013; 4 (3):31-44..
3. Latha K M U Uhumwangho S A Sunil RamanaSreekanth K V Murthy Chronobiology and chronotherapy of hypertension-A review International Journal of Health Research, 2010; 3(3): 121-131
4. Kaur M., Bala R. Chronotherapy : A review. Int J Pharm Sci Res2013;4(1):90-102.
5. Douglas JG. Compliance with antihypertensive therapy: Is it time for chronotherapy. Am J Hypertension, 2002;15(S3): 238.
6. Chaudhari MP, Chaudhari DP. Formulation and evaluation of multiparticulate system for chronotherapeutic delivery of salbutamolsulphate. J Pharm BioallSci 2012;4:71-3.
7. Mahajan AN, Pancholi SS. Formulation and evaluation of timed delayed capsule device for chronotherapeutic delivery of terbutaline sulphate. Ars Pharm,2010; 50(4):215-223.
8. Radke RS, Gangane PS, Kawtikwar PS, Rai Sudish. Formulation and Evaluation of Sustained Release System for an Effective Management of Motion Sickness. Research J. Pharm. and Tech. 2008;1(4): 426-429.
9. Gupta, V.K., Beckent, T.E., Price, J.C. (1993). A novel pH and time based multi unit potential colonic drug delivery system. I. development. Int J Pharm. 3:83–91.
10. Sinchaipanid N, Chitropas P, Mitrevej A, Influences of layering of theophylline pellets characteristics. Pharm Dev Tech 2004;9:163-170.
11. Rane, A.B., Gattani, S.G., Kadam, V.D., Tekade, A.R. (2009). Formulation and evaluation of press coated tablets for pulsatile drug delivery using hydrophilic and hydrophobic polymers. Chem Pharm Bull. 57:1213–7.
12. Bajwa1 PS, Sharma J, Sharma S., Bhargava A (2017). Development and In vitro In vivo Characterization of Chronomodulated Multi-Particulate Drug Delivery System of Terbutaline Sulphate for Treatment of Nocturnal Asthma by box–Behnken Statistical Design Journal of Applied Pharmaceutical Science. 7(11): 033-042.
13. Higuchi T. Mechanism of sustained action medication, Theoritical analysis of rate release of solid drugs dispersed in solid matrices, J Pharm Sci., 52: 1145-1149, (1963).
14. Korsmeyer RW, Gunny R, Peppas NA, Mechanism of solute release from porous hydrophilic polymers, Int J Pharmaceutics, 15: 25-35, (1983).