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Antihypertensive drug loaded PLGA nanoparticles: Impact of formulation variables on particle size distribution

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ABSTRACT

Carvedilol, an antihypertensive drug is a non-cardioselective beta blocker which is used for the treatment of symptomatic heart failure. Spherical Carvedilol-PLGA nanoparticles with controlled size were designed. Carvedilol, a hydrophilic molecule was entrapped into the nanoparticles with theoretical loading varying from 15-38 %(w/w).This study investigates the impact of some process variables on the mean diameter and size distribution of nanoparticles prepared by emulsion solvent- evaporation technique. The results shows that sonication time, PLGA content, Surfactant concentration, Aqueous and organic phase ratio and overall the method of solvent evaporation have significant influence on size distribution of the nanoparticles.

Key words: Nanoparticles; controlled release, PLGA, Carvedilol, solvent evaporation.

INTRODUCTION

Hypertension is the most common cardiovascular diseases. Elevated arterial pressure causes pathological changes in the vasculature and hypertrophy of the left ventricle. As a consequence, hypertension is the principle cause of stroke, leads to disease of the coronary arteries with myocardial infarction and sudden cardiac death and is a major contributor to cardiac failure, renal insufficiency, and dissecting aneurysm of the aorta [1, 2].

Carvedilol (Table 1) is a nonselective β-adrenergic receptor antagonist with an ∞_1 - adrenergic receptor antagonist activity that has been approved for the treatment of essential hypertension and for the treatment of symptomatic heart failure [3, 4, 5]. The ratio of ∞_1- to β- adrenergic receptor antagonist potency for Carvedilol is 1:10 [6].Carvedilol has bioavailability of about 25 to 35% because of extensive first-pass metabolism [7,8].Carvedilol undergoes oxidative metabolism and glucuronidation in the liver; the oxidative metabolism occurs via cytochrome CYP2D6 [9, 10].Interestingly Carvedilol also has an antioxidant activity [11].Carvedilol has a bioavailability of about 25% to 35% because of extensive first –pass metabolism. Carvedilol is eliminated by hepatic metabolism and has a terminal half-life of 7 to 10 hours [12, 13,14].

For nearly three decade, polymeric nanoparticles have been extensively studied because their unique and valuable physicochemical and biological properties. Indeed nanoparticles can protect the drug from degradation, enhance its transport and prolong its release; therefore they may improve the plasma –half life of the drug [15, 16].

Although a number of different polymer have been investigated for formulating biodegradable nanoparticles, Poly-DL-lactic –co- glycolic acid (PLGA), a synthetic non-toxic biodegradable copolymer have been extensively used for controlled drug delivery system [17,18].The lactide/glycolide polymer chains are cleaved by hydrolysis into natural metabolites (lactic and glycolic acids) which are eliminated from the body by citric acid cycle. PLGA provides a wide range of degradation rates from months to years, depending on its composition and molecular weight [19, 20,21].

Thus the goal of our study was to design a nanoparticulate drug delivery system with a drug controlled delivery, based on the biodegradable polymer (PLGA). The active substance used was Carvedilol, a lipophilic molecule and ideal model drug for incorporation in systems by emulsion-solvent evaporation technique.

Table 1

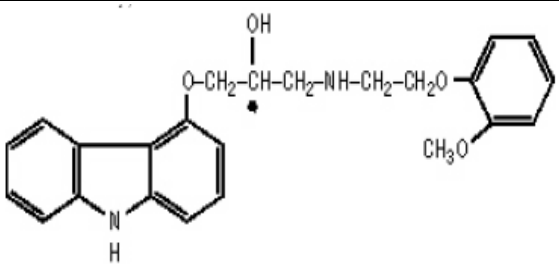

Figure1.Carvedilol (Chemical Abstracts Service No- 72956-09-3)

Molecular Weight: 406.5Da
Melting Point : 114-115^{0}C (at least two polymorphic forms)
pKa : 7.8
log p (octanol/water) at initial Carvedilol concentration of 6x10^{-7} M and room temperature
pH 9.0 : 3.10
pH 7.0 : 2.74
pH 5.0 : 1.93
pH of an aqueous 10% (w/v) suspension at room temperature 7.6-7.7.
Solubility in pure water at 25^{0}C (mg/ml)
Free base : 0.01
Hydrochloride : 1
Adipate : 0.3
Tartrate : 0.7
Methanesulfonate: 10

Reference: *European Pharmacopoeia 5.06, pp.1193-1194 and (23, 29 -32)*

MATERIALS AND METHODS

Material

Carvedilol was a kind gift of Zydas Cadila Limited (Ahmedabad, Gujrat, India). Poly (DL-lactide/glycolide copolymer) (PLGA,50:50 with average molecular weight 12000 and inherent viscosity 0.37 dL/g) was procured from Boehringer Ingelheim Co,(Ingelheim, Germany).Polyvinyl alcohol (PVA,Mv 30-70KDa,88% alcoholysis) was obtained from sigma chemicals (Mumbai, India).All the other reagent/chemicals were of the highest analytical/available grade.

Preparation of nanoparticles

Nanoparticles are prepared by using the method emulsification by sonication-evaporation. The method involve preparation of an organic phase consisting of polymer (PLGA) and drug (Carvedilol) dissolved in organic solvent (Dichloromethane).The organic phase is added to an aqueous phase containing surfactant (Polyvinyl alcohol) to form an emulsion. This emulsion is broken down into nanodroplets by applying external energy and these droplets form nanoparticles upon solvent evaporation and was isolated by centrifugation at 10,000xg at 4^{o}C for 45 minutes washed with water and dried under vacuum. The nanoparticles were recovered by centrifugation at 10,000xg at 4^{o}C for 45 minutes. The amount of non- entrapped Carvedilol in the supernatant was determined by HPLC method. The nanoparticles were washed (3x) time with water in order to remove the adsorbed Carvedilol. The washing solution was eliminated by further centrifugation as described above. The purified nanoparticles were freeze –dried.

Nanoparticles characterization

Particle size distribution was analysed by Master Sizer 2000 (Model: APA 2000, Malvern Instruments, Unighted Kingdom) equipped with a software (Version 1201).So to prevent clumping the dried powdered sample were diluted with duct free water to give the recommended scattering intensity as per Mie theory. Analysis was carried out at least for three times for each batch of sample and mean value were reported.

Determination of Carvedilol entrapment

The non-entrapped Carvedilol was determined by HPLC by UV detection at 240 nm.The mobile phase consisted of Phosphate buffer pH 3.0: acetonitrile: water (75:625:300 v/v/v) and the flow rate was set at 1 ml/minutes. Separation

was achieved using a Phenomenex Luna 5 µ C_{18} column (150x4.6mm, 250Å, SPD-W-20A, Shimadju, Japan).The free Carvedilol was separated from solid nanoparticles by ultracentrifugation as stated in section 2.2.The amount of Carvedilol entrapped in the nanoparticles was determined after their dissolution.(direct method).

In-vitro release profile

In-vitro release study of Carvedilol was determined by placing 10 mg nanospheres in 25 ml of release medium (PBS buffer,pH6.8).The sample was kept in a orbital shaker (SGM-300,Gallenkamp,Sanyo) maintained at 37^{o}C± 5 ^{o}C,stirring at 50 rpm.The study was carried out for 24 h with continuous stirring. At specified time intervals,1 ml aliquot of the release medium was removed and immediately replace with the identical volume of fresh medium. The aliquots were filtered and concentration of Carvedilol in the release medium was assessed by HPLC method. The chromatographic condition were: mobile phase comprised of Phosphate buffer pH 3.0: acetonitrile: water (75:625:300 v/v/v) and the flow rate was set at 1 ml/minutes, UV-vis detector set at 240 nm.The measurement were performed thrice for each batch.

RESULTS AND DISCUSSION

Impact of preparation variables on formulation characteristics.

The variables like time of sonication, PLGA content, surfactant content, evaporation rate, aqueous to organic phase ratio and drug content were assessed in order to obtain optimal formulation. For every series of experiment only one parameter was changed.

Sonication time

In order to obtain emulsified system, the incorporation of energy is a basic step. To see the impact of this factor on nanoparticles shape and size distribution, sonication time was varied from 1and 15 minutes. The results are depicted in Table 2.

Table 2 Impact of sonication time on nanoparticles mean diameters, Span value and granulometric size distribution.

Sonication Time	Mean Diameter (nm)	Span Value	Size distribution (nm)
1	370± 22	0.22	77% (242-355) 33%(920-1212)
5	321± 14	0.16	25% (95-210) 75% (470-560)
10	292± 24	0.22	15%(160-215) 85% (510-630)
15	257± 11	0.21	100% (246-254)

From the results it can be revelled that the increase in the sonication time leads to decrease in the mean diameter and narrower granulometric distribution of nanoparticles. The nanoparticles prepared with 15 minutes of sonication time showed a monomodal distribution where as particles prepared with 1, 5 and 10 minutes showed two ranges of particles with different sizes. With 15 minutes sonication time, a high energy released in the process leads to a rapid dispersion of polymeric internal organic phase and as a result no droplets of small size and monomodal distribution was observed. Emulsification is one of the key factors in the process to prepare nanoparticles because an insufficient dispersion of phases results in large particles with wide size distribution. The final particles sizes of nanoparticles depends on the globule size in entire emulsification process.

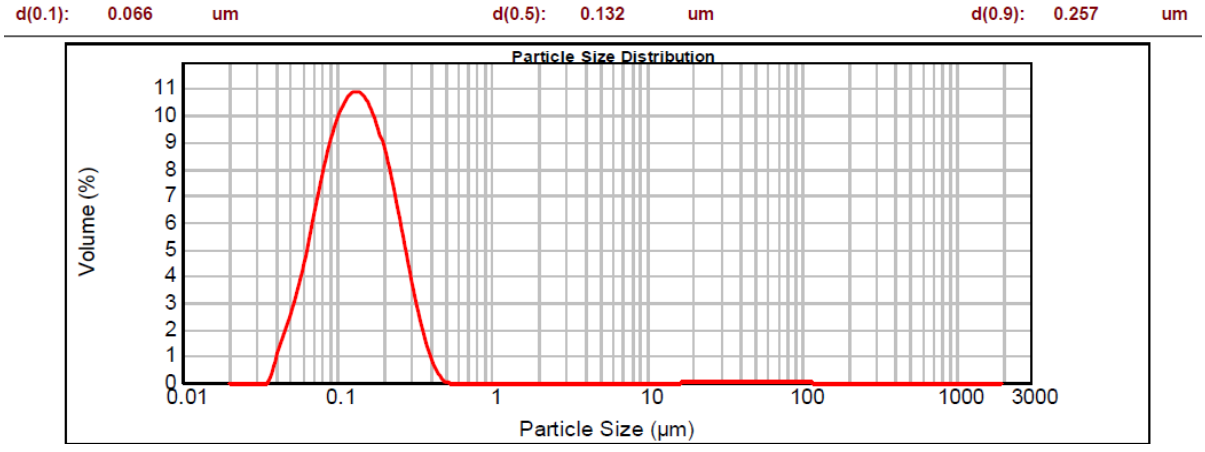

Figure 2. Nanoparticles particle size with 15 minutes sonication time.

PLGA content

PLGA content was varied from 25 mg and 50 mg, and the effects of weight of polymer on the particles morphology and size distribution were studied. The results are depicted in the Table 3. The nanoparticles prepared with 25 mg of PLGA were shown spherical shape and absence of agglomeration where as prepared with 50 mg of PLGA was shown non- spherical shape and presence of agglomeration. When the amount of polymer was increase from 25 mg to 37.5 mg, the nanoparticles diameter size increased from 256 to 267nm. The particles with 37.5 mg polymer were shown monomodal distribution profile. The size of nanoparticles increased as polymer concentration was also increased. This was caused by the increasing viscosity of dispersed phase (polymer solution) resulting a poorer dispersability of the polymer solution into the external aqueous phase .Coarse emulsion were obtained at higher polymer concentrations, which lead to the build of bigger particles during the diffusion process.

Table 3 Impact of PLGA content on nanoparticles means diameters, Span value and granulometric size distribution.

PLGA content (mg)	Mean Diameter (nm)	Span Value	Size distribution (nm)
25	253± 20	0.22	10% (180-210) 90%(240-275)
37.5	266± 12	0.16	100% (267-276)
50	340± 21	0.21	80% (260-365) 20% (840-1120)

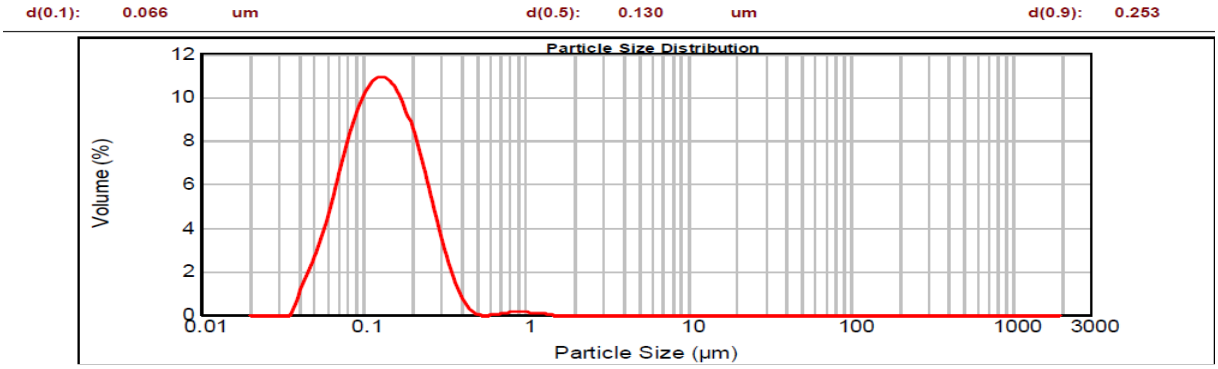

Figure 3. Nanoparticles particle size with 25 mg PLGA content.

Surfactant content

In order to study the impact of surfactant content on nanoparticles mean diameters, Span value and granulometric size distribution, few batches were prepared by using an external aqueous phase consisting of PVA at different concentration (0.25, 0.50 and 0.75).The results are shown in Table 4.We observed from the results that there was a decrease in particles size (325 -260 nm) when the surfactant concentration in the external aqueous phase was increased from 0.25 to 0.75 % w/v).The granulometric distribution became narrower as the amount of PVA was increased. This was probably caused by an insufficient amount of emulsifier would fail in stabilizing all the nanoparticles and thus some of them would tend to aggregate. As a result nanoparticles with larger size would be produced and nanoparticles with low surfactant concentration showed non-spherical shape and presence of agglomerates.

Table 4 Impact of Surfactant content on nanoparticles means diameters, Span value and granulometric size distribution

Surfactant content (% w/v)	Mean Diameter (nm)	Span Value	Size distribution (nm)
0.25	320± 20	0.089	100% (282-385)
0.50	275± 11	0.24	100% (258-281)
0.75	260± 14	0.17	100% (252-278)

Aqueous to organic phase volume ratio

The ratio between external aqueous Phase to internal organic phase is of great impact to its stability and influence the size of dispersed globules. The Internal organic phase was varied from 1 to 5 mL, its impact on mean diameter, span value and nanoparticles size distribution was observed. The results are presented in Table 5. It can be observed that an increase in the internal organic phase/external aqueous phase ratio leads to a slight decrease in nanoparticles mean diameter. This was probably caused by the coalescence of droplets can be prevented by a large amount of organic solvent available for diffusion in the O/W emulsion.

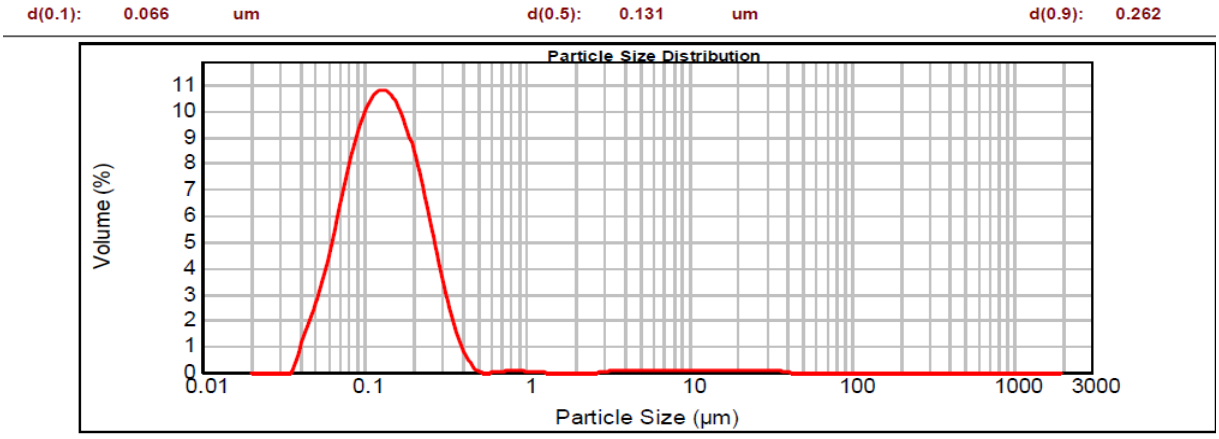

Figure 4. Nanoparticles particle size with 0.75(% w/v) Surfactant content

Table 5 Impact of Aqueous Phase to organic phase volume ratio on nanoparticles means diameters, Span value and granulometric size distribution

Internal phase volume (mL)	1	2.5	5
Mean Diameter (nm)	420± 15	277± 18	242± 21
Span Value	0.16	0.14	0.09
Size distribution (nm)	21% (175-240) 79% (520-630)	100% (261-271)	10% (110-125) 90% (235-284)

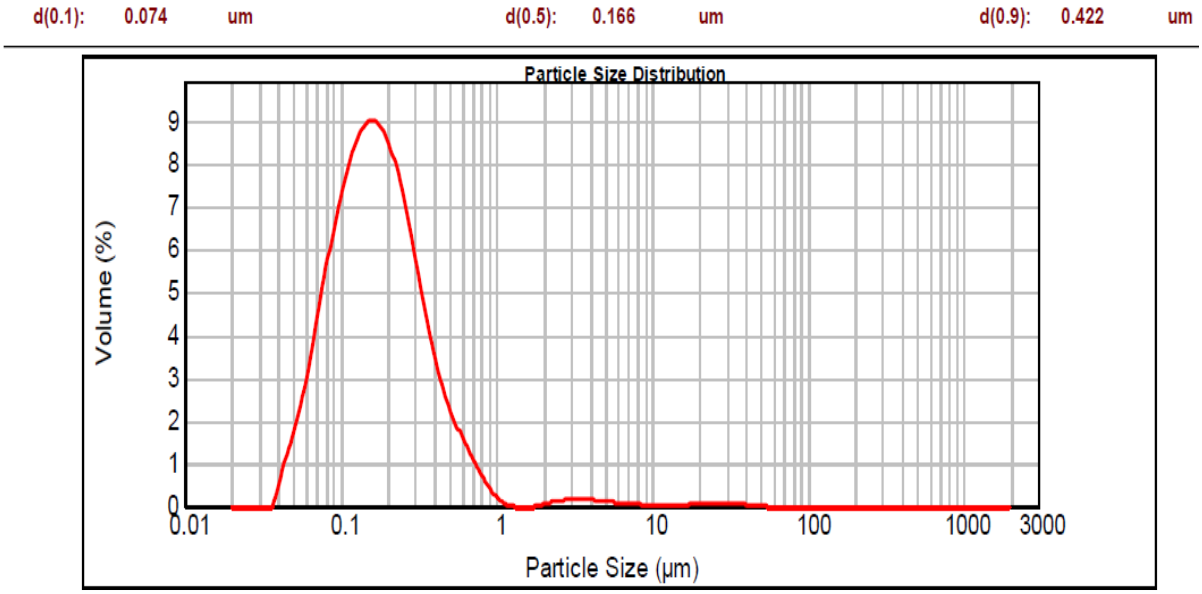

Figure 5. Nanoparticles particle size with internal phase volume 1 mL.

Table 6 Impact of solvent-evaporation rate on nanoparticles means diameters, Span value and granulometric size distribution.

solvent-evaporation	Vacuum evaporator (30 minutes)	Magnetic Stirring (5 hr)	
Mean Diameter (nm)	310±22	440±24	
Span Value	0.22	0.18	
Size distribution (nm)	21%(160-210nm) 79 %(502-635 nm)	35%(270-320nm) 65%(310-740 nm)	

Rate of solvent - evaporation

In the solvent- evaporation technique the impact of organic solvent evaporation rate on mean diameter and size distribution were assessed by using vacuum rotatary evaporator and the other was magnetic stirring under normal pressure. The results are given in Table 6. It was observed that nanoparticles showed smaller mean diameters when the vacuum evaporator method used in respect to magnetic stirring method. The reason for the formation of smaller

particles is that in rotative vacuum evaporator method the rate of diffusion of the organic phase is higher as compared to the magnetic stirring method.

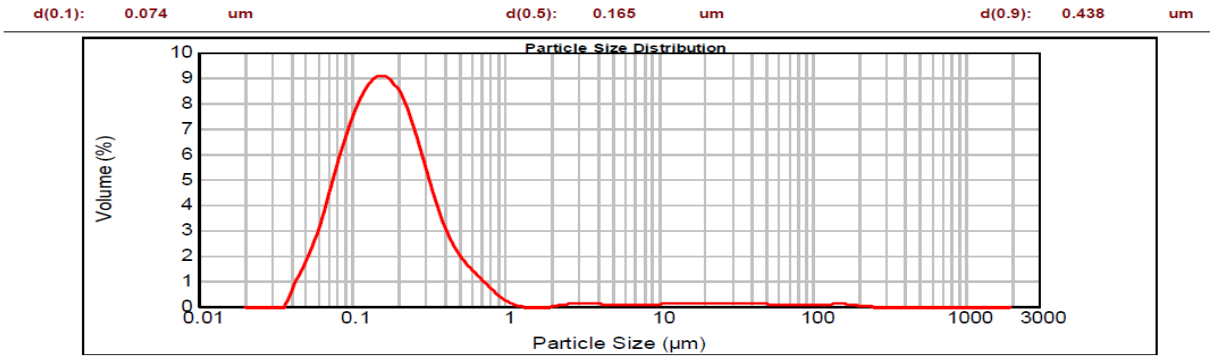

Figure 6. Nanoparticles particle size with magnetic stirring..

In vitro drug release profiles

In vitro drug release of carvedilol was studied as a function of time. Nanoparticles containing the (15%) (theoretical loading) and maximum carvedilol loading (38) (theoretical loading) were studied. The results are shown in Figure.7. It was observed that the nanoparticles containing higher amount of drug leads to quick the release and the particle with small drug amount exhibit a sustained manner release.

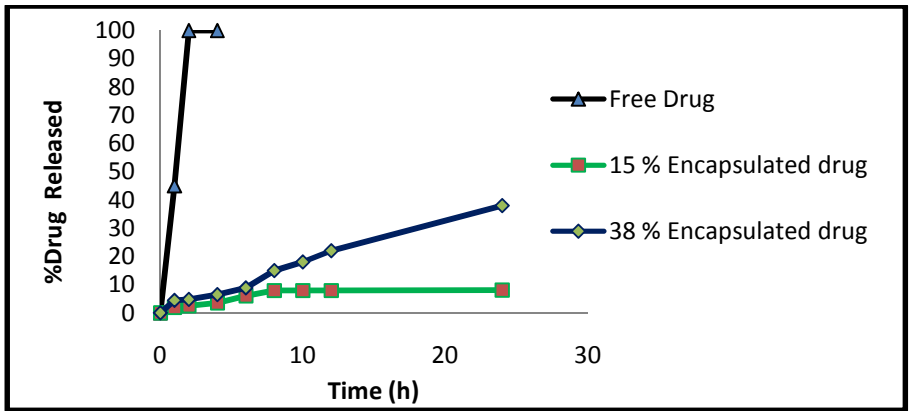

Figure 7.In vitro drug release kinetics of carvedilol loaded nanoparticles.

CONCLUSION

The emulsification by sonication-evaporation method allowed the preparation of spherical drug- loaded systems of biodegradable PLGA carriers containing an antihypertensive drug, carvedilol, incorporated in the polymer matrix. Preparative variables such as concentration of stabilizer and polymer, time of sonication, diffusion rate of organic solvent, and ratio between external and internal phases, showed to be important factor for the formation of PLGA nanoparticles. In release kinetics higher initial drug loading resulting in faster drug release.

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