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PREPARATION AND PHYSICO-CHEMICAL CHARACTERIZATION OF CARVEDILOL- POLY (LACTIDE-CO-GLYCOLIC ACID) LOADED NANOPARTICLES.

SOVAN LAL PAL[1], PRABAL KUMAR MANNA[2] , GURU PRASAD MOHANTA[3*]

[1,2,3]Department of Pharmacy, Annamalai University, Annamalainagar-608002, Tamil Nadu, India.

ABSTRACT

Carvedilol is a non-cardio selectivity β- blocker which is used for the treatment of essential hypertension and symptomatic heart failure. The objective of the present study was to prepare and determine the physicochemical characteristics of Carvedilol-Poly (Lactide-co-Glycolic acid) nanoparticles. The nanoparticles of Carvedilol with Poly (Lactide-co-Glycolic acid) were formulated following the solvent evaporation technique. The impact of various process parameters i.e. drug/polymer ratio, aqueous phase volume and speed of sonication time were evaluated and optimal conditions were established. The physicochemical characteristics of nanoparticles were studied applying Particle Size Analysis, Fourier Transform Infra Red Spectroscopy, Differential Scanning Calorimetry, Scanning Electron Microscopy and Atomic force microscopy. The release rate of carvedilol from various drug/polymer nanoparticles was observed as well. All the prepared formulation using Poly (Lactide-co-Glycolic acid) resulted in nano-range size particles (253-438nm) as detected by Zeta Sizer. The entrapment efficiencies were observed for all the nanoparticles in a range of 65.45 % to 85.56%.The nanoparticles of carvedilol- Poly (Lactide-co-Glycolic acid) observed no chemical interaction between drug and polymer molecules. The Scanning Electron Microscopy image revealed that particles were smooth, spherical in shape. The particle aggregation of the particles was observed by Atomic Force Microscopy study. The nanoparticles exhibited the slower release of drug in comparison with the intact drug and polymer molecules. The method was successfully applied in drug release studies from nanoparticles. The drug release kinetics was found to be fitted into the Higuchi model. According to the finding, formulation of carvedilol- Poly (Lactide-co-Glycolic acid) was able to improve physicochemical characteristics of the drug and possibly will enhance the antihypertensive effects of the drug following its oral administration.

KEYWORDS: ***Carvedilol; nanoparticles; physicochemical characteristics, Poly (Lactide-co-Glycolic acid), solvent evaporation.***

INTRODUCTION

Hypertension is an increased blood pressure. The elevation of blood pressure in the arteries leads to hypertension[1]. Arterial hypertension is a major risk factor for stroke, coronary events, and renal failure[2] in both industrialized and low- and middle-income countries, leading to substantial morbidity and mortality. The rationale for treating hypertension has achieved significant impetus with the finding that even small reductions in blood pressure can reduce associated morbidity and mortality risks. β-blockers have long been used as effective antihypertensive agents and, together with diuretics, have been the cornerstone of pioneering studies showing their benefits on cardiovascular morbidity and mortality as a consequence of blood pressure reduction in patients with hypertension[3]. Carvedilol is a vasodilating noncardioselective third-generation β- blocker, without the negative hemodynamic and metabolic effect of traditional β-blockers, carvedilol maintains cardiac output, has reduced prolonged effects on heart rate, and reduces blood pressure by decreasing vascular resistance[4]. Carvedilol is the first β- blocker to be approved for treating chronic heart failure. It is a lipophilic vasodilating noncardioselective β-blocker which lacks intrinsic sympathomimetic activity, thus having improved tolerability compared with older β- blockers. The older generation "traditional" β- blockers selectively antagonize β1-adrenergic receptors or antagonize

both β1-adrenergic and β2- adrenergic receptors. They also reduce blood pressure mainly through reduction in cardiac output, while systemic vascular resistance remains largely unchanged. In contrast, carvedilol blocks norepinephrine binding to α 1-adrenergic receptors in addition to both β1-adrenergic and β2- adrenergic receptors[4]. This results in a reduction in arterial blood pressure by maintaining cardiac output and decreasing total β-adrenoreceptor vasoconstrictor tone markedly superior to that of traditional β- blockers. Carvedilol has bioavailability of about 25 to 35% because of extensive first-pass metabolism[5].Carvedilol undergoes oxidative metabolism and glucuronidation in the liver; the oxidative metabolism occurs via cytochrome CYP2D6[6]. Interestingly Carvedilol also has an antioxidant activity. Carvedilol is a poorly water-soluble drug with a low bioavailability and short half life requiring 2-3 times administration per day. Rapid absorption coupled with the short elimination half-life can result in significant fluctuation in plasma drug concentrations during repetitive dosing. By controlling drug input from a modified release rate, modified release dosage form the problem of drug plasma level fluctuation in plasma may be overcome. This should have the advantage of providing a prolonged therapeutic effect with a reduced incidence of side effects. Biologically adhesive delivery systems may offer important advantages over conventional in the bioavailability of the drugs. 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[7]. Polymeric nanoparticles are the one of the effective way to address the issues of the drug. Once the drug is embedded with suitable polymeric materials the nature of the drug can possible be prevented. This polymeric nanoparticulate system have been considered a promising carrier for oral sustained release drug delivery followed through beneficial to the patient for the long-term treatment. The sub-cellular size of the nanoparticles could improve the stability of active drug and allows relatively higher intracellular uptake of native drug than the other particular system. 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[8].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. Its shown that Poly (DL-lactide/glycolide copolymer) (PLGA) has bio adhesive properties and binds with mucosa of gastrointestinal tract[9]. 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[10]. They can be taken up by enterocytes[11] and the lymphoid tissues in payers' patches. Thus, nanoparticles of PLGA have great potential as on oral bio adhesive, sustained drug delivery system for carvedilol in order to reduce the initial hypotensive peak and prolongation of antihypertensive effect of the molecule. Taking into consideration that the nanoparticles may confer capability for prolonged and effective drug delivery, the present study was to prepare carvedilol loaded PLGA polymeric nanoparticles and to study the physicochemical characteristic of prepared nanoparticles with a view to the slow release of carvedilol. We endeavor to formulate of carvedilol - PLGA nanoparticles by directing emulsion solvent evaporation / extraction technique. This method has initially described by RA Jain[12] to achieve particles with suitable size, charge and stability properties[13]. The physicochemical properties of the nanoparticles were characterized as well.

MATERIALS AND METHODS

Materials

Carvedilol sample was obtained from Zydas Cadila Health Care (Ahmedabad, India). Poly (DL-lactide/glycolide copolymer) (PLGA,50:50 with inherent viscosity 0.37 dL/g) was procured from Boehringer Ingelheim Co,(Ingelheim, Germany), Polyvinyl alcohol was generously supplied by Torrent Pharmaceuticals, Ahmedabad, Gujarat, India. Acetonitrile and methanol were of HPLC grade and purchased from Merck, India. All other chemicals were of analytical grade and procured from Merck. Milli-Q water was used for mobile phase preparation.

Preparation of nanoparticles

The nanoparticles of carvedilol with different ratio

of Drug / PLGA (1:1, 1:2 and 1:4) were prepared using emulsion solvent evaporation method as reported earlier[12,13,15] with some modification. The method involves preparation of an o/w emulsion between and organic phase (OP) consisting of carvedilol and PLGA in the dichloromethane and aqueous phase (AP), 1% w/v aqueous solution of Polyvinyl alcohol. Polyvinyl alcohol was used as a stabilizer during solvent evaporation technique. In the experiment, carvedilol was dispersed in the mixture of dichloromethane and acetone solution of PLGA by sonication (20 W, 40% duty cycle, 60s) using probe ultrasonicator (Branson Sonifier 450, USA). The resulted dispersion was added slowly into the aqueous phase (AP) with a constant flow rate (0.5 ml/min). During this process, the dispersion was homogenized at 14000 rpm using a probe homogenizer (Virtis Cyclone IQ, USA) to obtain o/w emulsion. The resultant (o/w) emulsion was kept at room temperature (25±2°C) for 24 hrs under stirring condition to evaporate the organic solvent. The resultant nanosuspension was centrifuged at 40,000 rpm for 25 minutes (Sorvall Ultracentriguge, USA). The pellets were collected and washed at least three times with double distilled water to remove unentrapped drugs. The recovered nanoparticulate suspensions were dried (-80°C and 10 mm mercury pressure) in a lyophilizer (Freezone 6 lt, Labconco Corp., MO) to get powdered nanoparticles and stored in a hermetically sealed container in a refrigerator. The influence of various process as well as formulation variables on entrapment efficiency was investigated in order to optimize the formulation with highest level of entrapment of drug in the nanoparticles formulation (Table 1).

Determination of particle size and zeta potential by Zetasizer[16]

Particle size and polydispersity were analysed by Photon Correlation Spectroscopy (PCS) with Zetasizer 3000 (Malvern Instrument, Malvern, UK). Each suspension was diluted to the appropriate concentration with double distilled water, previously filtered through a 0.22- µm filter (Millipore®). Photon Correlation Spectroscopy (PCS) is based on the measurement of the Brownian motion of particles. The Brownian motion is the random movement of particles in suspensions. The smaller the particles, the faster the Brownian motion. When the incident laser beam reaches the sample, light is scattered in such a way, depending on the Brownian motion, and then detected by a photomultiplier positioned at a determined angle (here at a fixed angle of 90°). Fluctuation in the intensity of scattered light are converted into output current, which is passed to an autocorrelator. In this way, a correlation function is generated and analyzed by software. The computer can provide the mean size and the distribution width of the nanoparticles in the batch. Analysis was carried out at least for three times for each batch of sample under identical condition and mean value were reported. The same colloidal suspension was used to carry out the characterization of Zeta potential of drug loaded nanoparticles with the help of same instruments.

Determination of drug entrapment efficiency and loading capacity by RP-HPLC[16]

The entrapment efficiency (EE) and loading capacity (LC) were estimated by reverse phase High performance liquid chromatography (RP-HPLC) method. The drug loaded nanoparticles solution of 1 mg/ml was prepared in mobile phase and 20 µl of the sample was injected manually to HPLC equipped with Shimadzu LC-20AD PLC Pump and SPD-M 20 A PDA detector. The output signal was monitored and integrated using Shimadzu Class-VP version 6.12 SPI software. The chromatographic separation was achieved by using Phenomenex 150 x 4.6 mm, 5µm (X-Terra; C18) analytical Column. The mobile phase used to consist of acetonitrile: acetate buffer pH 3.0: water (75:600:325) was passed through 0.45 µm membrane filter and degassed by ultrasonication. The flow rate was maintained at 1.0 mL/min and the measurement was made at 240 nm. The column was maintained in ambient condition using thermostat. The amount of the carvedilol in the sample was determined from the peak area correlated with the standard curve. The standard curve was prepared under the same identical condition.

Scanning Electron Microscopy (SEM) Studies[17]

The particle shape and surface morphology of Carvedilol loaded PLGA nanoparticles were studied using Scanning Electron Microscope (JSM 5610LV, SEM, JEOL, Datum Ltd, Tokyo, Japan). Freeze dried & moisture free samples were consigned on 10 x 10 mm brass stub using adhesive tapes and coated with gold using sputter coater (Joel auto fine coater, Japan) and observed for morphology at accelerated voltage of 20 KV at high vacuums mode.

Atomic Force microscopy (AFM) Studies [18]

The morphological characterization and direct visualization of the prepared nanoparticulate

formulations were performed using atomic force microscopy studies. It provides both quantitative and qualitative information on many physical properties, including size, surface area and volume of distribution. The nanoparticle suspension was prepared with milli-Q water and dried overnight in air on a clean glass surface and observation was performed with AFM (JPK Nano WizardII, JPK instrument, Berlin, Germany) with silicon tube by spin coat technique and immediately dried under vacuum. The scan speed of 2 kHz and 312 kHz resonant frequency was used for displaying amplitude, signal of the cantilever in the trace direction and to obtained image[14].

Fourier Transforms Infrared (FTIR) Spectroscopy analysis [19]

The chemical interaction and possible chemical integrity between the drug, polymer and prepared nanoparticles were characterized by FTIR analysis (Perkin Elmer, FT-IR Spectrometer, SPECTRUM RX 1, USA). Samples were mixed separately with potassium bromide (200-400 mg) and compressed by applying pressure of 200 kg/cm^2 for 2 minutes in hydraulic press to prepare the pellets. The pellets of the native drug, polymer and drug loaded nanoparticles were analysed by placing it on the light path. All the samples were scanned by averaging 32 interferograms with resolution of 2 cm^{-1} in the range of 4000-400 cm^{-1}.

Differential scanning calorimetry (DSC) studies[20]

The substantial status of the drug in-between the nanoparticles was ascertained by (DSC) analysis (Shimadzu, model no- DSC-60). The weighted quantity of 2 mg of drug, polymer & nanoparticles were placed separately into the different seal standard DSC aluminium pan, were scanned between 25°C and 300°C with heating rate of 10°C/minutes under an atmosphere of dry nitrogen. An empty aluminium pan served as reference.

Accelerated stability study

The nanoparticles were packed in borosilicate glass vials (USP Type I) and closed with Bromo butyl rubber stoppers & crimped with tear off clear lacquer aluminium seals and samples were stored in environmental simulation chambers for constant climatic conditions (Binder, Tuttlingen, Germany). Table 2 shows the storage conditions used in the stability study and samples were tested as per ICH (International Conference on Harmonization) guidelines[21]. Physicochemical characterization of the carvedilol loaded PLGA nanoparticles were studied over 6 months at regular intervals by dispersing 1 mg of carvedilol loaded Harmonization) guidelines. Physicochemical characterization of the carvedilol loaded PLGA nanoparticles in 10 ml of distilled water observes any degradation. The studies were carried out in triplicate from time to time. Particle size and zeta potential were measured using Zetasizer 3000 (Malvern Instrument, Malvern, UK). The chemical stability (drug content) of the formulation was determined by RP-HPLC at 240 nm.

In vitro drug release study[12,15]

In-vitro drug release studies were carried out by using rotating basket. The pure carvedilol, carvedilol loadedPLGA nanoparticles and marketed tablet formulation (each containing 10 mgcarvedilol) were suspended in glass bottlescontaining 100 ml of phosphate buffer pH 7.4. Glass bottles wereplaced in beaker and kept in incubator shaker throughout the study (37°C, 50 rpm). At specified time intervals 10 ml samples were collected and centrifuged at 13,500 rpm for 30 min. The supernatantswere collected for analysis and the precipitate resuspended in 10 ml of fresh phosphate buffer. The supernatant was lyophilised (Lab-conco Lyophilizer, USA) for 24 hr and the obtained dry powder was dissolved in mobile phase and analysed by RP-HPLC at 240 nm. All the measurements were carried out in triplicate.

STATISTICAL ANALYSIS

For statistical analysis the experimental data was tested by one-way analysis of variance (ANOVA). Data represented as mean values ± SD (standard deviation). A ρ value less than 0.05(*) was assumed for statistically significance difference and very significance difference if ρ<0.005(**).

RESULTS AND DISCUSSION

Particle size and Zeta potential measurement

Particle size and size distribution are two important micromeritic properties of drugs/drug products because they affect their dissolution rate, physical stability and in-vivo behaviour. Polydispersity Index (PDI) and Zeta-Potential of nano-dispersions are indicative of closeness of size distribution and stability of such systems. Results of the particle size and Zeta- Potential analysis obtained from Zetasizer 3000 (Malvern Instrument, Malvern, UK) are furnished in Table 1 and Figure. 1.

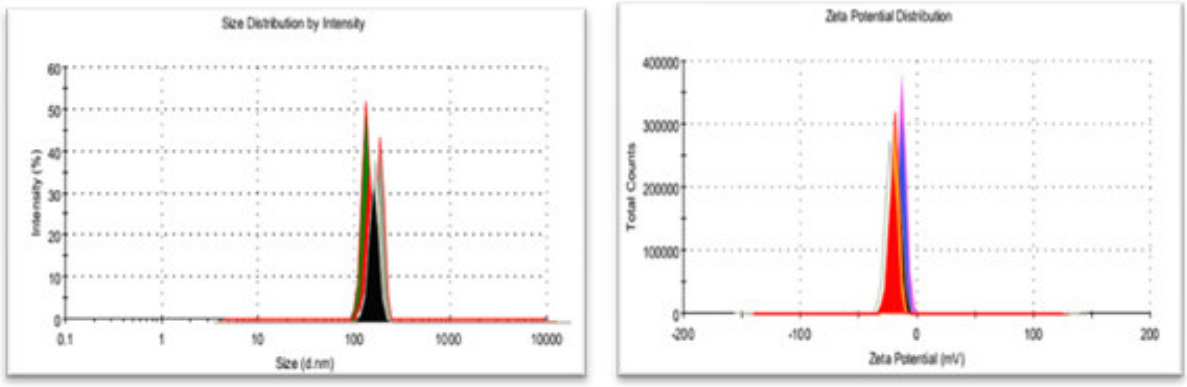
Table 1
Physico-chemical characterization of carvedilol loaded PLGA nanoparticles (data represents mean ± SD).

Sr. No	Formulation Code	Drug-Polymer (ratio)	Particle Size (nm) ± SD n=6	Poly Dis. Index (PDI)	Zeta Potential ± SD (mV) n=6	Loading Capacity[a] (% w/w)	Entrapment Efficiency[b] (% w/w)
01	CNPA1	1:1	253±3.12	0.326±0.05	-22.21±.62	21.65±0.72	66.41±0.75
02	CNPA2	1:2	262±3.46	0.403±0.03	-26.60± 0.58	22.41±0.38	85.56±0.72
03	CNPA3	1:4	422±5.12	0.436±0.08	-23.10±1.04	16.85±0.85	74.32±0.41
04	CNPA4	1:1	257±3.31	0.312±0.07	-22.80±0.76	23.75±0.46	65.45±0.91
05	CNPA5	1:2	271±4.32	0.410±0.03	-28.80±0.46	19.29±0.61	83.42±0.51
06	CNPA6	1:4	438±5.12	0.324±0.06	-18.21±1.10	18.56±0.82	69.32±0.62

[a]Drug Loading; [b]Entrapment efficiency; SD- standard deviation

$$[a]\text{Drug Loading (DL) \%w/w} = \frac{\text{Weight of the drug in nanopartic les}}{\text{Weight of the polymer and drug added}} \times 100$$

$$[b]\text{Entrapment efficiency (EE) \%w/w} = \frac{\text{Weight of the drug in nanopartic les}}{\text{Weight of the drug added}} \times 100$$



(a) ***(b)***

Figure 1
(a) Mean particle size distribution of the PLGA nanoparticles (CNPA1, CNPA2, CNPA3,CNPA4, CNPA5, CNPA6) prepared with various sonication drug-polymer ration and sonication speed. (b) Zeta Potential of the PLGA nanoparticles (CNPA1, CNPA2, CNPA3,CNPA4, CNPA5, CNPA6) prepared with various sonication drug-polymer ration and sonication speed.

The sharp and steep peaks in size distribution plots (Figure. 1) indicate narrow size range and uniformity in particle size in all the formulations. All the prepared formulations were in the nano-size range (253 nm to 438 nm) and the size distributions were relatively monodisperse in all of the formulations with the polydispersity index (PDI) values ranging between 0.312 and 0.436. A polydispersity index (PDI) value of less than 0.5 is indicative of very narrow size distribution range with very good control over particle size. The PDI values of all the nano-formulation in the present investigation are well below 0.5 and thus confirm that the prepared nano-formulations were of very controlled particle sizes with very narrow size distributions. During formulation development processing and formulation parameters were modulated to achieve the desired nano size range. The various parameters, influencing the product size, modulated were sonication intensity and duration, volume of aqueous phase (External phase) and drug/polymer ratio). The freeze-dried nanoparticles were found to be discrete, free flowing, spherical and were of the matrix type. The sizes of nanoparticles increased with increase in polymer ratio due to increased viscosity of the

coating solution phase. The volume of the aqueous phase (external phase) also influenced the size of the nanoparticles; the size was inversely proportional to the volume of aqueous phase. Significant difference was observed in mean particle size ($\rho<0.05$) in various formulations made with varying proportions of the aqueous phase. Formulations prepared with 15 ml of aqueous external phase produced smallest particles while formulation prepared with 45 ml of aqueous phase produced largest nanoparticles. Zeta potential provides information's about the surface charge properties and further the long-term physical stability of the nano suspensions. The value of particles surface charge indicates the strength of the interactive force between particle and particle at the nanosurfaces, which is the basis to nano suspensions stability at the macroscopic level. The Zeta potential is defined as the difference in potential between the surface of the highly bound layer (shear plane) and the electro-neutral region of the solution. The potential gradually decreases as the distance from the surface increases. In order to obtain an electrostatically stabilized nano suspension, a minimum zeta potential of ± 30mv is required. In the case of a combination of electrostatic and steric stabilization, a minimum zeta potential of ± 30mV is desirable. In the present investigation, zeta potential values of the carvedilol loaded PLGA nanosuspensions were between -18.21 to -28.80 mV (Table 1). In this case, the prepared nanosuspensions contains polymer embedded drugs and hence are stabilized by combination of electrostatic and steric stabilization. This result also suggests the external localization of free drugs which were adsorbed on the surface of the polymeric nanoparticles. Having '-'ve zeta potential, carvedilol-PLGA nanosuspensions are likely to facilitate an effective cohesion of the nanoparticles with the positively charged mucous membrane of the gastro-intestinal tract and this will help to prolong the effective residence time of the nanoparticles.

Surface morphological properties of nanoparticles

The assessment of the particle morphology helps in understanding the morphological changes that a drug might undergo when subjected to nanosizing. In order to get an actual understanding of particle morphology, the technique i.e., scanning electron microscopy (SEM) is preferred. SEM experiments revealed a spherical shape with a relative smooth surface for the resultant nanoparticles formulation.

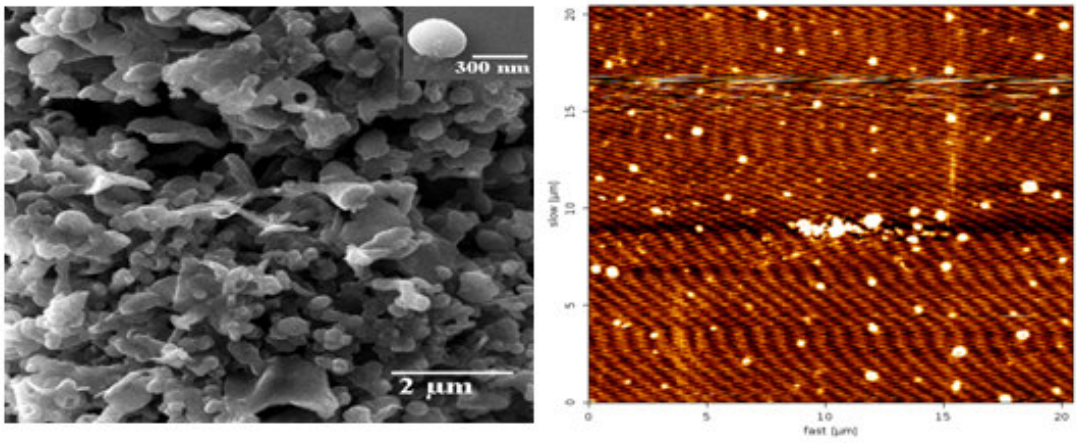


Figure 2

(a) Surface morphological properties of nanaoparticles. Scanning electron microscopy image of PLGA nanoparticles (CNPA2),

(b) Surface morphological properties of nanaoparticles. Size distribution PLGA nanoparticles as measured by AFM

The SEM image of nanoparticles revealed almost spherical in shape with relative smooth surface for all the formulations (Figure 2a). The AFM investigations revealed the disc like shape of the particles. Additionally, it was proven that the particles are surrounded by a soft layer (Figure 2b). The particle sizes obtained by SEM were relatively smaller than that of the particle sizes obtained by Zetasizer. The electron microscope picture (SEM) allows only the visualization of the nanoparticle surface, whereas the hydrodynamic layer was measured by Zetasizer.

Drug entrapment efficiency and loading capacity

The nature of polymer, drug and surfactant plays a vital role for higher entrapment efficiency. The

entrapment efficiency of nanoparticles is the function of the characteristics of the polymer, drug, surfactant etc. The high entrapment efficiency is observed when both drug and polymer have the high affinity to the same solvent. The low entrapment is due to the high affinity of drug and polymer to the different solvents. In our study drug loading and entrapment efficiency were influenced by drug and polymer ratio in the formulation. The maximum entrapment efficiency was observed with higher concentration of polymer in the formulation. The entrapment efficiency of nanoparticles were in the range of 66.41% to 85.56% and the drug loading were 16.85% and 23.75% respectively. Low values of standard deviation in percentage drug content indicate the uniformity of drug content in each batch of nanoparticles. The low yield in some cases could be attributed to losses occurring during various steps of processing, such as sticking of polymeric solution to glass container and due to washing steps .The nanoparticles formulation (CNPA2) prepared with drug-polymer ratio 1:2 with stabilizer concentration 3.0 % and sonication speed 20 W, 40% duty cycle, shows the entrapment efficiency 85.56 %, drug loading of 22.41 % and particle size of 262 nm with Zeta potential value -26.60 (Table 1, Figure 1.a, & Figure 1.b) . Based on the particle size and entrapment efficiency formulation (CNPA2) was selected and used for further studies.

Fourier transforms infrared spectroscopy

The FTIR spectral data were used to find out the interaction between the drug and polymer in the nanoparticles. FTIR spectra of pure carvedilol, polymer, carvedilol loaded PLGA nanoparticles are carried out for the interaction study between the drug and polymer (Figure 3). There were no distinctive changes in the FTIR spectrum of carvedilol and the physical mixture indicating that PLGA was not involved in intermolecular interaction. However, the intensity of the peak at 1505.17 cm-1 and 2418 cm-1 were slightly decreased in the FTIR spectrum of the related nanoparticles due to the intermolecular hydrogen bonding between carvedilol and PLGA, indicating the chemical stability of the carvedilol inside the nanoparticles.

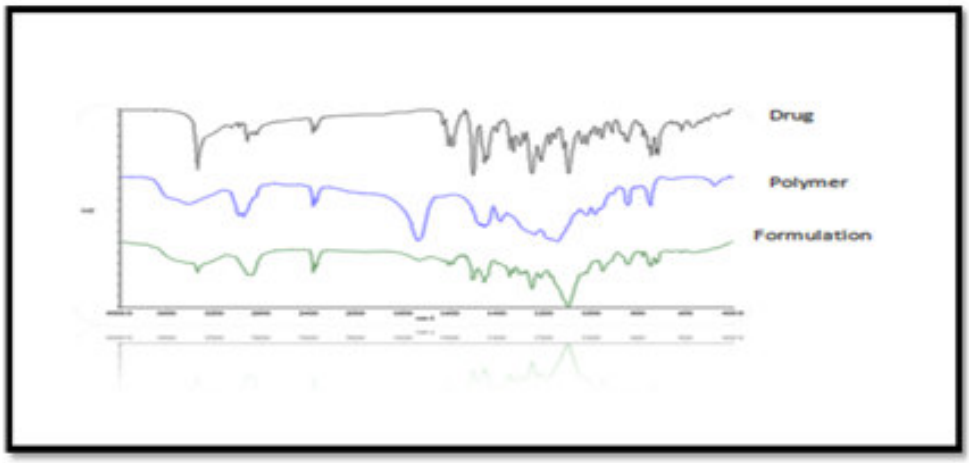


Figure 3
FTIR Spectra of Drug (carvedilol), Polymer (PLGA) and Formulation-Carvedilol Loaded PLGA Nanoparticles (CNPA2)

Differential scanning calorimetry

Differential scanning calorimetry used to analyse the physiochemical interaction of the drug encapsulated and the polymer. The analysis was performed for the pure carvedilol, polymer and carvedilol loaded PLGA nanoparticles (Figure 4). Different compounds show their characteristic peaks in DSC.

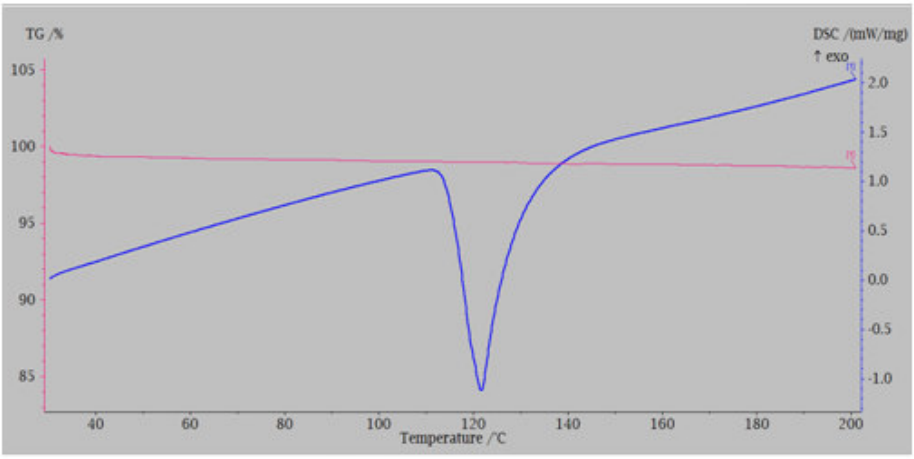


Figure 4
DSC thermogram of Drug (carvedilol), Polymer (PLGA) and Formulation-Carvedilol Loaded PLGA Nanoparticles (CNPA2)

The prominent and sharp endothermic peak at 120.3°C in the thermogram of native carvedilol represents its melting point. This sharp endothermic peak indicated that the intact carvedilol was in crystalline anhydrous state. The nanoformulation depicted no distinctive peak of the carvedilol in the DSC profiles owing to the decreased crystallinity in the formulations and/or drug salvation in the amorphous carrier as well as solid state interaction induced by heating. The glass transition temperature of the PLGA was found to be 64.50ºC.

In vitro drug release study

The drug release from pharmaceutical nanoparticles is a major determinant of its biological effects, thus evaluation of drug release pattern is of paramount importance in the field. Drug release profile of native drug powder and the best selected drug loaded nanoparticles formulation (CNPA2 with mean particle diameter 262 nm) and standard reference (a conventional markted product) in phosphate buffer pH 6.8 are furnished in Figure 5.

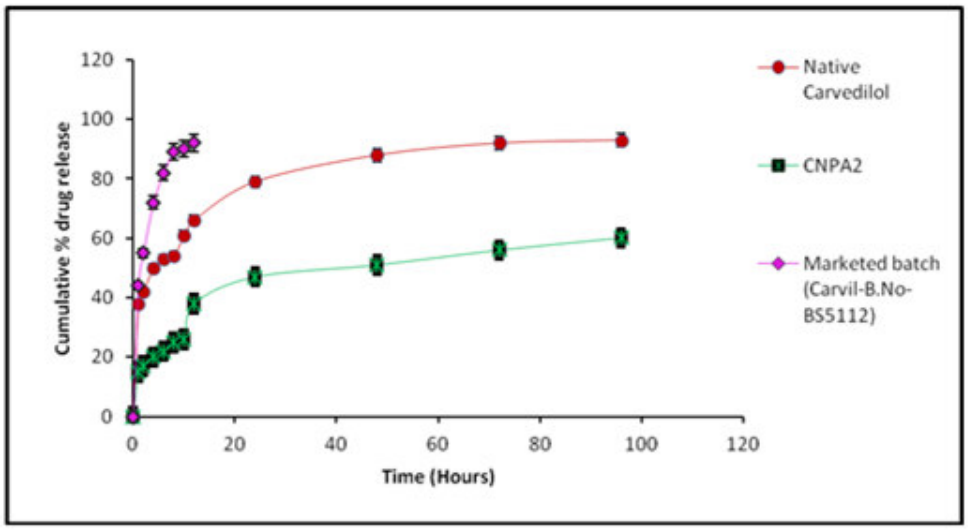


Figure 5
In Vitro Drug Release Profile of Native Carvedilol, Marketed Formulation and Carvedilol Loaded PLGA Nanoparticles in Phosphate Buffer pH 6.8

The in vitro release profile of carvedilol from drug loaded nanoparticles exhibited a biphasic release phenomenon. Initial burst release was observed and 38 % of the cumulative amount of carvedilol released during first 1hr. Afterwards, the drug was released slowly at constant rate and around 79 % drug was released at the end of 24 hrs. Beyond 24 hours the drug release was too slow and of insignificant amount. The rapid initial release of carvedilol was probably due to adsorbed and/or

embedded drug on the surface of the nanoparticles while the slow sustained release in second phase was due to diffusion of the drug from inner layers of the matrix. Extremely slow drug release beyond 24 hrs was probably due to depletion of drug from upper layers resulting in increased path length that the drug molecules had to travel coupled with various binding forces that opposed diffusion of the drug molecules. The drug release from pure drug as well as from Reference Standard (a conventional marketed formulation) was very fast in comparison to that from nanoparticles formulation. It suggests that the combination of dissolution, diffusion and erosion are the possible mechanism of drug release from the nanoparticles.

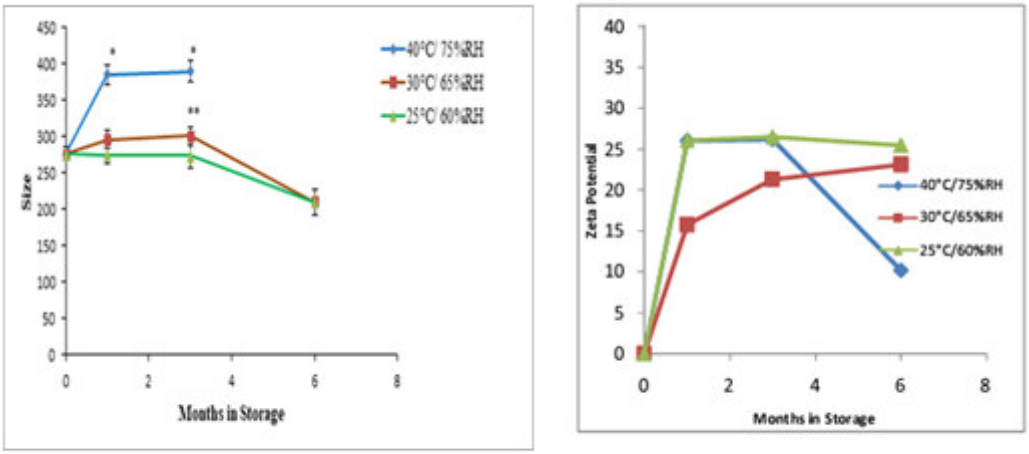
Accelerated stability study

The accelerated stability study of the carvedilol loaded PLGA nanoparticles were performed to establish stability of the formulation over the time period of 6 months of different storage condition (Table 2). The physical observation of the carvedilol loaded PLGA nanoparticles stored at 30°C/65%RH and 25°C/60%RH for 6 months showed a powdered aspect and were easily dispersed in phosphate buffer. The samples stored at 40°C/75%RH showed a clumping aspect at the time of dispersion in phosphate buffer. The size of carvedilol loaded PLGA nanoparticles stored at 40°C/75%RH for 1 and 3 months showed larger than at time 0 ($\rho<0.01$), and at 6 months formulation turns aggregates. At 30°C/65%RH and 25°C/60%RH, particle size (Figure 6a) hardly varied during the 6 months stability period. No significant changes were observed after completion of stability studies.

Table 2

Storage condition of the different groups included in the study (Temperature and Relative Humidity (RH)

Group	Storage conditions	Time point
Time 0	**Nil**	**0**
Long-term	25±2°C/60%RH±5% RH	3, 6 and 9 months
Intermediate	30±2°C/65%RH±5% RH	3, 6 and 9 months
Accelerated	40±2°C/75%RH±5% RH	1,3 and 6 months



(a) *(b)*

Figure 6

(a) And (b) Size and zeta potential of PLGA nanoparticles (CNPA2) stored at different storage conditions during different times from 1 to 6 months (n=6). X-axis indicates time of storage (months) .Error bars represent SD (n=3).*$\rho<0.05$ against time 0; ** $\rho<0.005$ against time 0.

The drug content estimation of carvedilol loaded PLGA nanoparticles following RP-HPLC analysis showed that there was no significant change in the content of carvedilol (97.12±0.212% at 0 time) after 6 months of storage condition. At ambient temperature, drug content was 98.12±0.212% after 6 month of storage. While the corresponding value at 40°C/75%RH was 95.37±0.282%. The similar

observations were observed for the formulation at 25°C/60%RH 98.54±0.232%) after 6 month of storage.

CONCLUSIONS

Carvedilol loaded PLGA nanoparticles were successfully formulated following emulsion solvent evaporation technique. The formulation was able to enhance the physicochemical characteristic of the drug. There was no significance difference observed between the sizes of nanoparticles obtained with drug polymer ratio 1:2. But particle size was increased with drug polymer ratio 1:4 due to increase the viscosity of organic phase. But significant changes were observed in the drug entrapment with the increasing of sonication time and concentration of polymer in the formulations. The FTIR and DSC study confirmed that there was no intermolecular interaction between the carvedilol and PLGA. It was observed that all nanoparticles exhibited a biphasic release phenomenon, initial burst release followed through a typical sustaining release pattern. So, the release of carvedilol from the nanoparticles follows the mixed order kinetics followed by diffusion and erosion mechanism. So the outcome of the study was to prepare drug loaded polymeric nanoparticles and to enhance the physicochemical characteristic of the drug loaded nanoparticles.

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet. 2005 Jan 15-21;365(9455):217-23.
2. Chobanian AV. National heart, lung, and blood institute; national high blood pressure education program coordinating committee. seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. Hypertension. 2003;42:1206-52.
3. Messerli FH, Grossman E. Beta- blockers in hypertension; is carvedilol different. Am J Cardiol. 2004 May 6;93(9):7-12.
4. McTavish D, Campoli-Richards D, Sorkin EM. Carvedilol. A review of its pharmacodynamics and pharmacokinetic properties, and therapeutic efficacy. Drugs. 1993 Feb 1;45(2):232–58.
5. Ubaidulla U, Reddy MV, Ruckmani K, Ahmad FJ, Khar RK. Transdermal therapeutic system of Carvedilol: Effect of hydrophilic and hydrophobic matrix on in vitro and in vivo characteristics. AAPS PharmSciTech. 2007 Mar 1;8(1):E13-E20.
6. Smith HS. Opioid Metabolism. Mayo Clin Pro. 2009 Jul 1;84(7): 613-24.
7. Aungst BJ. Novel formulation strategies for improving oral bioavailability of drugs with poor membrane permeation or presystemic metabolism. J Pharm Sci. 1993 Oct 1;82(10):979–87.
8. Budhian A. Siegel SJ, Winey KI. Haloperidol Loaded PLGA nanoparticles: Systematic study of particle size and drug content. Int J Pharm. 2007 May 24;336(2):367-75.
9. Shakwch M, Besnard M, Nicolas V, Fattal E. Poly (lactide-co-glycolide) particles of different physicochemical properties and their uptake by peyer's patches in mice. Eur J Pharm Biopharm. 2005 Sep 1;61(1-2):1-3.
10. Fallal E, Pecquet S, Couvreur P, Andremont A. Biodegradable microparticles for the mucosal delivery of antibacterial and antigens. Int J Pharm. 2002 Aug 21;242(1-2):15-24.
11. Damge C, Aprahamian M, Humbert W, Pinget M. Ileal uptake of Polyalkylcyanoacrylate nanocapsules in the rat. J Pharm Pharmacol. 2000 Sep1;52(9):1049-56.
12. Jain RA. The manufacturing techniques of various drug loaded biodegradable Poly (lactide-co-glycolide) (PLGA) devices. Biomaterials. 2000 Dec 1;21(23):2475-90.
13. Katare YK, Muthukumarn T, Panda AK. Influence of particle size, antigen load, dose and additional adjuvant on the immune response from antigen loaded PLA

microparticles. Int J Pharm. 2005 Sep 14;301(1-2):149-60.
14. Montasser I, Fessi H, Coleman AW. Atomic force microscopy imaging of novel type of polymeric colloidal nanostructure. Eur J Pharm Biopharm. 2002 Nov 1;54(3):281-4.
15. Raghuvanshi, Om Singh, Amulya K. Panda RS. Formulation and characterization of immunoreactive tetanus toxoid biodegradable polymer particles. Drug delivery. 2001 Jan 1;8(2):99-106.
16. Govender T, Stolnik S, Garnett MC, Illum L, Davis SS. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. J Control Release.1999 Feb 1;57(2):171-85.
17. Jain S, Saraf, S. Influence of processing variables and invitro characterization of glipizide loaded biodegradable nanaoparticles. Diabetes & Metabolic Syndrome. Cl Res Revie. 2009 Jun 1;3(2):113-7.
18. Calvo P, Vila-Jato JL, Alanso MJ. Comparative *in vitro* evaluation of several colloidal system, nanoparticles, nanocapsules and nanoemulsions as ocular drug carrier.J Pharm Sci, 1996 May 1;85(5): 530-6
19. Ramesh KD Sommer J. Pharmacokinetic & metabolism of nifedipine preparation. Hypertension.1983 Jul 1;5(4 Pt 2):1118-24.
20. Ramachandani M, RobinsonD.In vitro and in vivo release of ciprofloxacin from PLGA 50:50 implants. J Control release.1998 July 31;54(2):167-75.
21. ICH, Stability Testing of New Drug Substances and Products, International Conference on Harmonization Q1A(R2) .P 8 available at https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1A_R2/Step4/Q1A_R2__Guideline.pdf