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IN-VITRO STUDY OF AMLODIPINE AND NIFEDIPINE DRUGS OF PULSATILE DRUG DELIVERY SYSTEM

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ABSTRACT: To develop pulsatile microspheres of amlodipine and nifedipine drug release, a blend of polymer and solvent were used with pulsatile principles of drug delivery system to modified drug release pattern on and to release the drug at a particular time on the basis of lag time. In this study, we assessed the *in vitro* drug release pattern of the formulation prepared from different polymers ratio with the drugs of calcium channel blockers i.e. Amlodipine and Nifedipine. Different formulations of Amlodipine and Nifedipine were prepared using polymers Eudragit RS100, Eudragit S100, Carbopol 971P and polyvinyl alcohol. Optimum formulations were selected from both the drugs of pulsatile microsphere formulation such as formulation N12 from Nifedipine drug and A5 from amlodipine drug by conducting evaluation parameters for microsphere. *In vitro* studies were carried out for both the formulation and compared to each others. Kinetics drug released kinetics model fitted for both the drugs of optimum formulation for N12 and A5. Formulation N12 showed first order kinetics model and formulation A5 showed zero order kinetics models. Hence both the drugs showed a pulsatile effect, drug released from both the formulation at a particular lag time. The pulsatile dosage form of drug could be useful in chronopharmacotherapy of the treatment of hypertension.

INTRODUCTION: Pulsatile Drug Delivery System (PDDS) is an upcoming technique to combat patient's non-compliance, achieve optimum drug target actions and it leads to availability of the right amount of drug on right site at right time using right dosage [1]. These systems release their active moiety within a short period of time to produce its therapeutic action immediately after predetermined off release period [2]. The pulsatile effect in this system is to release the active drug in a pulsation form after lag time in such a manner that rapid drug release pattern should follow lag time [3, 4].

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Disparate studies enlighten the use of PDDS in xenobiotic having chronopharmacological behaviour (circadian rhythm), drug undergoing hepatic first pass metabolism, several ailments like asthma, allergic rhinitis, cardiovascular diseases, attention deficits in children, diabetes, gastric ulcers, cancer, neurodegenerative disease, infectious disease and hypercholestremia [5-7]. An underlying pathophysiology behind above disorders and their treatment strategies necessitates the development of Pulsatile Delivery System [8-10].

Amlodipine (AMD) and Nifedipine (NFD) are the drugs used for the hypertensive treatments and belong to calcium channel blockers. Both drugs are included in the World Health Organization (WHO) essential medicine list. Both of the drugs are taken orally. Both drugs having a lesser biological life so that both drugs initially show higher percentage drug release in shorter duration.

Pulsatile drug delivery systems are prepared of both drugs to extend the drug release at a particular time.

The aim of this research works to develop and compare the *in vitro* drug release formulation of antihypertensive drugs of calcium channel blockers such as amlodipine and nifedipine [11-12]. Pulsatile drug delivery systems of both drugs were prepared using different ratios of polymers, RPM and optimization. *In vitro* study is carried out of both drugs AMD and NFD formulation and selected formulation(s) *in vitro* study is compared.

MATERIALS AND METHODS: Nifedipine and Amlodipine was obtained as gift sample from Shasun Chemicals & Drugs, India, Eudragit RS100, Carbopol 971P (MW ~ 135 KDa, Rohm GmbH, Germany), polyvinyl alcohol, PVA,(MW ~ 70 KDa, 88% hydrolyzed were purchased from Sigma, (Germany). Eudragit S100 were obtained from Merck (Germany). Ethanol and dichloromethane of IP grade were used. The other additives and solvents were of analytical grade.

Preparation of AMD and NFD microspheres:
Pulsatile AMD and NFD microspheres were prepared using solvent evaporation methods. Different concentration of blend of Carbopol 971p and Eudragit RS100 as polymer mixed in a distilled water as a continuous phase with continuous stirring until the polymers dissolved in the water. To this mixture a NFD solution corresponding to 100 mg mixed thoroughly and injected drop wise into the continuous phase. At the beginning dichloromethane (DCM) and methanol was mixed uniformly at room temperature. Then a blend of polymer and drug was dissolved in various proportions in the above solutions as given in **Table 1**. Nine different formulations were prepared. Similarly in the case of AMD loaded pulsatile microsphere, seven different formulations were prepared. Formulations A1 to A3 were prepared with different concentration of Eudragit S100 (**Table 2).** After finalizing the optimum concentration of polymer by various evaluation parameters of microspheres variation of resolution per minute were studied from A4 to A7 such as 750 to 1500 RPM.

TABLE 1: COMPOSITION OF NFD FORMULATIONS WITH VARIOUS RATIOS OF POLYMERS

Sr. No.	Formulation Code	NFD (mg)	Carpobol 971P (mg)	Eudragit RS 100	Dichloromethane & Methanol (ml)	Stirring Rate (RPM)
1	N1	100	100	100	20	1000
2	N2	100	150	100	20	1000
3	N3	100	200	100	20	1000
4	N4	100	100	150	20	1000
5	N5	100	150	150	20	1000
6	N6	100	200	150	20	1000
7	N7	100	100	200	20	1000
8	N8	100	150	200	20	1000
9	N9	100	200	200	20	1000
10	N10	100	150	200	20	500
11	N11	100	150	200	20	750
12	N12	100	150	200	20	1000
10	N13	100	150	200	20	1250
11	N14	100	150	200	20	1500

TABLE 2: COMPOSITION OF AMD FORMULATIONS WITH VARIOUS RATIOS OF POLYMERS

Sr. no.	Formulation code	AMD (mg)	Eudragit S 100 (mg)	0.2 % PVA (mL)	Chloroform	RPM
1	A1	10	250	100	10	500
2	A2	10	500	100	10	500
3	A3	10	750	100	10	500
4	A4	10	500	100	10	750
5	A5	10	500	100	10	1000
6	A6	10	500	100	10	1250
7	A7	10	500	100	10	1500

***In-vitro* drug release studies of formulations of AMD and NFD:** An *in vitro* dissolution profile of NFD of optimized formulation microsphere was studied by employing USP XXIV dissolution apparatus II paddle type (Model DS-8000 Lab India) of all the formulation N1 to N14 (**Table 1).** Microspheres equivalent to 2.5 mg nifedipine was placed into the basket of the dissolution apparatus. Acid buffer of pH 1.2 for the first 2 h and phosphate buffer of pH 6.8 was used for the next 10 h. A 900 mL of buffer medium, speed 100 rpm and temperature 37±0.5 °C were maintained. Five millilitres of the sample was withdrawn from the dissolution media at suitable time intervals and the same amount was replaced with fresh buffer. Samples were filtered through membrane filter 0.45 µm (Millipore). The absorbance of the filtrate was determined spectrophotometrically at 238 nm against the respective buffer as blank. The amount of drug present in the filtrate was then determined from the calibration curve and cumulative percent of drug release was calculated and compared the final formulation with the conventional marketed dosage form and controlled release marketed formulation of nifedipine and same *in vitro* studies were carried for all formulations N1 to N14.

In vitro dissolution profiles of all formulations of AMD were performed employing USP 36-NF31 dissolution apparatus. Microspheres equivalent to 2.5 mg AMD of all formulations A1 to A7 as shown in **Table 2** were placed into the dissolution apparatus containing 900 ml of acid buffer of pH 1.2 for first 2 h and 900 ml of phosphate buffer of pH 6.8 was used for the next 10 h and speed at 100 rpm and temperature 37±0.5 °C were maintained. Five millilitres of the sample was withdrawn from the dissolution media at particular time intervals and the same amount was replaced with fresh buffer. Samples were filtered through membrane filter 0.45µm (Millipore). The absorbance of the filtrate was determined spectrophotometrically at 239 nm. Cumulative percent of drug release of the formulation were reported and all data were compared with formulation of NMD microspheres.

Kinetics of drug release of AMD and NFD: Model dependent methods are based on different mathematical functions, which describe the release profile. Once a suitable function has been selected, the release profiles are evaluated depending on the derived model parameters[14-16]. The results obtained from *in vitro* release studies were plotted in different model of data treatment as follows

Zero order kinetics: The zero order rates describe the systems where the drug release rate is independent of its concentration. A zero-order release would be predicted by the following equation;

$$A_t = A_0 - K_0 t$$

Where A_t is the amount of drug released in time t, A_0 is the initial concentration of drug (most times, A_0=0) and K_0 is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as amount of drug released 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: The first order describes the release from system where release rate is concentration dependent. A first-order release would be predicted by the following equation

$$\text{Log } C = \text{Log } C_0 - \frac{Kt}{2.303}$$

Where C is the amount of drug released in time t, C_0 is the initial concentration of drug and K is the first order rate constant.

Higuchi's Model: The first example of a mathematical model aimed to describe drug release from a matrix system was proposed by Huguchi in 1961. Initially conceived for planar systems, it was then extended to different geometrics and porous systems. This model is based on the hypotheses that (i) initial drug concentration in the matrix is much higher than drug solubility; (ii) drug diffusion takes place only in one dimension (edge effect must be negligible), (iii) drug particles are much smaller than system thickness, (iv) matrix swelling and dissolution are negligible, (v) drug diffusivity is constant, and (vi) perfect sink conditions are always attained in the release environment. Higuchi was the first to derive an equation to describe the release of a drug from an insoluble matrix as the square root of a time-

dependent process based on Fickian diffusion. Simplified Higuchi equation is following;

$$Q_t = K_H (t)^{0.5}$$

Where, Q_t is the amount of drug released in time t and K_H is the release rate constant for the Higuchi model.

Korsmeyer and Peppas Model: The release rates from controlled release polymeric matrices can be described by the equation proposed by Korsmeyer *et al.*

$$Q = Ktn$$

Where, Q is the percentage of drug released at time‘t’, K is a kinetic constant incorporating structural and geometric characteristics of the tablets and ‘n’ is the diffusional exponent indicative of the release mechanism.

Statistical analysis: The results were expressed as mean ± standard deviations (SD). Statistical analysis was carried out using analysis of variance (ANOVA) on Graphpad Prism 4.0 (Graphpad Software Inc. San Diego, CA, USA). $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION:

Comparison of *in vitro* studies of optimum formulation of AMD and NFD: Optimum formulation from the formulations of NFD, N1 to N14 were studied by conducting various parameters such as percentage yield, entrapment efficiency, drug loading capacity, micrometric studies, *in vitro* studies and kinetics studies. Formulations also compared with the marketed product and found N12 showed maximum percentage drug release at a particular time, so that N12 formulation of NFD was selected for the comparison with the formulation of AMD. Optimum formulation of AMD, A1 to A7 were studied in same way as conducted for NFD and also compared with the marketed product (Conventional dosage form) and found A5 showed maximum drug released at a particular lag time. Formulation A5 was selected from AMD formulations for the comparison with the optimum formulation N12 of NFD.

The release profile of NFD indicated that after 1 h 8.73 % amount of NFD was released from the microsphere and after 4 h, 22.88 % amount of NFD (N12) released from the microsphere. First 4 h dissolution was constant pattern release of an average drug release from the microsphere. From the 4 h to 6 h were the lag time in which drug released and containing burst and drug released reached to 90.25% that was the lag time from the microsphere.

In the case of release profile of AMD indicated that after 1 h 3.94% amount of AMD was released from the microsphere which was very less as compared to NFD formulation and after 4 h, 19.71% amount of AMD (A5) released from the microsphere. Lag time in the case of AMD is 6 h to 8 h in which drug released was 90.21% and after 12 h, it was 94.92%. Hence polymer coating and type decides the lag time of the formulation.

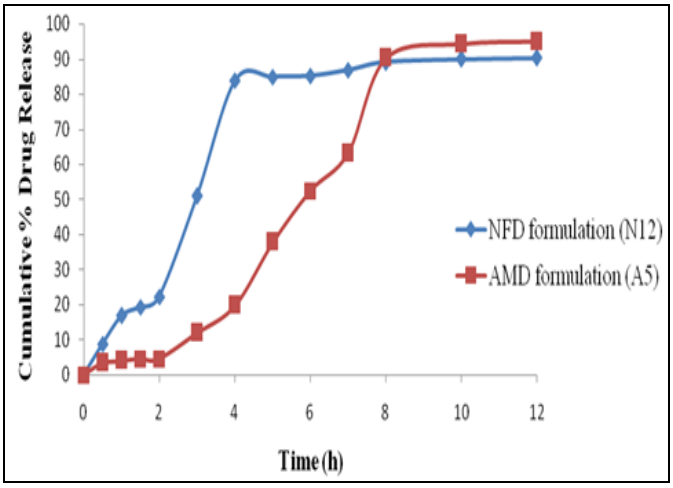

FIG. 1: COMPARISON OF *IN-VITRO* DRUG RELEASE OF OPTIMUM FORMULATIONS OF AMD AND NFD

The data obtained from *in vitro* release studies of final formulation NFD (N12) and AMD (A5) were fitted to various kinetics equations such as zero-order, first-order, Higuchi model and Korsmeyer and Peppas model to find out the mechanism of drug release from microspheres. The drug kinetics models are represented in **Table 3.** R^2 values of different release kinetic models were shown in **Table 3**. From the **Table 3**, it was found that Formulation NFD followed First order kinetics because R^2 was found to be more for first order kinetics model as compare to other model and for the formulation AMD followed Zero order kinetics because R^2 was found to be more for Zero order kinetics as compared to other models.

TABLE 3: COMPARISON *IN VITRO* DISSOLUTION KINETICS PARAMETERS OF OPTIMUM FORMULATION OF AMD AND NFD

Formulation Code	R^2 (Regression Coefficients)			
	Zero Order	First Order	Higuchi Model	Korsmeyer-Peppas
NFD (N12)	0.906	0.959	0.881	0.647
AMD (A5)	0.922	0.893	0.743	0.815

Pulsatile microspheres have been investigated for improving AMD and NFD release after a particular lag time which initially release slightly and then maximum while marketed products do *vice-versa*. AMD and NFD are recommended as first line for patients with high blood pressure. Those patients who are suffering from early morning blood pressure problem have problem to take a medicament, so for such patient these type of drug delivery is very beneficial. Drug release showed a pulsatile effect of the optimum formulation of NFD (N12) and compared with the effect of the optimum formulation of AMD (A5). The pulsatile release effect were increasing at a particular lag time and later shows First order model kinetics in the case of NFD (N12) as comparison with AMD (A5) pulsatile release effect shows Zero order kinetics i.e. time independent.

CONCLUSION: The present investigation from the research concluded that microsphere formulation NFD (N12) with blend of Carbopol 971P and Eudragit RS100 showed release at particular lag time and showed first order kinetics as compared to microsphere formulation AMD (A5) with the blend of Eudragit S100 and 0.2% PVA showed released at a particular lag time and showed zero order kinetics. Both microspheres formulations of AMD and NFD showed pulsatile effect and time controlled properties. Thus, the result form this study of microspheres provided a potential pulsatile drug delivery effect for the delivery of nifedipine and amlodipine in the treatment of hypertension.

CONFLICT OF INTEREST: No conflict of interest associated with this work.

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