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Original Article

Formulation and Evaluation of Nanoparticles Containing Antihypertensive Agent and Hypolipidaemic Drug

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ABSTRACT

The nanoparticles containing Antihypertensive agent (Amlodipine besylate) and Hypolipidaemic drug (Atorvastatin calcium) were prepared by nanoprecipitation technique using PLGA, Eduragit RLPO as polymers and pluronic F 68 as tribloere polymeric stabilizer. The preformulation studies were carried out to confirm the compatibility of with excipients by FT – IR and for drug, solubility studies, Hygroscopicity, loss on drying for drug identification. The prepared nanoparticles were assayed by HPLC to determine the drug content. The morphological shape was confirmed by using Scanning Electron Microscope. The particle size distribution was analyzed by using particle size analyzer. The average mean particle size of F1, F2, F3, F4 were 50nm, 70nm, 80nm and 100nm respectively. For Atorvastatin: The entrapment efficiency of formulation F2 containing PLGA 5 mg and pluronic F68 was found to be 69% which showed maximum percent drug entrapment where as those containing (F1) PLGA 10mg, (F3) Eudragit 10 mg and (F4) Eudragit 5 mg were found to be 65, 45,47 respectively. For Amlodipine: The entrapment efficiency of formulation F2 containing PLGA 5 mg and pluronic F68 was found to be 74% which showed maximum percent drug entrapment where as those containing PLGA 10 mg (F1), Eudragit 10 mg (F3) and Eudragit 5 mg (F4) were found to be 72, 51, 54 respectively. Therefore, the formulation F2 showed maximum drug entrapment efficiency for both drugs Atorvastatin and Amlodipine. For Atorvastatin: The % amount released for F1, F2, F3, F4 at 48 hours were found to be 90.15%, 97.44% 74.07%, 76.32% respectively. The maximum % amount released was observed for F2 when compared to all other formulation. For Amlodipine: The % amount released for F1, F2, F3, F4 at 48 hours were found to be 92.58% 95.33%, 84.19% 84.19% respectively. The maximum percentage amount release was more for F2 when compared to other three formulations. So formulation F2 produced more drug release when compared to F1, F3 and F4. From the release studies, the polymer Eudragit was more sustaining action when compared to PLGA. According to my work, the formulation containing antihypertensive agent should not be sustaining for long time. So I conclude that the PLGA was best polymer for maximum % drug release at 48 hours than the Eudragit RLPO.The formulation F1 showed zero order release3 kinetics, and F3, F4 did not fitted for any other release kinetics. But, release kinetics of F2 indicated that it follow zero order and Higuchi model rather than first order. These findings indicated that the drug release from formulated nanoparticles were diffusion controlled.

Keywords:

1. INTRODUCTION

Cardiovascular disease (DVD) is still the leading cause of mortality and morbidity in society. Atherosclerosis is the etiologic factor underlying CVD. Atherosclerosis

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is a condition in which fatty material collects along the walls of arteries. Hypertension and hyperlipidaemia are major risk factors for the development of atherosclerosis. Calcium channel blockers (CCBS) have been used for decades and have established antihypertensive effects. Stations have been extensively used because of their potent lipid lowering properties. Amongst other factors, inflammation and oxidation are involved in enhanced progression of atherosclerosis and new lesion development. Therefore, research has been focusing on the antioxidant and anti- inflammatory properties of CCBs and Stations, beyond their primary effect, in order to evaluate the possible additive effects of combined treatment of CCBS with stations as antiatherosclerotic therapy. Atorvastatin calcium is a second generation 3- hydroxy-3-methyl glutarylCoA reducatase inhibitor approved for clinical use as a lipid lowering agent. Atorvastatin calcium, the world's best selling drug is associated with poor oral bioavailability (12%) and serious adverse effects like rhabdomyolysis on chronic administration. Side effect of Atorvastatin was reduced 60% by combining with Amlodipine. The Amlodipine has potency to promote the activity of Atorvastatin. Therefore, Atorvastatin and Amlodipine combination was taken for this research. A biodegradable nanoparticulate approach was introduced here with a view to improving the efficacy and safety of atorvastatin calcium. Particulate systems like nanoparticles have been used as a physical approach to alter and pharmacodynamic properties of various types of drug molecules. The nanoparticluate suspension of amlodipine is to improved its absorption rate and therapeutic efficacy.

Thus, the need for the present study is to prepare nanoparticles containing atorvastatin and amlodipine for effective ant atherosclerotic therapy by improving their bioavailability and therapeutic efficacy.

2. MATERIALS AND METHODS

Atorvastatin and Amlodipine were procured as gift sample from Micro Labs, Hosur, PLGA and Pluronic F68 was purchased from Sigma-Aldrich Cheme.

PREFORMULATION STUDIES:

Preformulation may be described as a stage of development process during which the researchers characterize the Physical, chemical and mechanical properties of the drug substance to form effective, stable and safe dosage form.

Description:

About 1g of sample is taken in a dry Petridish and the sample is observed for compliance against the specification.

Observation: Atorvastatin: White powder and Amlodipine: White powder

Melting point: Capillary tube, which is sealed at one end is charged with sufficient amount of dry powder to form a column in the bottom of the tube 2.5mm to 3.5mm when packed down as closely as possible by moderate tapping on a solid surface. The apparatus is operated according to the standard operating procedure. The block is heated until the temperature is about 30°c below the expected melting point. The capillary tube is inserted into the heating block, and the heating is continued at a rate of temperature increased of about 1°c to 2°c per minute until melting is complete. The temperature at which the detector signal first leaves its initial value is defined as the beginning of melting, and the temperature at which the detector signal reaches its final value is defined as the end of melting, or the melting point. The two temperature fall within the limits of melting range. The melting point of the Atorvastatin was found to be 168°C[168-170°C] The melting point of the Amlodipine was found to be 196°C[190-200°C]

Solubility studies: The spontaneous interaction of two or more substance to form a homogeneous molecular dispersion is called as solubility (sethi 1985). The solubility of Atorvastatin and Amlodipine were studied in various solvents separately. The drug (10 mg) was suspended separately in a 10ml of different solvents at room temperature in tightly closed test tubes and shaken on wrist action shaker (Yarco, New Delhi) for 8 hours.

Water Content By Loss On Drying: 2 g of the substance is mixed well and weighed accurately. Glass stoppered, shallow weighing bottle that has been dried for 30 minute under the same conditions to be employed in the determination was tared. The test specimen was transferred to the bottle, and closed. The bottle and the contents were weighed accurately.The loaded bottle was placed in the drying chamber (LOD Oven). Then stopper was removed and kept in the chamber itself. The test specimen was dried for one hour and the stopper was replaced and again it was weighed.

Hygroscopic nature:

Procedure: 2 gm of the two test specimens were weighed accurately in Petridish and the weight were noted down. Then the test specimens were exposed to 75%RH at 40°C in environment stability testing chamber and the other was kept at room temperature for 7 days period. The specimen was weighed after 7 days and the difference in weight was noted down.

Absorption maxima for amlodipine

Standard curve: 100mg of drug was dissolved in 100ml of solvent. From this stock solution, 2.5 ml was pipette. Out and made up to 25ml with solvent. From this solution, serial dilutions were made to produce 5,10,15,20,30,40,50 Mg/ ml concentrations. These samples were analyzed spectrophotometrically at the λmax of drug using solvent as blank.

Identification Test FTIR (With Working Standard) Atorvastatin Sample + Standard Amlodipine Sample + Standard

The FT-IR spectrum for Atorvastatin and Amlodipine was obtained by mixing equal proportion of the drug with their working standards. A overlapping spectrum is obtained hence it is confirmed that the samples obtained are Atorvastatin and Amlodipine.

Preparation of Nanoparticles

Nanoparticles were prepared by nanoprecipitation technique: Polymer was dissolved in acetone. The drugs were soluble in polymer/acetone Solution. This organic phase was added to an aqueous solution containing pluronic-f68.The final nanosuspension was centrifuged to separate the drug polymeric aggregates. Then it filtered through 0.22µm membrane filter.

Drug Entrapment Efficiency: The nanoparticles were separated from the aqueous medium by ultracentrifugation at 10,000 rpm for 30 min at 5^0C. then the resulting supernatant solution was decanted and dispersed into phosphate buffer saline pH 7.4. Thus the procedure was repeated twice to remove the unentrapped drug molecules completely. The amount of drug entrapped in the nanoparticles was determined as the difference between the total amount of drug used to prepare the nanoparticles and the amount of drug present in the aqueous medium.

$$\text{Drug Entrapment Efficiency} = \frac{\text{Amount of drug released from the lysed nanoparticles}}{\text{Amount of drug initially take to prepare the nanoparticles}} * 100$$

3. RESULTS AND DISCUSSION

DRUG CONTENT

Instrument used: Schimadzu prominence- LC-20AT binary Pump system.

Standard addition method: Mobile phase : acetonitrile (43%) / triethylamine (0.5%, pH-4 adjusted with orthophosphoric acid) Wavelength of detection : 243nm Column : Phenomenex C18 column, 5 micron

particle Size) Flow rate : 1ml/min Sample volume : 20 micro litre

Separately 20µl of the solutions (1) and (2) were injected, chromatograms were recorded and the response for the principal peak was measured

% Content == Peak area of sample X Sample X SampleX Average

Peak area of standard Dilution Dilution Weight

Particle Shape: The nanoparticles were subjected to microscopic examination (SEM) for characterizing size.

Particle size distribution of nanoparticles: Nanoparticles were subjected to Particle size analyzer for particle size distribute on of nanoparticles. It showed the particle size range of 10-150nm. For optimized formulation. The average mean particle size was 75nm.

In Vitro Drug Release Studies: The *in vitro* release rate of nanoparticles was evaluated by the dialysis bag method in distilled water up to 120 hr incubation period. The nanoparticulate suspension equivalent to 10 mg of Atorvastatin and 5mg of Amlodipine was placed in a dialysis membrane-70 (HIMEDIA, CA393 Mumbai, India) and sealed at both the ends. The dialysis bag which act as a donor compartment was immersed in the Receptor compartment containing 200 ml of diffusion medium which was stirred at medium speed and maintained at 37 ±2ºC. The receptor compartment was cover to prevent the evaporation of diffusion medium. Samples were withdrawn at regular time intervals and the same volume was replaced by fresh diffusion medium. The samples were analyzed (simultaneous analysis) using a UV-visible spectrophotometer (Shimadzu UV 1700) at 246nm and 360nm by using phosphate buffer 7.4.

Release Kinetics: Data obtained from *in vitro* release studies were fitted to various kinetic equations. The kinetic models used are zero order equation (Q=k0t), First order equation { ln (100 – Q) = ln Q – k1t}, Higuchi equation (Q= kt 1/2), Hixson and Crowell model Qt 1/3 Vs t and Qt 2/3 Vs t -Modified root cube equation. Further, to find out the mechanism of drug release, first 60% drug release was fitted in Korsmeyer and Peppas equation (Q = kpt n). Where, Q is the percent of the drug release at time t and k0 and kt are the coefficients of the equations and n is the release exponent. The n value is used to characterize different release mechanism.

Table 1: Solubility Profile of Atorvastatin

S. NO	SOLVENT	SOLUBILTIY
1	Distilled water	Very Soluble
2	PBS (pH 7.4	Very Soluble
3	Methanol	Sparingly Soluble
4	Ethanol	Slightly Soluble
5	Acetone	Practically insoluble or Insoluble.
6	Chloroform	Practically insoluble or Insoluble.

Table 2:Solubility Profile of Amlodipine

S. NO	SOLVENT	SOLUBILTIY
1	Distilled water	Very Soluble
2	PBS (pH 7.4	Very Soluble
3	Methanol	Sparingly Soluble
4	Ethanol	Slightly Soluble
5	Acetone	Practically insoluble or Insoluble.
6	Chloroform	Practically insoluble or Insoluble.

Table 3: Formulae of Nanosuspension

code	Atorvastatin (mg)	Amlodipine (mg)	Polymer (mg)	Pluronic f68 (mg)	Acetone (ml)	Water (ml)
F1	1	0.5	PLGA5	10	5	15
F2	1	0.5	PLGA7.5	10	5	15
F3	1	0.5	EUDRAGIT 5	10	5	15
F4	1	0.5	EUDRAGIT 7.5	10	5	15

Table 4: Drug Content Value

S.No	Formulation	Drug Content Value	
		Atorvastatin	Amlodipine
1	F1	100.01%	100.05%
2	F2	99.99%	99.99%
3	F3	99.98%	99.98%
4	F4	99.96%	100.01%

Table 5: Parameters of the model equations applied to the release of Atorvastatin from nanoparticles formulation

Formulation	Model	r2	Slope	K
F1	Zero order equation	0.9902	1.1603	0.9902
	First order equation	0.9188	0.0401	0.9585
	Higuchi model	0.9880	4.902	0.9902
	Peppas	0.8863	0.5891	1.1972
	Hixson-Crowell	0.9769	0.0567	2.047
F2	Zero order equation	0.9841	1.1885	0.9920
	First order equation	0.9480	0.0299	0.9737
	Higuchi model	0.9839	5.0019	0.9919
	Peppas	0.8848	0.4356	0.9839
	Hixson-Crowell	0.9658	0.0429	2.3753
F3	Zero order equation	0.9474	0.9733	1.139
	First order equation	0.8878	0.9422	0.032
	Higuchi model	0.9307	0.9647	4.752
	Peppas	0.8867	1.0498	0.4752
	Hixson-Crowell	0.9513	2.2859	0.0452
F4	Zero order equation	0.9685	0.9641	1.499
	First order equation	0.9470	0.9731	0.0241
	Higuchi model	0.9454	0.9723	6.234
	Peppas	0.8721	0.7541	0.3649
	Hixson-Crowell	0.9741	2.7924	0.041

Table 8: Parameters of the model equations applied to the release of Amlodipine from nanoparticles formulation

Formulation	Model	r2	Slope	K
F1	Zero order equation	0.9857	2.138	0.9928
	First order equation	0.8950	0.0647	0.9460
	Higuchi model	0.9733	8.943	0.9865
	Peppas	0.9791	0.6922	1.2124
	Hixson-Crowell	0.8508	0.0569	2.2775
F2	Zero order equation	0.9807	0.9609	0.9903
	First order equation	0.7162	0.8431	0.9344
	Higuchi model	0.9577	0.9786	0.9956
	Peppas	0.9663	0.9300	1.0177
	Hixson-Crowell	0.7473	2.7889	2.6248
F3	Zero order equation	0.9234	0.9609	2.876
	First order equation	0.7162	0.8431	0.0564
	Higuchi model	0.9577	0.9786	12.33
	Peppas	0.9663	0.9300	0.5471
	Hixson-Crowell	0.7473	2.7889	0.0449
F4	Zero order equation	0.9648	2.0511	0.9822
	First order equation	0.9271	0.0421	0.9629
	Higuchi model	0.9499	8.5661	0.6746
	Peppas	0.9471	0.5379	0.9452
	Hixson-Crowell	0.6806	0.0518	2.6069

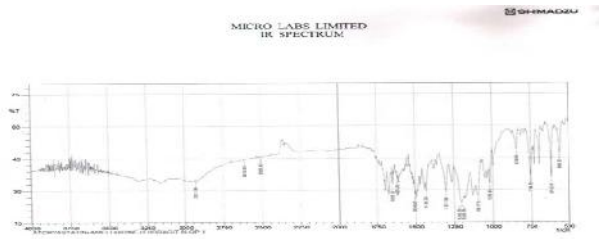

Fig 1: FTIR of Atorvastatin+Amlodipine + Eudragit+Pluronicf 68

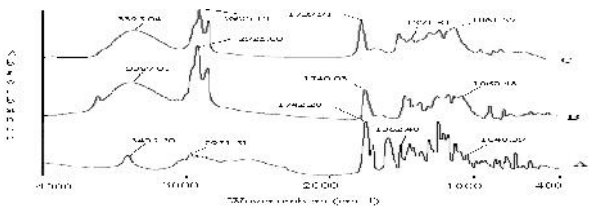

Fig 2: FT-IR of atorvastatin+amlodipine + PLGA+pluronic f68

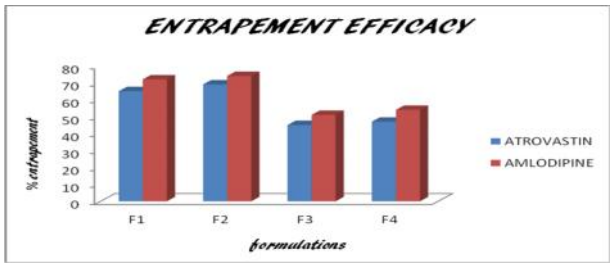

Fig 3: % Drug entrapment efficiency

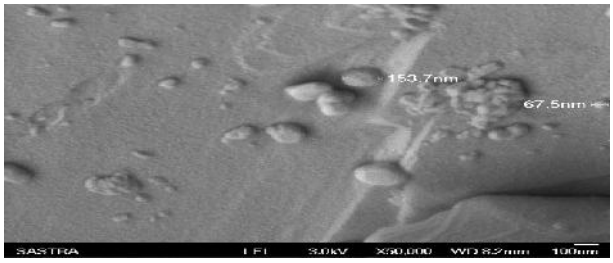

Fig 4: SEM of Formulation F2

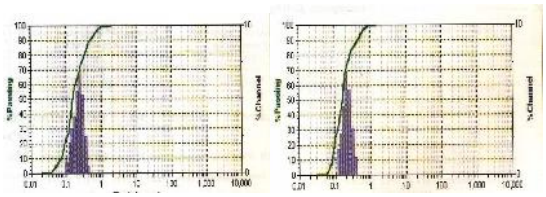

Fig 5: Particle Size Distribution

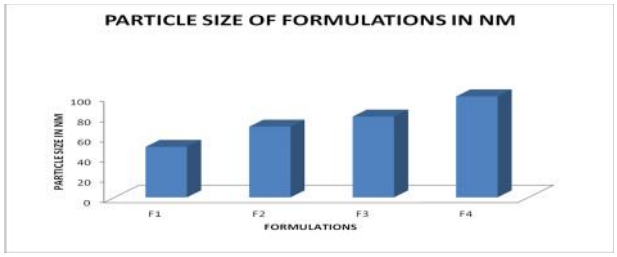

Fig 5: Particle size Formulation

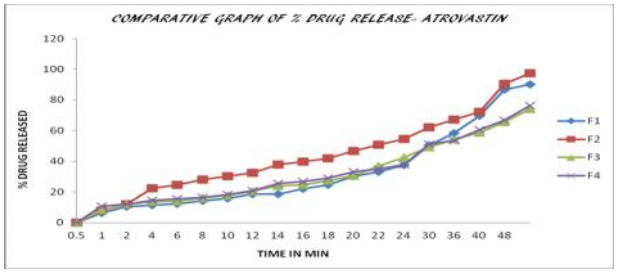

Fig 6: Comparative Graph of % Drug Release-Atrovastin

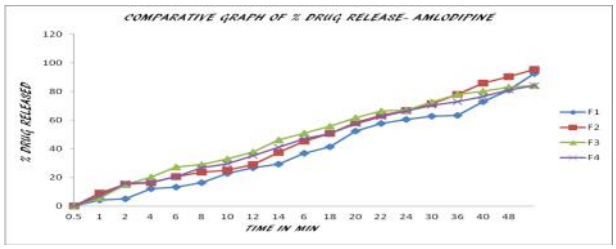

Fig 7: Comparative Graph of % Drug Release-Amlodipine

4. SUMMARY AND CONCLUSION

The nanoparticles containing Antihypertensive agent (Amlodipine besylate) and Hypolipidaemic drug (Atorvastatin calcium) were prepared by nanoprecipitation technique using PLGA, Eduragit RLPO as polymers and pluronic F 68 as tribloere polymeric stabilizer. The preformulation studies were carried out to confirm the compatibility of with excipients by FT – IR and for drug, solubility studies, Hygroscopicity, loss on drying for drug identification. The prepared nanoparticles were assayed by HPLC to determine the drug content. The morphological shape was confirmed by using Scanning Electron Microscope. The particle size distribution was analyzed by using particle size analyzer. The average mean particle size of F1, F2, F3, F4 were 50nm, 70nm, 80nm and 100nm respectively.

Entrapment Efficiency: For Atorvastatin: The entrapment efficiency of formulation F2 containing PLGA 5 mg and pluronic F68 was found to be 69% which showed maximum percent drug entrapment where as those containing (F1) PLGA 10mg, (F3) Eudragit 10 mg and (F4) Eudragit 5 mg were found to be 65, 45,47 respectively. For Amlodipine: The entrapment efficiency of formulation F2 containing PLGA 5 mg and pluronic F68 was found to be 74% which showed maximum percent drug entrapment where as those containing PLGA 10 mg (F1), Eudragit 10 mg (F3) and Eudragit 5 mg (F4) were found to be 72, 51, 54 respectively. Therefore, the formulation F2 showed maximum drug entrapment efficiency for both drugs Atorvastatin and Amlodipine.

Invitro Drug Release: For Atorvastatin: The % amount released for F1, F2, F3, F4 at 48 hours were found to be 90.15%, 97.44% 74.07%, 76.32% respectively. The maximum % amount released was observed for F2 when compared to all other formulation.

For Amlodipine: The % amount released for F1, F2, F3, F4 at 48 hours were found to be 92.58% 95.33%, 84.19% 84.19% respectively. The maximum percentage amount release was more for F2 when compared to other three formulations. So formulation F2 produced more drug release when compared to F1, F3 and F4. From the release studies, the polymer Eudragit was more sustaining action when compared to PLGA. According to my work, the formulation containing antihypertensive agent should not be sustaining for long time. So I conclude that the PLGA was best polymer for maximum % drug release at 48 hours than the Eudragit RLPO.

Release Kinetics: The data obtained from the first 90% release were fitted to various kinetic equations to determine the mechanism of drug release and release rate as indicated by higher correlation coefficients (r2), the drug release from nanoparticles followed zero order equation and Higuchi model diffusion controlled rather than the first order. Hixson- Crowell and modified cube root equation showed poor correlation.The formulation F1 showed zero order release3 kinetics, and F3, F4 did not fitted for any other release kinetics. But, release kinetics of F2 indicated that it follow **zero order and Higuchi model** rather than first order. These findings indicated that the drug release from formulated nanoparticles were diffusion controlled.

F2 has more drug release and its release kinetics follow zero order and Higuchi. Thus, it is concluded that F2 releases at diffusion control. F2 is stable at or below $4^{o}C$

The specific properties of the prepared nanoparticles such as stability, carrier capacity, sustained release upto 48 hrs enable the formulation to improve the bioavailability, enhance the therapeutic effect and reduces the side effects in controlling atherosclerosis. Therefore, F2 had achieved to increased bioavailabity, Prolonged action, Decreased dose and Frequency of administration.

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