ORIGINAL RESEARCH ARTICLE

Preparation, Characterization And Evaluation Of Nanoparticles Containing Hypolipidemic Drug And Antihypertensive Drug

M.Suganeswari*[1], Anto Shering[2], K.Azhagesh raj [3], P.Bharathi[4], B.Sathish[5]

[1]Sri Ramachandra College of Pharmacy, Vedaraniam, Tamilnadu, India.
[2]Medisas Pharmaceuticals, Puducherry, Puducherry, India
[3]E.G.S.Pillay College of Pharmacy, Nagapattinam, Tamilnadu, India.
[4]Sri Ramakrishna Institute of Paramedical sciences, College of Pharmacy, Coimbatote, Tamilnadu, India.
5Greater Institute of Hyderabad, Hyderabad, Andhra Predesh, India

ABSTRACT

The main aim of this study is to prepare, characterize and evaluate nanoparticles containing Hypolipidaemic drug (Atorvastatin calcium:D1N) Antihypertensive agent (Amlodipine besylateD2N) loaded by nanoprecipitation method using tribloere polymeric stabilizer (Pluronic F68). Biodegradable nanoparticles formulated from poly (D,L-lactide-co-glycolide) (PLGA) polymers are being extensively investigated for various drug delivery applications. Nanoparticles using PLGA polymers were formulated using nanoprecipitation technique, and were characterized for size, drug loading, and in vitro release. 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 improve its absorption rate and therapeutic efficacy.

Key Words: Nanoparticles, Atorvastatin, Amlodipine

INTRODUCTION:

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm.The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix [1]. Novel drug delivery systems (NDDS) are revolutionizing the drug discovery, development and creating R&D focused pharmaceutical industries to increase the momentum of global advancements [2]. To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. A successful nanoparticulate system should have a high drug-loading capacity thereby reduce the quantity of matrix materials for administration [3,4,5]. Drug loading can be done by two methods:

❖ Incorporating at the time of nanoparticles production (incorporation method)
❖ Absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution (adsorption / absorption technique)[6,7,8]

Atherosclerosis 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.

*Corresponding Author: M.Suganeswari, **Email:** suganeswari2011@gmail.com

The main objective of this work is to improve the bioavailability of Atorvastatin calcium and Amlodipine besylate, to improve absorption of the drugs than the conventional dosage form and to alter the pharmacokinetics and pharmacodynamics of drug substances in order to improve the therapeutic efficacy and safety through the use of novel drug delivery system (nanopatricles).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 [9].

MATERIALS AND METHODS:

Atorvastatin calcium, Amlodipine besylate were gift sample of Microlabs, Hosur. PLGA (50:50) and Eudragit RLPO were procured from Sigma-Aldrich Cheme, Germany. The surfactant Pluronic f68 was obtained from Microfine chemicals, Delhi.

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.

1. Melting point:

The melting point of the Atorvastatin was found to be 168^{C}[168-170^{0C}].The melting point of the Amlodipine was found to be 19^{0C}[190 -200^{0}C] by using capillary method.

2. Water content by Loss on Drying

$$\% \text{ Loss on drying} = \frac{W2 - W3}{W2 - W1} \times 100$$

3. Hygroscopic nature:

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. There was no weight gain for both drugs of Atorvastatin and Amlodipine indicating the non-hygroscopic nature.

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.

Table – 1 Formulae Of Nanosuspension:

Formulation code	Amount of Atorvastatin(mg)	Amount of Amlodipine(mg)	Amount of polymer(mg)	Amount of Pluronic f68 (mg)	Amount of acetone(ml)	Amount of water(ml)
F1	1	0.5	PLGA 5	10	5	15
F2	1	0.5	PLGA 7.5	10	5	15
F3	1	0.5	EUDRAGIT 5	10	5	15
F4	1	0.5	EUDRAGIT 7.5	10	5	15

Evaluation Of Prepared Nanoparticles:
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.

$$\text{Drug Entrapment Efficiency (\%)} = \frac{\text{Amount of released from the lysed Nanoparticles}}{\text{Amount of Drug Initially taken To prepare the nanoparticles}} \times 100$$

Particle Shape:

The nanoparticles were subjected to microscopic examination (SEM) for characterizing size. The nanosuspension was characterized by SEM (Hitachi model S-3000 H, Japan). Before going

950

for evaluation, the nanosuspesion was lyophilized to form solid particles. The solid particles were coated with platinum alloy using a sputter coater.

Invitro Drug Release Studies

The *invitro* 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 ±2°C. 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.

RESULTS & DISCUSSION
Pre formulation studies
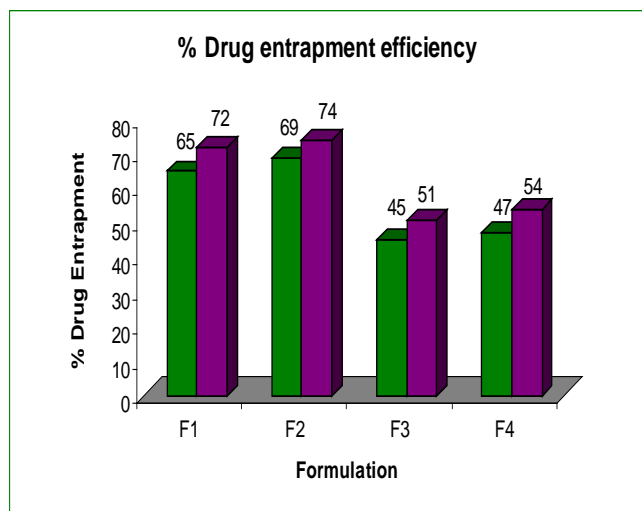#### Water content by Loss on Drying

% LOD [Atorvastatin] =   0.099%.
% LOD [Amlodipine]   =   0.129%

Hygroscopic nature:

There was no weight gain for both drugs of Atorvastatin and Amlodipine indicating the non-hygroscopic nature.

Drug 1: Atorvastatin
Entrapment Efficiency:

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.

Invitro Drug Release:

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.

Drug 2: Amlodipine
Entrapment Efficiency:

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.

Invitro Drug Release:

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.

**Fig 1: Entrapment Efficiency of Prepared Nanoparticles**

Fig 2: SEM Photograph
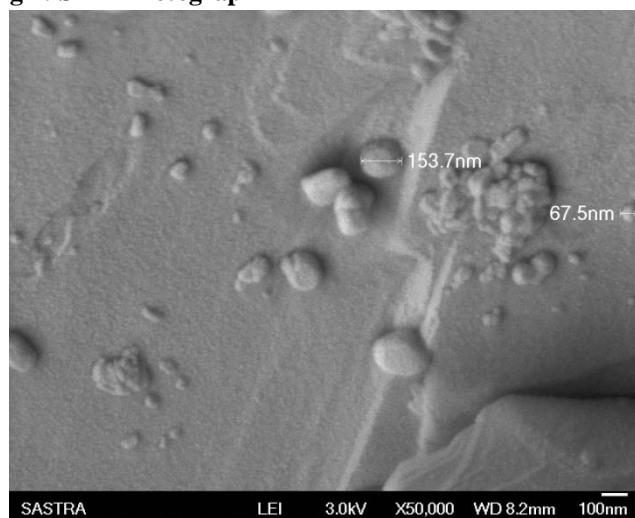
Table -2   Comparative Percentage Drug Release

TIME(min)	% AMOUNT DIFFUSED							
	F1		F2		F3		F4	
	D1N	D2N	D1N	D2N	D1N	D2N	D1N	D2N

1\2	6.40	4.31	7.78	9.08	8.24	5.78	10.61	6.94
1	10.35	5.00	12.06	15.14	11.89	15.02	11.86	15.92
2	11.22	12.07	22.29	16.46	13.48	20.34	14.52	16.50
4	12.37	13.16	24.64	20.62	13.84	27.30	15.53	20.46
6	14.33	16.34	28.00	23.72	16.19	28.89	16.27	26.67
12	18.65	29.20	37.87	37.51	24.02	46.13	25.43	41.08
24	50.44	62.64	62.20	71.37	49.14	72.54	51.08	70.21
36	69.68	72.93	72.27	85.86	58.77	80.07	60.68	76.42
48	90.15	92.58	97.44	95.33	74.07	84.19	76.32	84.19

Figure 3 Drug Release Study
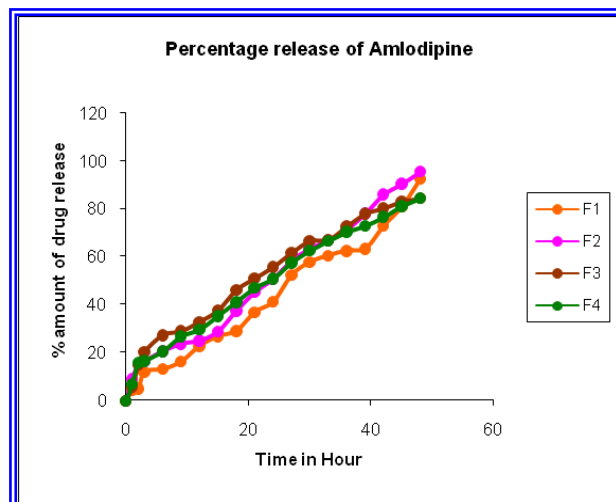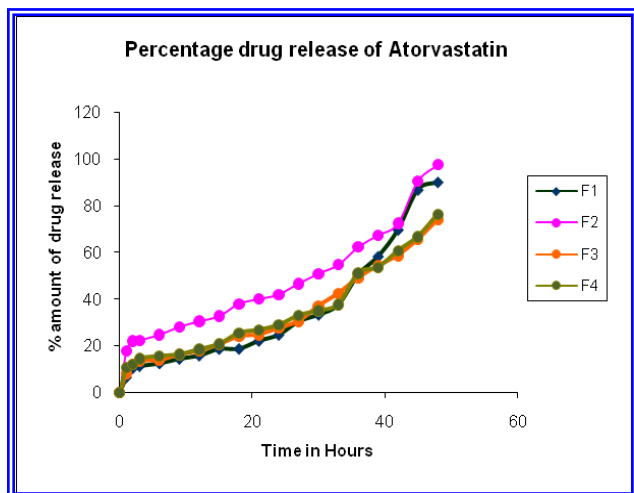
CONCLUSION

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 solubility studies, Hygroscopicity and 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. The formulation F2 showed maximum drug entrapment efficiency for both drugs Atorvastatin and Amlodipine. We are going to study the invivo release of drugs in a animal model. We hope this formulation will be successful till we market it for medical use.

REFERENCES:

1. Biopharmaceutics & Clinical Pharmacokinetics- Milo Gibaldi Pharma book Syndicate fourth Edition PP 147-175.

2. Langer R. Biomaterials in drug delivery and issue engineering; one laboratory's experience. Acc chem. Res 2000; 33: 94-101.

3. Bhadra D, Bhadra S, Jain P, NK. Pegnology; a review of PEG – ylated systems. Pharmazie 2002; 57: 5-29.

4. Kommareddy s, Tiwari SB, Amiji MM. Long – circulating polymeric nanovectors for tumor - selective gene delivery. Technology of cancer Treatment 2005; 4 : 615 – 25.

5. Lee M, Kim SW. Polyethylene glycol – conjugated copolymers for plasmid DNA delivery. Pharm Res 2005; 22: 1-10.

6. Desai MP. Labhasetwar V, Amidon GL, Levy RJ. Gastronitestinal uptake of biodegradable microparticles; effect of particle size, Pharm Res 1996; 13: 1838 – 45.

7. Kroil RA, Pagel MA, Muldoon LL, Roman – Goldstein S, Flamengo SA, Neuwelt EA. Improving drug delivery to intracerebral tumor and surrounding brain in a rodent model; a comparison of osmotic versus bradykinin modification of the blood – brain and / or blood –

tumor barriers. Neurosurgery 1998; 43: 879 – 86; discussion 886-9.

8. Kreuter J, Ramage P, V.Hamm S, Gelpernia SE, Engeltardt B, Alyautdin R, von Briesen H, Begley DJ, Direct evidence that polysorbate – 80 - Coated poly (butycyanocrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of

drug to the nanoparticles. Pharm Res 2003: 20; 409 – 16.

9. Das S, Ray R, Snehlata, Das N, Srivastava LM (2006). "Effect of ascorbic acid on prevention of hypercholesterolemia induced atherosclerosis". Mol Cell Biochem 285(1-2) (1-2): 143–7. doi:10.1007/s11010-005-9070-x. PMID 16479321.