

**PREPARATION AND PHYSICO-CHEMICAL CHARACTERIZATION OF CARVEDILOL-EUDRAGIT® RS100 NANOPARTICLES''****Sovan Lal Pal\*<sup>1</sup>, U. Jana, G. P. Mohanta and P. K. Manna**

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**\*Corresponding Author****Sovan Lal Pal**Department of Pharmacy,  
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Tamil Nadu, India.**ABSTRACT**

The objective of the present study was to formulate carvedilol-eudragit® RS100 nanoparticles and investigate the physico-chemical characteristics of the prepared nanoparticles. The nanoparticles of carvedilol with eudragit® RS100 were formulated using the emulsion solvent evaporation. Several process parameters, i.e., drug/polymer ratio, aqueous phase volume and speed of sonication were considered to achieve optimal preparation condition. The physicochemical characteristics of nanoparticles were studied applying particle size analysis, differential scanning calorimetry, Fourier transform infrared spectroscopy, scanning electron microscopy and atomic force microscopy. The release rate of carvedilol from various drug/polymer

nanoparticles was investigated as well. All the prepared formulation using eudragit® RS100 resulted in nano-range size particles (280-467 nm) as detected by Zeta Sizer. The entrapment efficiencies were observed for all the nanoparticles in a range of 67% to 91%. The nanoparticles of carvedilol- eudragit® RS100 displayed no chemical interaction between drug and polymer molecules. The SEM image revealed that particles were smooth, spherical in shape. The particle aggregation of the particles was observed by AFM study. The nanoparticles exhibited the slower release of drug in comparison with the intact drug and polymer molecules. According to the finding, formulation of carvedilol- eudragit® RS100 nanoparticles 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; physico-chemical characteristics, eudragit® RS100, solvent evaporation.

## INTRODUCTION

Hypertension is high blood pressure. The elevation of blood pressure in the arteries leads to hypertension.<sup>[1]</sup> Arterial hypertension is a major risk factor for stroke, coronary events, and renal failure<sup>[2,3,4]</sup> in both industrialised and low- and middle-income countries, leading to substantial morbidity and mortality.<sup>[5]</sup> 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.<sup>[6,7]</sup>  $\beta$ - 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.<sup>[8,9]</sup> The beneficial effects of  $\beta$ - blockers in myocardial infarction (atenolol, metoprolol, nebivolol, timolol) and heart failure (nebivolol, metoprolol succinate, bisoprolol) have been reported.<sup>[10]</sup> Carvedilol is a vasodilating noncardioselective third-generation  $\beta$ - blocker, without the negative hemodynamic and metabolic effect of traditional  $\beta$ - blockers, carvedilol maintains cardiac output, has reduced prolonged effects on heart rate, and reduces blood pressure by decreasing vascular.<sup>[11,12,13]</sup> Carvedilol is the first  $\beta$ - blocker to be approved for treating chronic heart failure.<sup>[14]</sup> It is a lipophilic vasodilating noncardioselective  $\beta$ - blocker which lacks intrinsic sympathomimetic activity, thus having improved tolerability compared with older  $\beta$ - blockers.<sup>[15]</sup> The older generation “traditional”  $\beta$ - blockers selectively antagonize  $\beta$ 1-adrenergic receptors or antagonize both  $\beta$ 1-adrenergic and  $\beta$ 2- adrenergic receptors.<sup>[16]</sup> They also reduce blood pressure mainly through reduction in cardiac output, while systemic vascular resistance remains largely unchanged.<sup>[9]</sup> In contrast, carvedilol blocks norepinephrine binding to  $\alpha$  1- adrenergic receptors in addition to both  $\beta$ 1-adrenergic and  $\beta$ 2- adrenergic receptors.<sup>[11,13,17]</sup> This results in a reduction in arterial blood pressure by maintaining cardiac output and decreasing total  $\beta$ - adrenoreceptor vasoconstrictor tone.<sup>[9,18]</sup> markedly superior to that of traditional  $\beta$ - blockers. Carvedilol is highly protein bound (>98%), BCS Class-II drug is rapidly absorbed after oral administration from the gastrointestinal tract.<sup>[19]</sup> Additionally it is subject to significant first-pass metabolism by cytochrome P450 in the liver resulting in only about 25-35% absolute bioavailability with peak plasma concentration occurring within 1- 2 hrs after drug administration.<sup>[20,21,22]</sup> Carvedilol is a poorly water-soluble drug with a low bioavailability and short half life requiring 2 to 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.<sup>[23]</sup> Biologically adhesive delivery systems may offer important advantages over conventional in the bioavailability of the drugs.

In the recent decades nanoparticles containing therapeutic agents have gained much interest in scientific and commercial fields owing to their potential for site -specific drug delivery, and according the optimization of drug therapy.<sup>[24,25,26]</sup> Specially, polymers take part of an important role as drug carrier devices. Pharmacologically active agents possibly will be incorporated into a polymer matrix, or covalently bind to the polymer backbone, otherwise from polyelectrolyte complexes of oppositely charged polymer/drug systems. Applying such polymeric carriers, some critical objectives such as stabilization of the therapeutic agent, enhancement of drug solubility, and decrease of side-effect can be attained.<sup>[27]</sup>

Eudragit<sup>®</sup> RS100 polymer is commonly used for the preparation of controlled release dosage form.<sup>[28]</sup> Eudragit<sup>®</sup> RS100, a co-polymer of Poly (ethylacrylate, methyl-methacrylate and chlorotrimethyl-ammonioethyl methacrylate), contains 4.48-6.77% of quaternary ammonium groups.<sup>[29]</sup> The latter chemical groups provide positive surface charge to the polymer, by which it may interact with negatively charged drugs or cellular surface of the target tissues.<sup>[30]</sup> Moreover, eudragit<sup>®</sup> RS100 appears to be insoluble in aqueous media, is permeable and have pH independent release profiles. The permeability of eudragit<sup>®</sup> RS100 in aqueous media is due to the presence of quaternary ammonium groups (4.48% - 6.77%) in their structure.<sup>[31]</sup> Consequently, eudragit<sup>®</sup> RS100 seems to be promising polymer for controlled and prolonged localized delivery of desired medicine to some physiological fluid.<sup>[32]</sup>

The particle size reduction of the native drug has gained great interest to increase the oral bioavailability. Various technique (bottom up and bottom down) used by the scientist to reduce particle size in an attempt to enhance bioavailability. For nearly three decades, 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.<sup>[33]</sup> 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. These polymeric nanoparticulate systems

have been considered a promising carrier<sup>[34]</sup> 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.<sup>[35]</sup> Thus, nanoparticles of eudragit<sup>®</sup> RS100 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. Taking into consideration that the nanoparticles may confer capability for prolonged and effective drug delivery, the present study was to prepare carvedilol loaded eudragit<sup>®</sup> RS100 polymeric nanoparticles and to study the physicochemical characteristic of prepared nanoparticles with a view to the slow release of carvedilol. We endeavour to formulate of carvedilol- eudragit<sup>®</sup> RS100 nanoparticles by directing emulsion solvent evaporation / extraction technique. This method has initially described by RA Jain<sup>[36]</sup> to achieve particles with suitable size, charge and stability properties.<sup>[37]</sup> The physicochemical properties of the nanoparticles were characterized as well.

## 2. MATERIALS AND METHODS

### Material

Carvedilol sample was obtained from Zydus Cadila Health Care (Ahmedabad, India). Eudragit<sup>®</sup> RS100 (Evonik Industries AG, Germany) was obtained from Panacea Biotech, Lalur, Chandigarh, India). Poloxomar 188 (Lutrol<sup>®</sup> F-68) 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/ eudragit<sup>®</sup> RS100 (1:1, 1:2 and 1:4) were prepared following emulsion solvent evaporation reported earlier<sup>[36,37,38]</sup> with some modification. The method involves preparation of an o/w emulsion between and organic phase (OP) consisting of carvedilol and eudragit<sup>®</sup> RS100 in the mixture of dichloromethane and acetone (10:90) and aqueous phase (AP), 1% w/v aqueous solution of Poloxomar-188. Poloxomar-188 was used as a stabilizer during solvent evaporation technique. In the experiment, carvedilol was dispersed in the mixture of dichloromethane and acetone solution of eudragit<sup>®</sup> RS100 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 ( $25\pm 2^{\circ}\text{C}$ ) for 24 hrs under stirring condition to evaporate the organic solvent. The resultant nanosuspension was centrifuged at 40,000 rpm for 25 minutes (Sorvall Ultracentrifuge, USA). The pellets were collected and washed at least three times with double distilled water to remove untrapped drugs. The recovered nanoparticulate suspensions were dried ( $-80^{\circ}\text{C}$  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**

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-  $\mu\text{m}$  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 will be. 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  $90^{\circ}$ ). 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**

The entrapment efficiency (EE) and loading capacity (LC) were estimated by reverse phase High performance liquid chromatography (RP-HPLC) method.<sup>[39]</sup> The drug loaded nanoparticles solution of 1 mg/ml was prepared in mobile phase and 20  $\mu\text{l}$  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, 5 $\mu$ m (X-Terra; C18) analytical Column. The mobile phase used consisting of acetonitrile/acetate buffer pH 3.0/water (75:600:325) was passed through 0.45  $\mu$ m 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**

The particle shape and surface morphology of Carvedilol loaded eudragit® RS100 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**

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.<sup>[40,41]</sup>

### **Fourier Transforms Infrared (FTIR) Spectroscopy analysis**

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<sup>2</sup> 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<sup>-1</sup> in the range of 4000-400 cm<sup>-1</sup>.

### Differential scanning calorimetry (DSC) studies

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.

### In vitro drug release study

In-vitro drug release studies were carried out by using rotating basket method.<sup>[38,42]</sup> The pure carvedilol, carvedilol loaded eudragit® RS100 nanoparticles and marketed tablet formulation (each containing 10 mg carvedilol) were suspended in glass bottles containing 100 ml of phosphate buffer pH 6.8. Glass bottles were placed 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 supernatants were 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  $\pm$  SD (standard deviation). A  $\rho$  value less than 0.05(\*) was assumed for statistically significance difference and very significance difference if  $\rho < 0.005(**)$ .

## 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. 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 (280 nm to 467 nm) and the size distributions were relatively monodisperse in all of the formulations with the polydispersity index (PDI) values ranging between 0.232 and 0.385. 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.<sup>[43]</sup> 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 ( $p < 0.05$ ) 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 informations about the surface charge properties and further the long – term physical stability of the nanosuspensions. 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 nanosuspensions 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 nanosuspension, a minimum zeta potential of  $\pm 30$ mv is required.<sup>[44]</sup> In the case of a combination of electrostatic and steric stabilization, a minimum zeta potential of  $\pm 20$ mV is desirable.<sup>[45]</sup> In the present investigation zeta potential values of the carvedilol loaded eudragit nanosuspensions were between +17.90 to + 26.80 mV (Table 1). In this case, the prepared nanosuspensions contains polymer embedded drugs and hence are stabilized by combination of electrostatic and steric stabilization. All the prepared carvedilol-eudragit nanosuspensions except two (NPA1 and

NPA6) have Zeta potential  $> +20$  mV and thus are likely to be stable. 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-eudragit nanosuspensions are likely to facilitate an effective adhesion of the nanoparticles with the negatively charged mucous membrane of the gastro-intestinal tract and this will help to prolong the effective residence time of the nanoparticles.

**Table 1: Physico-chemical characterization of carvedilol loaded eudragit® RS100 nanoparticles (data represents mean  $\pm$  SD).**

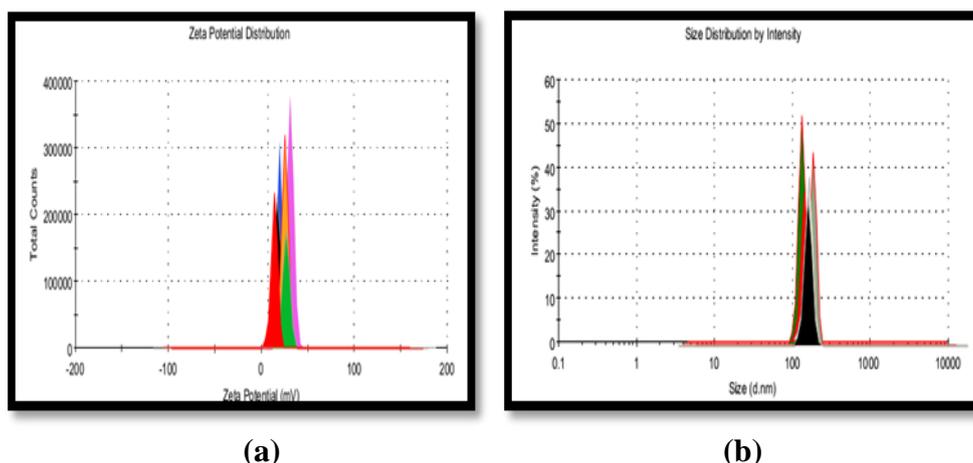
Sr. No	Formulation Code	Drug-Polymer (ratio)	Particle Size (nm) $\pm$ SD n=6	Poly Dis. Index (PDI)	Zeta Potential $\pm$ SD (mV) n=6	Loading Capacity <sup>a</sup> (% w/w)	Entrapment Efficiency <sup>b</sup> (% w/w)
01	NPA1	1:1	280 $\pm$ 3.67	0.335 $\pm$ 0.016	18.7 $\pm$ 0.82	10.65 $\pm$ 0.22	67.72 $\pm$ 0.341
02	NPA2	1:2	310 $\pm$ 4.26	0.232 $\pm$ 0.006	23.7 $\pm$ 0.66	9.94 $\pm$ 0.08	72.41 $\pm$ 0.831
<b>03</b>	<b>NPA3</b>	<b>1:4</b>	<b>330<math>\pm</math>5.78</b>	<b>0.275<math>\pm</math>0.008</b>	<b>26.8<math>\pm</math>0.72</b>	<b>12.60<math>\pm</math>0.22</b>	<b>90.12<math>\pm</math>0.212</b>
04	NPA4	1:1	295 $\pm$ 4.03	0.356 $\pm$ 0.088	21.6 $\pm$ 0.53	14.62 $\pm$ 0.06	86.45 $\pm$ 0.645
05	NPA5	1:2	391 $\pm$ 3.05	0.354 $\pm$ 0.072	24.7 $\pm$ 0.71	9.93 $\pm$ 0.41	76.45 $\pm$ 0.361
06	NPA6	1:4	467 $\pm$ 5.56	0.385 $\pm$ 0.012	17.9 $\pm$ 0.92	18.56 $\pm$ 0.82	88.01 $\pm$ 0.325

Weight of the drug in nanoparticles

$$^a\text{The LC (\% w/w)} = \frac{\text{Weight of the drug in nanoparticles}}{\text{Weight of the polymer and drug added}} \times 100$$

Weight of the drug in nanoparticles

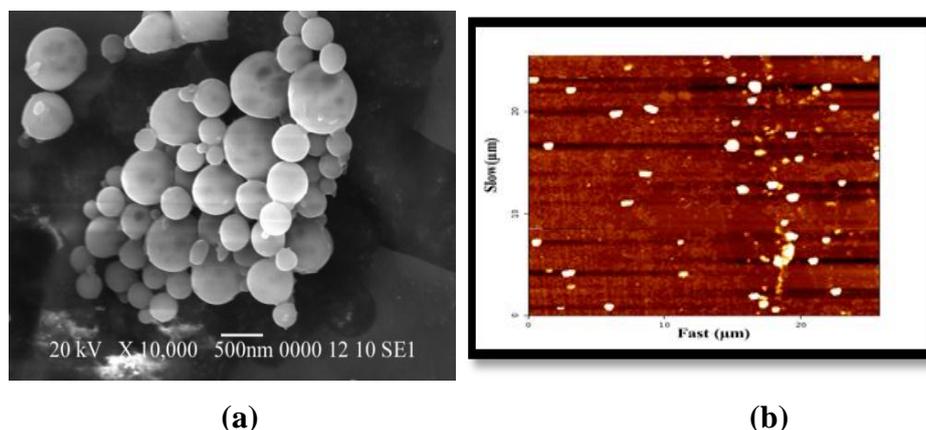
$$^b\text{The EE (\% w/w)} = \frac{\text{Weight of the drug in nanoparticles}}{\text{Weight of the drug added}} \times 100$$



**Fig. 1: (a) Mean particle size distribution of the carvedilol –eudragit ® RS100 nanoparticles (NPA1, NPA2, NPA3, NPA4, NPA5, NPA6) prepared with various sonication drug-polymer ration and sonication speed. (b) Zeta Potential of the carvedilol- eudragit ® RS100 nanoparticles (NPA1, NPA2, NPA3, NPA4, NPA5, NPA6) prepared with various sonication drug-polymer ration and sonication speed.**

### 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. 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 allows only the visualization of the nanoparticle surface, whereas the hydrodynamic layer is measured by Zetasizer.



**Fig. 2: (a) Surface morphological properties of nanoparticles. Scanning electron microscopy image of carvedilol- eudragit<sup>®</sup> RS100 nanoparticles (NPA3). (b) Surface morphological properties of nanoparticles. Size distribution carvedilol- eudragit<sup>®</sup> RS100 nanoparticles (NPA3). as measured by AFM.**

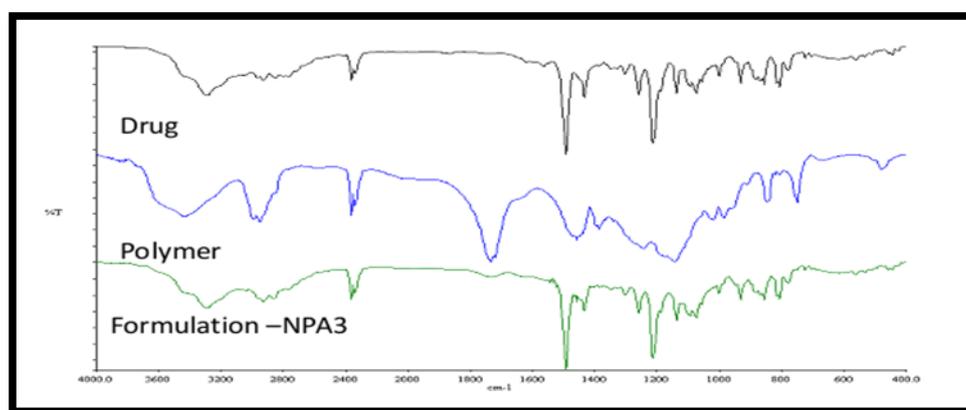
### Drug entrapment efficiency and loading capacity

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 67.72% to 91.01% and the drug loading were 9.93% and 18.56% 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.<sup>[46]</sup> The nanoparticles formulation (NPA3) prepared with drug-polymer ratio 1:4 with stabilizer concentration 3.0% and sonication speed 20 W, 40% duty cycle, shows the entrapment efficiency 90.12%, drug loading of 12.60% and particle size of 330 nm with Zeta potential value 26.8 (Table 1, Figure 1.a, & Figure 1.b). The nanoparticles formulation (NPA1) showed smaller particle size (250 nm) along with entrapment efficiency and drug loading 10.65% and 67.72% respectively. Based on the particle size and entrapment efficiency formulation (NPA3) was selected and used for further studies.

#### Fourier transforms infrared spectroscopy

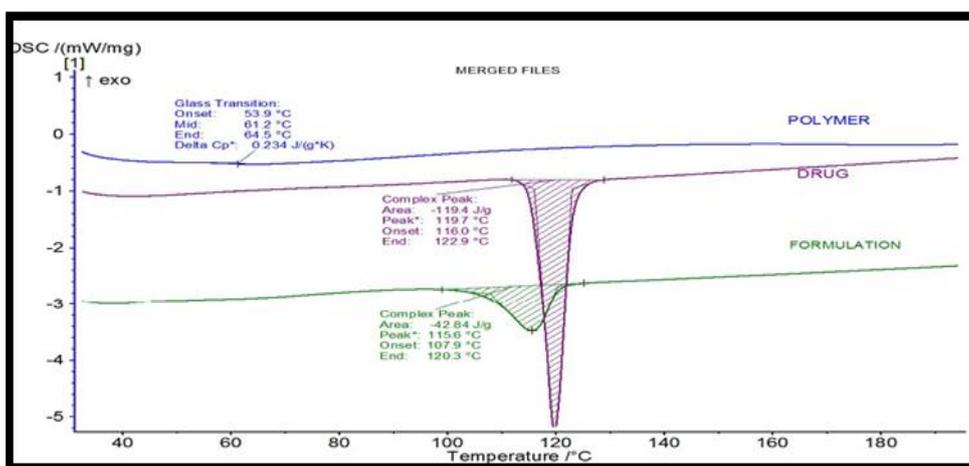
The FTIR spectral data were used to find out the interaction between the drug and polymer in the nanoparticles. FTIR spectra of pure carvedilol, polymer, carvedilol loaded eudragit<sup>®</sup> RS100 nanoparticles are carried out for the interaction study between the drug and polymer (Figure 3). There were no distinctive changes in the FTIR spectrum of carvedilol and the physical mixture indicating that eudragit<sup>®</sup> RS100 was not involved in intermolecular interaction. However, the intensity of the peak at 1505.17 cm<sup>-1</sup> and 2418 cm<sup>-1</sup> were slightly decreased in the FTIR spectrum of the related nanoparticles due to the intermolecular hydrogen bonding between carvedilol and eudragit<sup>®</sup> RS100, indicating the chemical stability of the carvedilol inside the nanoparticles.



**Fig. 3:** Fourier transforms infrared of (a) Drug- carvedilol, (b) Polymer- eudragit<sup>®</sup> RS100 and (c) Formulation- carvedilol- eudragit<sup>®</sup> RS100 nanoparticles (NPA3).

### 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 eudragit® RS100 nanoparticles (Figure 4). Different compounds show their characteristic peaks in DSC. 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.<sup>[47]</sup> 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 eudragit® RS100 was found to be 64.50°C.

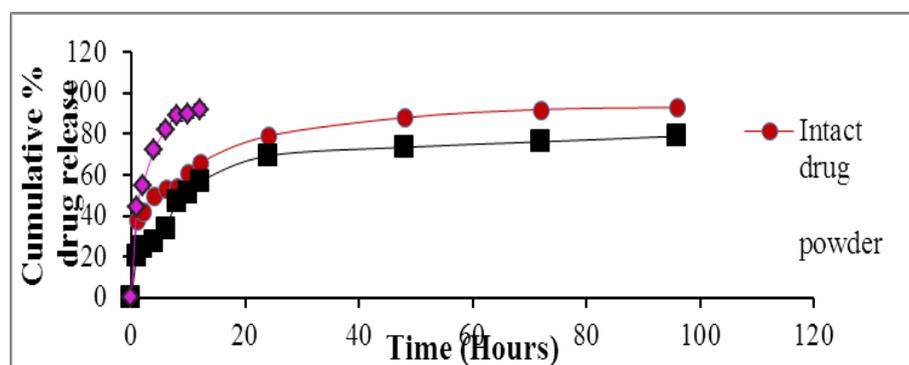


**Fig. 4:** DSC thermogram of drug –carvedilol, Polymer- eudragit® RS100 and formulation- carvedilol- eudragit® RS100 nanoparticles (NPA3).

### 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 (NPA3 with mean particle diameter 324 nm) and standard reference (a conventional marketed product) in phosphate buffer pH 6.8 are furnished in Figure 5. The in vitro release profile of carvedilol from drug loaded nanoparticles exhibited a biphasic release phenomenon. Initial burst release was observed and 20.3% of the cumulative amount of carvedilol released during first 1hr. Afterwards, the drug was released slowly at constant rate and around 70% 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. Similar release phenomenon with eudragit polymers are reported in the literature.<sup>[48,49]</sup> 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.



**Fig. 5:** *In vitro* drug release study of native carvedilol, carvedilol- eudragit<sup>®</sup> RS100 nanoparticles (NPA3) and Marketed formulation, Data as mean  $\pm$  standard error of mean (n=3).

## CONCLUSION

Carvedilol loaded eudragit<sup>®</sup> RS100 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 eudragit<sup>®</sup> RS100. 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.

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