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Development and charecterization of PLGA-nanoparticles containing carvedilol

N.Jawahar*, D.Nagasamy Venkatesh, R.Sureshkumar, V.Senthil, G.N.K.Ganesh,. P.Vinoth, Sumeet Sood and M.K.Samanta.

Pharmaceutics research laboratory, J.S.S.College of Pharmacy, Ooty-643001, Tamilnadu, India

Abstract:

The objective of this study was to prepare PLGA nanoparticles of Carvedilol that will improve the bioavailability of Carvedilol and sustain the release to reduce the initial hypotensive peak and to prolong the antihypertensive effect of the drug. Carvedilol encapsulated by Nanoprecipitation method using PLGA and Pluronic F-68.Prepared nanoparticles were examined for physicochemical characteristics, *in vitro* release kinetics and invivo biodistribution studies. Average size of the nanoparticles were in range of 132-234nm. The drug encapsulation efficiency was 77.6% at 33% drug loading. *In vitro* cumulative release from the nanoparticles was 72% at 24hr. *In vivo* biodistribution studies in rats revealed that these particles are distributed in heart, liver and kidney at higher concentration may allow their delivery to target sites.

Keywords: Carvedilol, Nanoparticles, PLGA, Sustained release, Biodistribution.

Introduction

Carvedilol is an adrenergic β-antagonist and has demonstrated clinical benefit in the management of patients with coronary artery disease, heart failure and in the post-myocardial settings[1]. Carvedilol, which is lipophilic in nature with less bioavailability due to its extensive first pass metabolism. Since it has short biological half life, multiple daily dosing is necessary for the maintenance of its therapeutic effect throughout the day. The drug Carvedilol suffers from severe drawbacks of hypotensive, bradycardia, dizziness, fatigue and upper respiratory tract infection. Hence, a sustained oral drug delivery will be useful for the long term treatment.

Biologically adhesive delivery systems may offer important advantages over conventional in the bioavailability of the drugs. Its shown that Poly (DL-lactide/glycolide copolymer) (PLGA) has bioadhesive properties and binds with mucosa of gastrointestinal tract[2,3,4.] This may increase the residency time and may enhance the drug absorption time due to intimate contact with the epithelium cells. Also, biodegradable nanoparticles are of particular interest as they provide protection of fragile molecules against enzymatic and hydrolytic degradation in the gastrointestinal tract[5]. They can be taken up by enterocytes[6] and the lymphoid tissues in payer's patches[7,8].

Thus, nanoparticles of PLGA have great potential as on oral bioadhesive, sustained drug delivery system for Carvedilol in order to reduce the initial hypotensive peak and prolongation of antihypertensive effect of the molecule.

Materials and Methods

Chemicals and Reagents:

Carvedilol was a gift sample from Cipla labs, Mumbai. Poly (D,L-lactide-co-glycolide) (PLGA) and Pluronic F-68 were purchased from Sigma Chemicals, Mumbai. Dialysis bag for invitro release studies was procured from HiMedia, Mumbai. All other reagents used were analytical grade.

Preparation of Carvedilol PLGA Nanoparticles:

Nanoparticles were prepared by Nanoprecipitation method[9]. Carvedilol and PLGA were dissolved in 10 ml of acetone. This organic phase was injected at the rate of 10 ml/min in 20 ml of water containing Pluronic F-68 under stirring at room temperature. Acetone was evaporated under reduced pressure. After that the aqueous colloidal mixture was centrifuged and lyophilized to obtain the dry powder nanoparticles. The composition of Nanoparticles is given in Table 1.

Evaluation of Nanoparticles

a) **Particle Size Distribution:** To analyse particle size,

nanosuspension was diluted with filtered (0.22µm) ultra pure water.

Table No.1

Composition of Nanoparticles

Batch	Amount of Carvedilol (mg)	Amount of PLGA (mg)	Amount of Pluronic F-68 (mg)	Amount of Acetone (ml)	Amount of Water (ml)
F1	5	125	250	10	20
F2	10	125	250	10	20
F3	5	175	250	10	20
F4	10	175	250	10	20
F5	5	125	350	10	20
F6	10	125	350	10	20
F7	5	175	350	10	20
F8	10	175	350	10	20

Samples were analysed using Zeta sizer

b) 2000 (Malvern instrument, UK) which allows sample measurement in the range of 0.020-2000.00 µm and particle size distribution[10].

b) Polydispersity index: Polydispersity was determined according to the equation,

Polydispersity = D (0.9)-D (0.1)/ D (0.5)

Where,

D (0.9) corresponds to particle size immediately above 90% of the sample. D (0.5) corresponds to particle size immediately above 50% of the sample. D (0.1) corresponds to particle size immediately above 10% of the sample[11].

c) External Morphological Study: External morphology of nanoparticles was determined using Scanning Electron Microscopy (SEM). Samples were diluted with ultrapurified water to obtain a suitable concentration. Then the samples were spread on a sample holder and dried using vacuum. They were subsequently coated with gold (JFC 1200 fine coater, Japan) and examined by a Scanning Electron Microscopy.

d) Determination of drug loading: A known amount of freeze-dried nanoparticles was taken in trilicate,and dissolved in a known volume of acetone. The amount of Carvedilol was quantified by measuring the absorbance at 241.8 nm[12].

e) Invitro release kinetics: The dialysis bag diffusion technique was used to study the *in vitro* drug release of Carvedilol nanoparticles. The prepared nanoparticles were placed in the dialysis bag and immersed in to 50ml of Phosphate buffered saline (PBS) at pH 6.8. The entire system was kept at 37 ± 0.5°C with the continuous magnetic stirring. Samples were withdrawn from the receptor compartment at predetermined intervals (30 min, 1, 2, 3, 4, 8 and 24hr) and replaced by fresh medium. The samples were withdrawn and diluted to 10 ml with Phosphate buffered saline pH (6.8) and the amount of drug dissolved was analysed by UV-Spectrophotometer at 241.8 nm[12].

f) Invivo biodistribution studies: *In vivo* biodistribution studies were carried out in Wistar rats weighing 200-250gm and were divided into 2 groups containing 6 animals. The 2 groups namely group 1 treated with free drug and group 2 treated with Carvedilol loaded nanoparticles. Once a day each group of animals were administered orally with free drug and Carvedilol loaded nanoparticle with a dose of 10mg/kg body weight of rats. After 24hrs of drug administration, the animals were sacrificed, then different organs like heart, liver and kidney were extracted out

and homogenised in 10% Potassium Chloride followed by centrifugation. Supernatant of the tissue were analysed by HPLC to estimate the biodistribution[13].

Results and Discussion

Particle Size Distribution: The mean particle size of Carvedilol loaded nanoparticles is shown in Table 2. The particle size distribution curves for all the samples are unimodel. Average nanoparticles sizes of the formulations were range from 132-234 nm (F1-F8) respectively. The nanoparticle size dependant on PLGA concentration. The smallest particle size of were found in batch F2 (132 nm) and largest particle of was found in batch F7 (234 nm). The data suggest that in an increase in polymer concentration increase the particle size.

Table No.2

Evaluation of PLGA Nanoparticles of Carvedilol

Formulation Code	Drug Content (mg)	Entrapment Efficiency (%)	Polydispersity	Average Particle size (nm)
F1	2.31	46.31	0.095	145
F2	5.61	56.10	0.214	132
F3	2.17	43.39	0.259	174
F4	5.43	54.32	0.164	151
F5	2.05	41.06	0.123	168
F6	5.37	53.74	0.192	137
F7	2.13	42.52	0.208	234
F8	5.18	51.84	0.109	197

Polydispersity index: The Polydispersity index of the data revealed that the particle size distributions of all the formulations are unimodel. The results are shown in table 2.

External Morphological Study: The External morphological studies (SEM) revealed that maximum nanoparticle were nearly spherical in shape (Fig.1). The nanoparticle size observed by SEM correlated well with the Particle size measured by Zeta sizer (Malvern instrument).

Drug content and Entrapment efficiency: The total drug content in nanoparticle were varied from 2.05-2.31 mg for 5 mg (F1, F3, F5, F7) and 5.18-5.61 mg for 10 mg (F2, F4, F6, F8) shown in Table 2. The drug content increased with an increase in the concentration of drug. The amount of free dissolved drug in nanoparticle ranged from 0.61-1.17 mg, this is due to the limited solubility of Carvedilol in aqueous phase. The percentage entrapment efficiency varied from 41.06-56.10%. Entrapment efficiency of the formulation increased due to the high concentration of drug.

***In vitro* drug release:** The *in vitro* release of the drug from the polymeric nanoparticles was studied by the dialysis bag diffusion technique. The dialysis bag retained nanoparticles and allow the diffusion of the drug immediately into the receiver compartment. The nanoparticle size was also associated with change in drug release kinetics. The smaller size nanoparticles prepared with lower amount of PLGA exhibited higher drug release rate (72.72%, batch F2), this is may be due to the increased nanoparticle surface resulting in larger drug fraction exposed to the dissolution medium and also the higher

amount of drug loading. The larger size nanoparticles prepared with higher amount of PLGA exhibited lower drug release rates (56.50%, batch F3), this is may be due to the decreased nanoparticle surface resulting in smaller larger drug fraction exposed to the dissolution medium and also the lower amount of drug loading. The cumulative % drug release from all the drug loaded batches was found to be in the range between 56.50-72.72 % which may be considered to be satisfactory. Fig 2 shows the comparative release of drug from various drug loaded batches.

Table No.3
Biodistribution of free drug, Carvedilol loaded Nanoparticles

S. No	Organs	Amount of drug distributed (ng)	
		Free drug	Carvedilol loaded nanoparticles
1.	Heart	473.64	873.18
2.	Liver	375.80	730.10
3.	kidney	325.10	680.20

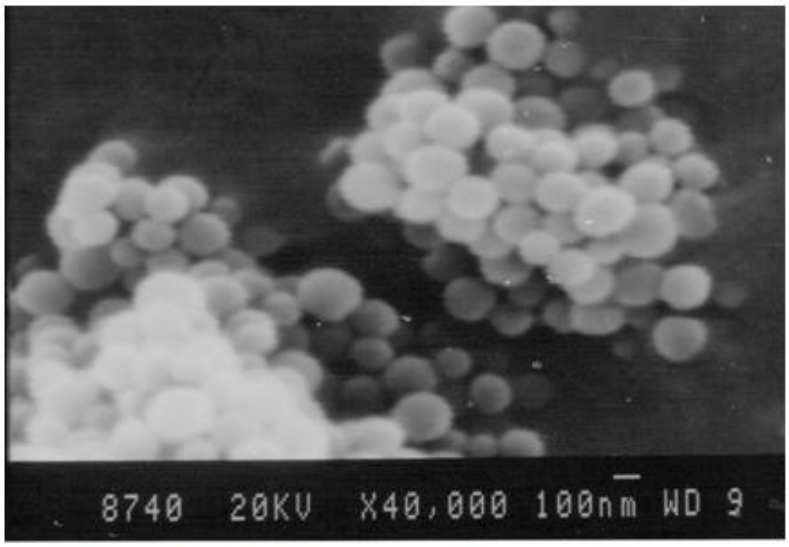


Fig No. 1: SEM of Carvedilol nanoparticles prepared by Nanoprecitation method Batch F4

***In vitro* release kinetics**: The data analysis revealed that a good regression (Fig.3) was obtained for First order kinetics and Higuchi's equation, which indicated that almost all formulations released the drug in idealistic sustained release concentration dependant mode(First order) and the mechanism of drug release from the polymer matrix was Higuchi's diffusion.

***In vivo* biodistribution studies:** The batch of nanoparticles with high drug loading and high drug release (F2) was subjected for *in vivo* biodistribution studies two different groups of animals were treated with free drug and Carvedilol loaded nanoparticles. From the results (Table 3) it is clear that Carvedilol loaded nanoparticles showed a comparatively better amount of drug distribution to organs like heart, liver and kidney. Thus it can be concluded that Carvedilol loaded nanoparticles are

relatively more effective in targeting the drug to the respective organs.

Conclusions

There are numerous preparation methods available for produsing nanoparticles. In the present study Carvedilol loaded PLGA nanoparticles were prepared by nanoprecipitaion technique. The chosen method was simple,safe,reproducible and minimize the loss of drug or its pharmacological activity. The results for

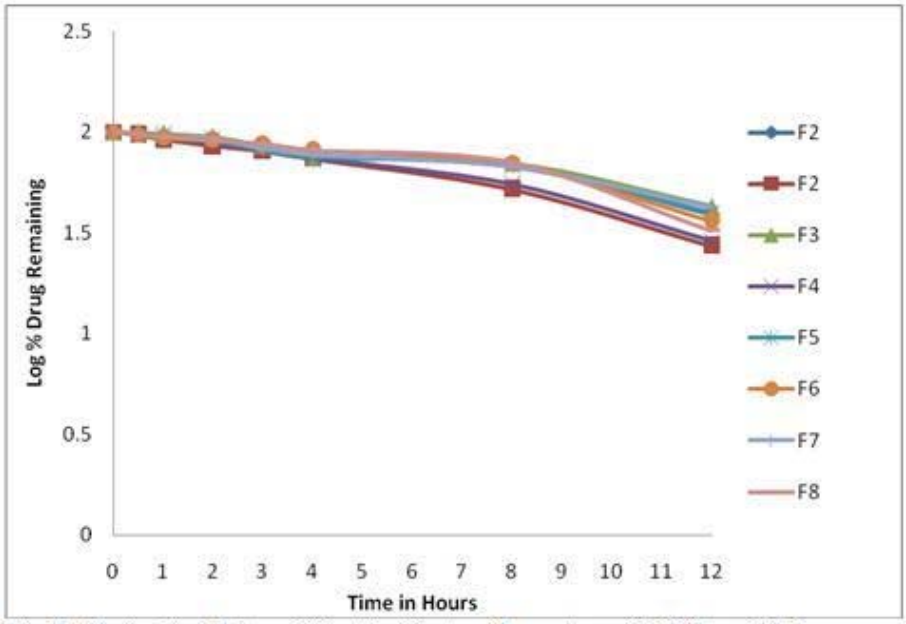
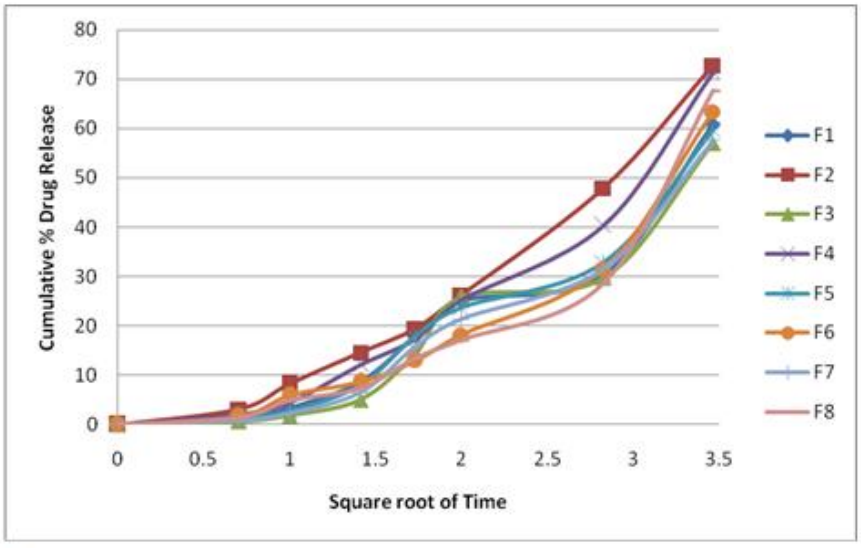


Fig No.3 - *Invitro* Release Kinetics First order values of all Carvedilol Nanoparticles batches



Higuchi's profile of Carvedilol nanoparticles batches

Carvedilol indicates that the PLGA nanoparticles have potential as a drug adrug delivery system.Furthemore,they may have utility for site specific drug delivry since the small size of the particle and its biodistribution properties may allow their delivery to target sites[14]. Sustained release of nanoparticles might extend the

circulation time of drug will suitable for reducing the initial hypotensive peak and prolong the antihypertensive effect.

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