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Formulation, optimization and evaluation of oral nanosuspension tablets of nebivolol hydrochloride for enhancement of dissoluton rate

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ABSTRACT

Nebivolol hydrochloride is a poorly water soluble drug falls under class II biopharmaceutical classification system, which is β_1 receptor antagonist that leads to vasodilatation, decreased peripheral vascular resistance, lowers blood pressure and heart rate. The rate of its oral absorption is often controlled by dissolution rate in the gastro intestinal tract. The aim of the present investigation was to improve the solubility and dissolution rate of poorly soluble drug, nebivolol hydrochloride by nanosuspension tablet prepared using microcrystalline cellulose PH101 (MCC) as diluent, povidone k 30 as binder and croscarmellose sodium as disintegrating agent. The formulation development work was performed by wet granulation method. The prepared granules and tablets were evaluated for various pre and post compression parameters as per IP and all the formulations are as per standards. Nebivolol nanosuspensions were prepared using solvent displacement/nanoprecipitation method. Among all the formulations F6 formulation has given the best dissolution studies (98.93%) and disintegration time (12.80 sec). In-vitro dissolution studies showed maximum (98.93%) release of drug within 15 minutes (F6) and mechanism of drug release from the tablets was followed first order kinetics. The optimized formulation (F6) is further selected and compared with the in-vitro drug release of innovator product (Nebilet) it showed 98.37% release of drug within 60 minutes and pure drug 27.34% within 60 minutes. Enhanced drug release rates were observed by nanosuspension tablets when compared to pure drug and innovator product (Nebilet). The physicochemical compatibility of the drug and excipients were studied by infrared spectroscopy and differential scanning calorimetry. The crystalline state of nebivolol hydrochloride drug state was changed to amorphous state due to nanosuspension formation and was confirmed by powderd X-ray diffraction study. Fourier transform infrared spectroscopy results revealed that there was no interaction between drug and excipients and results showed that there were no known chemical interactions of drug with excipient in formulation. It is concluded nanosuspension tablets were successfully prepared and they have demonstrated dramatic improvement in dissolution rate of the active drug.

Keywords*:* Nebivolol hydrochloride, eudragit RL-100, tween 80, solvent displacement/nanoprecipitation method, dissolution rate, MCC PH 101, croscarmellose sodium, povidone k 30, wet granulation, nanosuspension tablets.

INTRODUCTION

The conventional oral drug delivery has been known for decades is the most widely utilized route of administration among all the routes that have been employed for the systemic delivery of drug via various pharmaceutical products of different dosage forms. It remains the preferred route of administration in the discovery and development of new drugs candidates and formulation. The popularity of oral route is attributed to most versatile, convenient, patient acceptance, ease of administration, accurate dosing, cost effective manufacturing methods, generally improve shelf life of the product and commonly employed route of drug delivery for systemic action. In fact the development of a pharmaceutical product for oral drug delivery, irrespective of its physical form (solid, semisolid, or liquid dosage form) involves varying contents of optimization of dosage form characteristics within the inherent constraints of gastrointestinal physiology. The reasons that the oral route achieved such popularity may be in part attributed to its

ease of administration as well as the traditional belief that by oral administration the drug is as well absorbed as the food stuffs that are ingested daily. Oral solid dosage forms such as tablets has been formulated and developed nowadays since they are most effective routes of administration of a new drug. Till date oral route is major and safest route of administration for majority of drugs. Although these new systems are in fast progression, for many drugs and therapeutic indications, conventional oral solid release drug delivery systems provided satisfactory clinical performance with an appropriate balance of efficacy and safety. Recent advances in novel drug delivery aims to enhance safety and efficacy of drug molecule by formulating a convenient dosage form for ease of administration and to achieve better patient compliance. Oral drug delivery is still preferred way of administration for most of the active drug molecules due to its several advantages such as greater flexibility in design, high patient compliance, greater stability, accuracy in dose, easy of production, formulation of tablets is preferred oral dosage form. But the poor dissolution of water insoluble drugs is the major problem for pharmaceutical formulators to prepare in the form of tablets. The absorption rate of a poorly water-soluble drug, formulated as an orally administered solid dosage form, is controlled by its dissolution rate in the fluid at the absorption site. The dissolution rate is often the rate-determining step in drug absorption. Since they exhibit poor and erratic dissolution profiles, most water-insoluble drugs are included by the FDA in the list of drugs having a high risk for therapeutic in equivalence due to differences and inconsistencies in bioavailability. Therefore, the solubility and dissolution behavior of a drug are the key determinants of the oral bioavailability.[1-4]

A biopharmaceutical Classification System (BCS) was introduced by Amidon et al as a basis for predicting the likelihood of *in vitro-in vivo* correlations for immediate release dosage forms, based on the recognition that drug solubility/dissolution properties and gastrointestinal permeability are the fundamental parameters controlling the rate and extent of drug absorption. According to Biopharmaceutical Classification System (BCS) which is incorporated in the guidelines of the Food and Drug Administration (FDA), Class II drugs are with high permeability and low solubility. Regulatory agencies have utilized the BCS to allow the use of in-vitro dissolution data for establishing the in-vivo bioequivalence of drug products [5-6]. Recently, the concept of the BCS has been used not only for the biowaiver but also for formulation design from early to clinical stages. The bioavailability of a BCS class II drug is rate-limited by its dissolution, so that even a small increase in dissolution rate sometimes results in a large increase in bioavailability. Therefore, an enhancement of the dissolution rate of the drug is thought to be a key factor for improving the bioavailability of BCS class II drugs. Poor water soluble drugs are associated to slower rate of absorption from oral route; therefore, it is required to enhance the dissolution of these drugs to ensure maximum therapeutic utility of these drugs. To improve the dissolution rate various techniques have been introduced to enhance the dissolution rate and solubility of the drug [7-8].

Compounds with poor aqueous solubility are increasingly posing challenges in the development of new drugs, since a large number of drugs coming directly from synthesis or from high throughputs screenings have a poor solubility. The ability to increase aqueous solubility is thus a valuable aid to increase the efficacy for poorly water soluble drugs. Solubility is an important parameter for absorption of drugs especially for those which are water insoluble and poorly soluble drugs. Dissolution of such drugs limits their absorption through oral route. Consideration of the modified Noyes-Whitney equation provides some hints as to how the dissolution rate of even very poorly soluble compounds might be improved to minimize the limitations to oral availability. According to Noyes-Whitney equation, increase in saturation solubility leads to an increase in dissolution velocity in the lumen and blood, thus accelerating drug diffusion, promoting absorption and therefore enhancing bioavailability of drugs. Due to poor solubility and limited dissolution rate Class II drugs suffer less bioavailability thereby decreased therapeutic effect. Several techniques have been reported to improve the solubility and dissolution properties of poorly soluble drugs, which can also improve absorption and bioavailability. Over the years, various techniques have been employed to formulate drug delivery systems which would enhance the dissolution profile and, in turn, the absorption efficiency of water-insoluble solid drugs such as digoxin, digitoxin, prednisolone, hydrocortisone, prednisone, spironolactone, hydrochlorothiazide, polythiazide, and/or liquid lipophilic medications such as clofibrate, chlorpheniramine, water-insoluble vitamins, fish oil, etc [9-12]

Different approaches have been attempted to increase aqueous solubility of poorly soluble drugs, such as conversion of crystalline molecule to its amorphous state, a particle size reduction via micronization, solubilization in surfactant systems, co-solvency, hydrotropic solubilization, cyclodextrin complexation. The principles are generally based on the modification of the physicochemical properties of the drug, these includes the conventional approach such as salt formation, p^H solubility, micronization, solubilization using co-solvents and micellar solubilization, solid dispersions, oily solutions, complexation with beta-cyclodextrin, self-emulsifying and liquisolid compacts[13-15]. The methods are not universal suffers from limitations such as large amount of excipients and sophisticated equipment. The addition of novel functionality to the molecular structure of the drug to enhance intestinal wall penetration and modification of pharmaceutical formulation technology by the use of novel drug delivery carrier systems such as microparticles, microemulsions, liposomes, nanoemulsions, nanoparticles and dry emulsions. In

recent years, nanosuspension processes have become a promising approach for the enhancement of dissolution rates of poorly aqueous soluble drugs [16-17]. Most of these various approaches have been successfully employed to improve oral drug delivery which has often translated in enhanced drug absorption. In spite of significant advances in other areas of drug delivery such as pulmonary or topical, oral drug delivery remains the most favored route of administration.

From the last few years, the pharmaceutical scientists were working to develop patient compliance and safe dosage forms due to enhanced demand in the market for them. As a result developing the new technologies has been increasing annually because the development of new drug molecule requires high cost rather than new technology. So the current trend in most of pharmaceutical industries is development of dosage form with new formulation technology using old drug molecules to improve safety, efficacy and patient compliance. Development of nanosuspensions is one such technology to enhance dissolution rate of poorly soluble drugs, thereby improving efficacy of drug molecule [18].

Nebivolol is a new antihypertensive drug, that it is a competitive and highly selective Beta-receptor antagonist and does not show any intrinsic sympathomimetic activity. Nebivolol is the newer drug among those β_1-Adrenergic blockers and also used as monotherapy for initial management of uncomplicated hypertension. The drug is poorly soluble of BCS II, the wettability, solubility, dissolution velocity and bioavailability of drug are low which is taken as model drug. Nanosuspension tablets of nebivolol hydrochloride are developed to enhance the dissolution rate of poorly soluble drug thereby improving oral bioavailability[19-20].

A nanosuspension is a submicron colloidal dispersion of drug particles which are stabilized by surfactants, for either oral and topical use or parenteral and pulmonary administration. Nanosuspensions are biphasic systems consisting of pure drug particles dispersed in an aqueous vehicle, stabilized by surfactant in which the diameter of suspended particle is less than 1µm in size. The particle size distribution of the solid particles in nanosuspension is usually less than one micron with an average particle size ranging between 200 and 600 nm. Nanosuspension technology can be used to improve the stability as well as the bioavailability of poorly soluble drugs. Nanosuspensions can be used to enhance the solubility of drugs that are poorly soluble in aqueous as well as lipid media. Nanosuspensions are prepared by bottom up and top down technologies. In bottom up technologies the low soluble drugs are dissolved in a solvent and then precipitated in different ways in surfacatant solution. The top down technologies are based on particle fragmentation to submicron with high pressure homogenizer. Techniques such as wet milling, high pressure homogenizer, emulsification evaporation and supercritical fluid have been used in the preparation of nanosuspensions. Nanosuspensions have revealed their potential to tackle the problems associated with the delivery of poorly water-soluble and poorly water- and lipid-soluble drugs, and are unique because of their simplicity and the advantages they confer over other strategies[21].

Tablets dosage form is mostly preferred because of their accurate dose good physical and chemical stability, competitive unit production cost and an elegant distinctive appearance results in high level of patient acceptability. Tablets, on the other hand, currently account for well over two third of the total number and cost of medicines produced all over the world. Tablet is defined as a compressed solid dosage form containing medicaments with or without excipients. According to the Indian Pharmacopoeia Pharmaceutical tablets are solid, flat or biconvex dishes, unit dosage form, prepared by compressing a drugs or a mixture of drugs, with or without diluents. They vary in shape and differ greatly in size and weight, depending on amount of medicinal substances and the intended mode of administration. It is the most popular dosage form and 70% of the total medicines are dispensed in the form of Tablet. All medicaments are available in the Tablet form except where it is difficult to formulate or administer[22,23].

MATERIALS AND METHODS

Materials: Nebivolol hydrochloride as a gift sample from MSN Pharma Pvt. Ltd, eudragit RL 100, tween 80, microcrystalline cellulose PH101 (MCC), pregelatinised starch, sodium starch glycolate, magnesium stearate, talc**,** methanol, acetone are from SD fine chemicals.

Preformulation Studies with the Drug

The preformulation studies with the Nebivolol obtained were performed using conventional and reported techniques. The UV-Visible spectrum, solubility, flow properties, drug crystallinity were determined.

Methods: Nebivolol nanosuspensions were formulated and prepared into nanosuspension tablets:

1. Solvent displacement/ Nanoprecipitation method:
Preparation of Nebivolol Nanosuspensions and Optimization of Preparation Process
Several batches of nebivolol nanosuspensions were prepared by Solvent displacement/ Nanoprecipitation method. Required quantity of Nebivolol and Eudragit RL 100 as polymer were weighed and dissolved in appropriate quantity of organic phase acetone and 1% tween 80 as stsbilizer in required quantities of distilled water was dissolved in a 250ml Buchner flask as aqueous phase. Organic phase is injected into aqueous phase dropwise with a constant stirring on a magnetic stirrer using a butterfly syringe and subjected sonication for 15-30 minutes. Vaccum was applied by placing paper on buchner flask while stirring till the solvent evaporates for 5-6 hours. The obtained product was filtered and dried at room temperature in a dessicator over night. Finally the product was filtered and centrifuged at 7000 rpm for 30 min. Particle size of drug nanosuspension after bath sonication mixing was measured. The drug nanosuspension was freeze dried using Mini Lyodel Freeze Dryer (Chennai, India)[24,25].

2. Preparation of nanosuspension tablets
Nanosuspension tablets were prepared by a wet granulation method, each tablet (average weight 150 mg) for *in-vitro* drug release studies consisted of nanosuspension, microcrystalline cellulose (MCC), cross carmellose sodium, talc and magnesium stearate (Table 1). Cross carmellose sodium was added to get fast disintegration tablets of nebivolo hydrochloride nanosuspension. The materials were weighed, mixed and passed through a sieve no.60 to ensure complete mixing and prepared into granules by wet granulation method using povidone k 30 as binder. The prepared granules are dried in tray dryer (sisco scientific instruments, thane, india) and compressed into 100mg tablet using 6 mm convex punches on 16 station rotary tablet punching machine (cadmach, ahmedabad). Tablet quality control tests such as weight variation, hardness, friability, thickness, disintegration and dissolution in different media were performed on the nanosuspension tablets[26,27].

Table: 1 Formulations of nanosuspension tablets of nebivolol hydrochloride

Ingredients in mg	F1	F2	F3	F4	F5	F6
Nebivolol hydrochloride Nanosuspension	5	5	5	5	5	5
Microcrystalline cellulose PH 101	89.5	89.5	89.5	89	89	89
Croscarmellose sodium	1.5	1.5	1.5	2	2	2
Povidone K 30 (PVP K-30)	1	1	1	1	1	1
Sodium lauryl sulphate	1	1	1	1	1	1
Talc	1	1	1	1	1	1
Magnesium stearate	1	1	1	1	1	1
Total	100	100	100	100	100	100

Saturation Solubility:
Saturation solubility is defined as the maximum quantity of a compound (solute) that can be dissolved in a certain quantity of a specific solvent at a specified temperature. Saturation solubility of pure drug nebivolol hydrochloride and nanosuspensions in different non-volatile solvents was estimated by conducting saturation solubility studies. The saturation solubility of the prepared nanosuspension and the pure drug were determined by using rotary shaker (Neo labs instruments, Mumbai). Excess amount of drug was added to 10 ml of solvent and 1.5 ml of nanosuspension in different vials and subjected to continous stirring using rotary shaker for 72 hrs at 25^{o}C. Then each solution was filtered and the filtrates wre analysed for drug content and solubility using U.V. spectrophotometer (Merck, Thermo scientific Evoluation 201) at 281 nm.

Scanning Electron Microscopy:
In order to examine the particle surface morphology and shape, scanning electron microscopy (SEM) was used. A concentrated aqueous suspension was spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with gold layer 20 nm thick. Photographs were taken using a JSM-5200 Scanning Electron Microscope (Tokyo, Japan) operated at 20 kV. The smallest size nanosupension was used for determining surface morphology.

Micromeritic properties of pure drug[28]:
Angle of repose:
Angle of repose of pure drug was determined using fixed funnel method. The blend was poured through funnel that can be raised vertically until a maximum cone height (h) was obtained. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the powder blend. The powder blend was allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose

was calculated using the following equation. Radius of the heap (r) was measured and angle of repose was calculated using the formula:

$$\tan \theta = h/r$$

Where, θ is the angle of repose, h is height of pile; r is radius of the base of pile.

The angle of repose of powder blend was determined by the funnel method.

Bulk density:
The drug powder was weighed and transferred into measuring cylinder and the volume occupied by the powder was noted. Apparent bulk density (ρb) was determined by pouring the blend into a graduated cylinder. A quantity of 2 gm of powder blend from each formula, previously shaken to break any agglomerates formed, was introduced in to 10 ml measuring cylinder. After that the initial volume was noted and the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5cm at second intervals. The bulk volume (Vb) and weight of powder (M) was determined. The bulk density was calculated using the formula

$$\textit{Bulk density} = \frac{\textit{weight of the powder blend/powder mass}}{\textit{Bulk volume}}$$

Tapped density:
The drug was weighed and transferred into a measuring cylinder. Then it was tapped on a bulk density-tapped density apparatus (sisco scintific instuments, thane, India) for 500 taps and the final volume was noted. Tapping was continued until no further change in volume was noted. The minimum volume (Vt) occupied in the cylinder and weight (M) of the blend was measured. The tapped density (ρb) was calculated using the following formula

$$\textit{Tapped bulk density} = \frac{\textit{weight of the powder blend/powder mass}}{\textit{Tapped volume}}$$

Hausner's Ratio:
Bulk density and tapped density were measured and Hausner's ratio was calculated. It indicates the flow properties of the granules and is measured by the ratio of tapped density to the bulk density. Hausner ratio is an indirect index of ease of powder flow. It is calculated by the following formula:

$$\textit{Hausner's Ratio} = \frac{\textit{Tapped density } (\rho t)}{\textit{Bulk density } (\rho d)}$$

Lower Haunser ratio (< 1.25) indicates better flow properties than higher ones6 (>1.25).

Compressibility index (Carr's Index):
Compressibility index is an important measure that can be obtained from the bulk and tapped densities. The simplest way of measurement of free flow of powder is compressibility, an indication of the ease with which a material can be induced to flow is given by compressibility. In theory, the less compressible a material is the more flowable it is. A material having values of less than 20% has good flow property. The compressibility index of the granules was determined by Carr's compressibility index, which is calculated by using the following formula

$$C_I = \frac{(\textit{ Tapped density – Balk density }) \; x\; 100}{\textit{Tapped density}}$$

Evaluation studes of nanosuspension tablets of nebivolol hydrochloride[29]:
Pre-compression parameters:
All the formulations were estimated for flow properties like angle of repose, bulk density, tapped density, hausner's ratio and carr's index for all the formulations and calculated as per the procedures followed for the pure drug.

Post-compression parameters:
The tablets were evaluated for in-process and finished product quality control tests i.e. appearance, thickness, hardness, weight variation, friability, drug content uniformity, disintegration time and in-vitro drug release.

Appearance:
The tablet should be free from cracks, depressions, pinholes etc. The color and the polish of the tablet should be

uniform on whole surface. The surface of the tablets should be smooth.

Thickness:
The dimensions of the tablets are thickness and diameter. The tablets should have uniform thickness and diameter. The manufacturer normally states these. Thickness and diameter of a tablet were measured using vernier calipers. These values were checked and used to adjust the initial stages of compression.

Hardness:
The resistance of tablets to shipping or breakage under conditions of storage, transportation and handling before usage depends on its hardness. The hardness of each batch of tablet was checked by using Monsanto hardness tester (sisco scintific instuments, thane, India). The hardness was measured in terms of kg/cm^2. Six tablets were chosen randomly and tested for hardness. The average hardness of six determinations was recorded.

Weight Variation:
Twenty tablets from each formulation were selected at a random and average weight was determined. Then individual tablets were weighed and was compared with average weight. The weight of the tablet being made was routinely determined to ensure that a tablet contains the proper amount of drug. The tablets should met the I.P specification that not more than 2 tablets are outside the percentage limits and no tablet differs by more than 2 times the percentage limit as in I.P official limits.

$$\textit{\% weight variation} = \frac{\textit{individual weight-average weight}}{\textit{Average weight}} \; X\,100$$

Table: 2 Standards of uniformity of weight as per I.P

S. No	Average weight of tablets (mg)	% of deviation
1	130 or less	10
2	From 130 to 324	7.5
3	More than 324	5

Friability:
Six nanosuspension tablets were randomly selected, weighed and friabilated for 100 revolutions using Roche friabilator (sisco scintific instuments, thane, India) for 15 mins at 25 rpm. Then tablets were removed from the friabilator, dusted off the fines and again weighed and the weight was recorded. Friability determines the resistance of tablets to shipping or breakage under conditions of storage transportation and handling before usage. Friability generally refers to loss in weight of tablets in the containers due to removal of fines from the tablet surface. Friability generally reflects poor cohesion of tablet ingredients. If there is any chipping, capping, cracking or breaking of tablet; then the batch should be rejected.

$$\textit{Friability} = \frac{(w_1 - w_2)}{w_1} \; x\,100$$

Where: w_1= weight of the tablet before test, w_2= weight of the tablet after test.

Drug content uniformity:
Six tablets were randomly selected and grinded to powder. The powder equivalent tio dose of nebivolol hydrochloride was dissolved in methonal and filtered the solution through the whatman filter paper. The filtrate was analyzed for drug concentration using U.V. spectrophotometer (Merck, Thermo scientific Evoluation 201) at 281 nm. Each sample was analyzed in triplicate.

Disintegration time:
Six tablets were placed individually in each tube of USP disintegration test apparatus (sisco scintific instuments, thane, India) and discs were placed and run the appratus for 10 mins at a temperature of 37°±2°C and time taken for the entire tablet to disintegrate completely was noted. Then each nanosuspension tablet was checked for complete disintegration.

In-vitro drug release studies:
The release of nebivolol hydrochloride from nanosuspension tablets were studied using USP dissolution apparatus II paddle method (Electro lab TDT-082, Model-ETC 11L Type II apparatus-USP XIV Dissolution test apparatus) and the dissolution media used was 500 ml of 7.4 P^H phosphate buffer. The temperature and speed of rotation maintained was $37^0C \pm 5^0C$ and 50 rpm. An aliquot of 5ml was withdrawn at different time intervals5, 10, 15, 30, 45, 60 and the equal volume of fresh dissolution medium was replaced at predetermined time points. The filtered samples were

analysed using U.V. spectrophotometer (Merck, Thermo scientific Evoluation 201) at 281 nm. The dissolution of nanosuspension tablets were compared with the dissolution of equivalent amount of innovator (Nebilet) and pure drug.

Compatibility studies:
The objective of drug/excipient compatibility considerations and practical studies is to delineate, as quickly as possible, real and possible interactions between potential formulation excipients and the API. This is an important risk reduction exercise early in formulation development. The drug-excipient incompatibility can alter the stability and/or the bioavailability of drugs, thereby, affecting its safety and/or efficacy.

Fourier transform Infra-red spectrophometer (FT-IR studies):
The FT IR spectra of drug, physical mixture, optimized nanosuspension and nanosuspension tablet were obtained. Sample about 5 mg was mixed thoroughly with 100 mg potassium bromide IR powder and compacted under vacuum a pressure of about 12 Psi for 3 minutes. The resultant disc was mounted in a suitable holder in Perkin Elmer IR spectrophotometer and the IR spectrum was recorded from 4000 cm^{-1} to 625 cm^{-1} in a scan time of 12 minutes. IR helps to confirm the identity of the drug and to detect the interaction of the drug with the carriers.

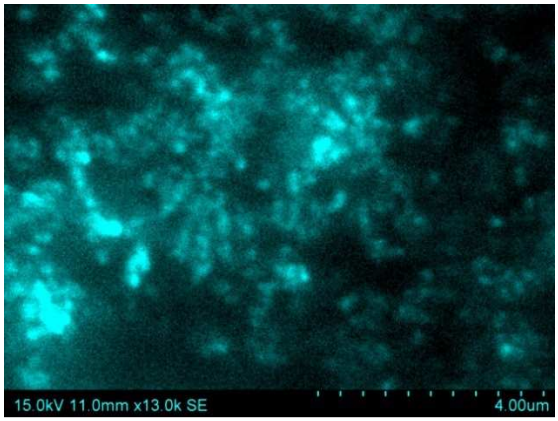
Differential Scanning Calorimetry (DSC): *Differential Scanning Calorimetry (DSC)*:
Differential Scanning colorimetry is used to determine drug excipient compatibility studies, investigate and predict any physiochemical interactions between components in a formulation and, therefore, can be applied to the selection of suitable chemically compatible excipients. It is also used to observe more phase changes such as glass transition, crystallization, amorphous forms of drugs and polymers. Differential scanning calorimeter has been proposed as a rapid method for evaluating the drug-excipient interaction. The physical state of drugs and polymer was analyzed by Differential Scanning calorimeter (Schimadzu). Thermal analysis and properties of the powder samples of drug, physical mixture, optimied nanosuspension and nanosuspension tablet were investigated with a DSC (DSC shimadzu model 60, Japan). Approximately 10 mg of sample was analyzed in an open aluminum pan, and heated at scanning rate of 10°C/min between 0°C and 400°C under nitrogen atmosphere, was performed by increasing the temperature from 40^0C to 200^0C at 5^0C/min. Magnesia was used as the standard reference material.

Powder X-Ray Diffraction (XPRD) analysis:
The drug crystalline state in the polymer sample was evaluated by Powder X-Ray Diffraction (XPRD) analysis. X-ray spectra were recorded with X'Pert-PRO multipurpose X-Ray diffractometer (PANalytical, Tokyo, Japan) using Ni-filtered, Cu Ka radiation, a voltage of 45 kV, and a current of 40 mA with a scintillation counter. The instrument was operated in the continuous scanning speed of 4°/min over a 2θ range of 5° to 40°. The samples were grinded using a wedgwood mortar and pestle, placed into the cavity of an aluminum sample holder and packed smoothly using a glass slide. The smallest size nanosuspension was used in the study.

RESULTS AND DISCUSSION

Preformulation study for nebivolol hydrochloride has been performed to know the drug physical properties so as to design it to a suitable formulation. Nebivolol is a poorly soluble drug of BCS class II with low solubility and high permeability. Nebivolol is poorly soluble in water and soluble in organic solvents such as N, N-dimethylformamide, methanol and dimethylsulfoxide found in our lab is same as that what was reported in literature. Drug is crystalline in nature. UV spectrum obtained matched that of reported results previously. Poor solubility leads to poor dissolution, therefore to enhance the dissolution rate of the drug, nanosuspension technology which is a novel technique where saturation solubility has been higher compared to micronization, additionally surfactant and stabilizer are employed to enhance the solubility of the drug which improved dissolution rate and thereby enhances the bioavailability. Nanosuspensions were prepared by solvent displacement/nanoprecipitation method. Different batches of nebivolol nanosuspensions are prepared by two methods considering the concentration of polymer and surfacatant to obtain nanoparticles with reduced particle size. The saturation solubility of the particles is essential as it affects the bioavailability of the drug, the rate of drug release into dissolution medium and consequently, the therapeutic efficiency of the pharmaceutical product. The saturation solubility was measured for the pure drug is 0.62 ± 0.73 µg/ml and nebivolol nanosuspension is 12.65 ± 0.61 µg/ml in solvent system and found to be higher for nanosuspensions. Reduction in the particle size of to nanometer range led to the increase in saturation solubility of nanosuspensions. Optical microscopy was used in the determination of particle size of nanosuspensions, the particle size was verified using SEM and zetasizer. The average particle size for all the batches is between 150-300 nm shown in figure 1. From the SEM it was concluded that the average particle size was found to be in nano range (< 1 µm). Surface morphology and shape were visualized. The particles are smooth and spherical. From this study it has been concluded that there is a size reduction of particles. Increase in polymer and surfactant concentration reduced the particle size. The particle size was also reduced due to effect of solvent ratio. Within the nanosuspensions

prepared solvent displacement/ nanoprecipitation method reduction in particle size was found with formulation F6 with particle size 0.91µ. Nebivolol hydrochloride was shown poor flow properties with an angle of repose > 45^{o}, carr's index of >25%, and hausner's ratio of >1.25. The values of pre-compression parameters evaluated were found to be angle of repose 22.87 ± 0.19 to 25.45 ± 0.15, carr's index 12.04 ± 0.11 to 17.30 ± 0.18 and hausner's ratio 1.14 ± 0.04 to 1.16 ± 0.09 i.e > 1.25 all the values within prescribed limits of I.P and indicated good free flowing property as shown in table 3. The data obtained from post-compression parameters such as weight variation, hardness, friability, drug content and disintegration time for nanosuspension tablets were shown in table 4 .In all the formulations, hardness test indicated good mechanical strength, as the hardness of the nanosuspension tablets was found in the range of 3.16 to 3.76 kg/cm^{2}. Friability was observed less than 1%, indicated that nanosuspension tablets had a good mechanical resistance. Drug content was found to be high (≥99.72%) and uniform in all the nanosuspension tablets. The nanosuspension tablets were subjected for evaluation of disintegration time. The disintegration time for all the formulations varies from 12.80 to 53.20 seconds. The in-vitro dissolution studies of different formulations are shown in table 5 and figure 2, Among all the formulations F6 formulation has given the best dissolution studies (98.93%) and release of drug within 15 minutes (F6) and mechanism of drug release from the tablets was followed first order kinetics. The optimized formulation (F6) is further selected and compared with the in-vitro drug release of innovator product (Nebilet) it showed 98.37% release of drug within 60 minutes and pure drug 27.34% within 60 minutes. Enhanced drug release rates were observed by nanosuspension tablets when compared to pure drug and innovator product (Nebilet). From the above spectra of nebivolo hydrochloride, physical mixture and polymers and optimized formulations F6 shown in figures 3,4,5,6, it was found that FTIR spectra of nebivolol hydrochloride and optimized formulation F6. Pure drug showed characteristic absorption bands at 3184.25 cm^{-1} (N-H Stretching), 2319.46 cm^{-1} (C=C stretching), 1536.44 cm^{-1} (C=O stretching), 1490 cm^{-1} (C-H stretching), 1073 cm^{-1} (C-N stretching) and the F6 optimized formulation showed characteristic absorption band at 3015.94 cm^{-1} (N-H Stretching), 2378 cm^{-1} (C=C stretching), 1551.15 cm^{-1} (C=O stretching), 1519 cm^{-1} (C-H stretching), 1082 cm^{-1} (C-N stretching). The FTIR spectra of pure nebivolol hydrochloride and F6 optimized formulation revealed that all characteristic peaks of nebivolol hydrochloride were present in the combination spectrum and there is no shift in peaks, thus indicating compatibility of the nebivolol hydrochloride and polymer. There is no physical and chemical interaction of drug and polymers. Hence there is no drug and excipient incompatibility, Thus drug & excipients are compatable. XRPD was used to investigate the physical nature of the encapsulated drug, the Powder X-ray. XRPD was used for analysis of a variety of transformations during pharmaceutical processing and storage such as: polymorphic transformations; alterations in crystallanity, changes in state and degree of hydration. From the figure 7, XRPD Graphs it was observed that the crystallanity of the drug was changed in the nanosuspensions. The peaks obtained for pure drug was very clear and sharp the intensity of the peaks was very high when compared to peaks of nebivolol nanosuspensions. Reduction in the peak intensity indicates the change in crystal structure. From this we can conclude that there was reduction in the crystallanity and change into amorphous structures. Hence the nanosuspension was amorphous nanosuspension. DSC studies were performed to understand the nature of the encapsulated drug in the polymer. The physical state of drug in the polymer matrix would also influence its release characteristics. To probe this effect, DSC analysis was performed. Nebivolol hydrochloride and optimized formulation F6 as shown in the Figure 8. Nature of thermogram is totally changed and the sharp peaks are shifted, the peaks of pure drug have change to broad peaks with reduction of the height of each peak. These changes indicate that the dehydration of pure drug and change in the partical size giving more amorphous type of the product this may help in increasing the dissolution rate of nanosuspension tablets.



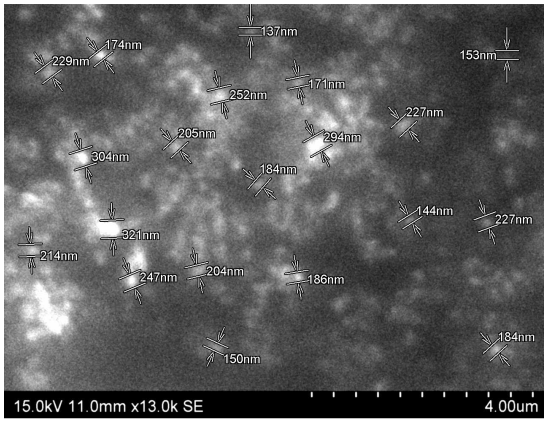


Fig: 1 Sem Pictograms of optimized nanosuspension (F6)

Table: 3 Pre-compression parameters of nanosuspension tablets of Nebivolol hydrochloride

Batch	Angle of repose(θ)	Bulk density (gm/cm^3)	Tapped density (gm/cm^3)	Hausner`s ratio	Compressability (%)/Carr's Index
F1	22.87 ± 0.19	0.4735 ± 0.03	0.5623± 0.013	1.14 ± 0.04	12.04 ± 0.11
F2	24.20 ± 0.09	0.4933 ± 0.027	0.5714 ± 0.012	1.14 ± 0.08	13.25 ± 0.20
F3	25.18 ± 0.11	0.5057 ± 0.004	0.5821 ± 0.0034	1.16 ± 0.06	13.81 ± 0.25
F4	23.25 ± 0.08	0.4864 ± 0.025	0.5516 ± 0.002	1.15 ± 0.08	14.36 ± 0.14
F5	25.45 ± 0.15	0.483 ± 0.021	0.553 ± 0.003	1.16 ± 0.06	15.69 ± 0.16
F6	24.17 ± 0.12	0.5198 ± 0.031	0.5987 ± 0.011	1.16 ± 0.09	17.30 ± 0.18

**Angle of repose, n=3*

Table: 4 Post- Compression parameters of nanosuspension tablets of Nebivolol hydrochloride

Formulation code	Thickness (mm)	Hardness Kg/cm^2	Weight variation(mg)	Friability (%)	Drug content uniformity	Disintegration time(sec)
F1	2.88 + 0.12	3.54 ± 0.13	98.29 ± 1.7	0.39 ± 0.8	99.56 ± 0.4	21.18 ± 1.2
F2	2.88 + 0.13	3.34 ± 0.12	99.84 ± 0.8	0.43 ± 0.4	99.49 ± 0.9	42.39 ± 0.5
F3	2.89 + 0.11	3.16 ± 0.13	99.98 ± 0.4	0.52± 0.5	99.72 ± 0.7	53.20 ± 2.2
F4	2.91 + 0.12	3.58 ± 0.14	99.86 ± 1.5	0.62 ± 0.2	99.22 ± 10	14.22 ± 0.8
F5	2.85 + 0.12	3.70 ± 0.11	99.83± 1.9	0.51 ± 1.4	99.43± 0.8	13.21 ± 0.6
F6	2.91 + 0.14	3.76 ± 0.14	99.59 ± 2.1	0.42 ± 0.9	99.52 ± 0.4	12.80 ± 0.9

**Angle of repose, n=3*

Table: 5 Comparative % drug release studies of nanosuspension tablets, innovator and pure drug nebivolol hydrochloride

Time in mins	Pure drug	F1	F2	F3	F4	F5	F6	Innovator (Nebilet)
0	0	0	0	0	0	0	0	0
5	9.20	69.13	70.18	68.17	66.14	65.13	73.33	33.16
10	12.23	81.44	83.67	81.64	80.38	82.63	89.54	49.73
15	15.17	92.18	91.43	92.58	91.36	90.74	100.56	61.12
30	18.32	93.67	94.18	96.32	93.13	95.39	101.23	73.25
45	23.56	97.90	96.13	98.15	97.58	99.56	101.89	82.71
60	27.34	99.67	99.88	100.32	99.86	100.73	102.14	98.97

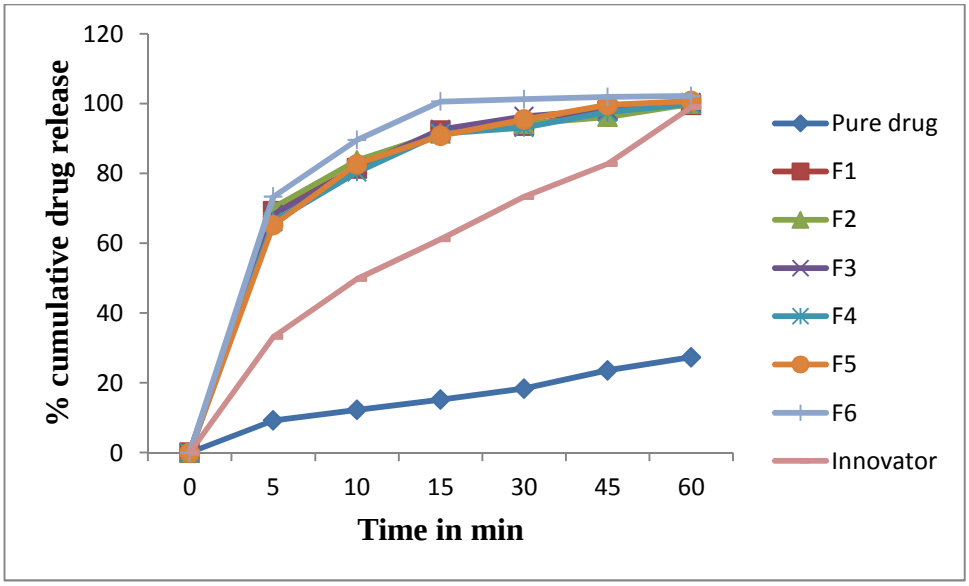


Fig: 2 Comparative % drug release studies of nanosuspension tablets, innovator and pure drug nebivolol hydrochloride

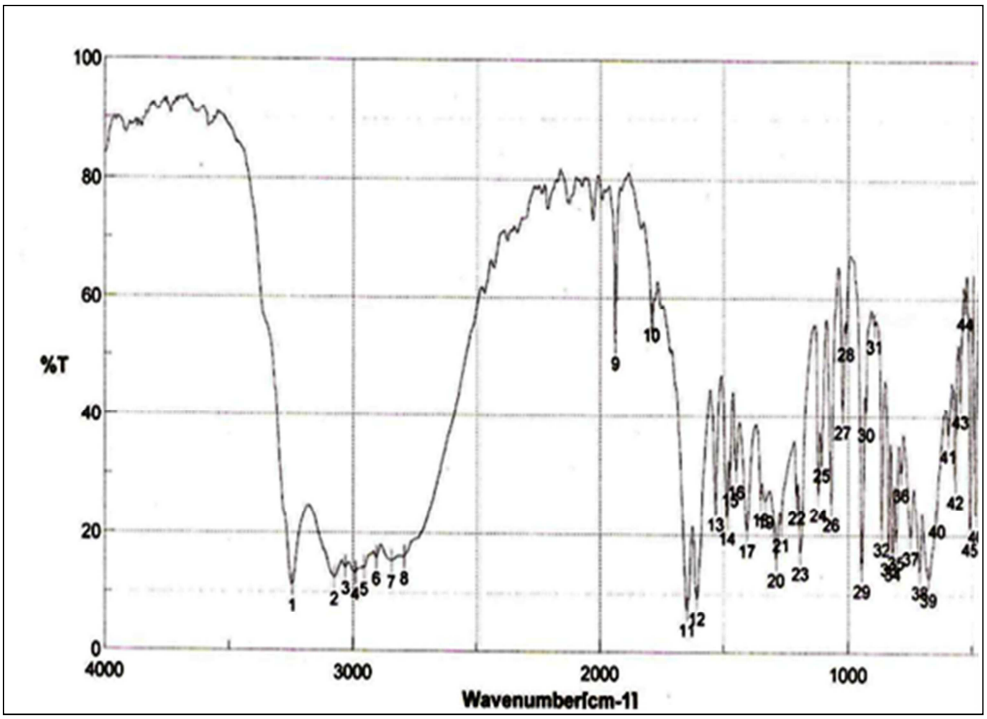


Fig: 3 IR spectrum of Nebivolol hydrochloride

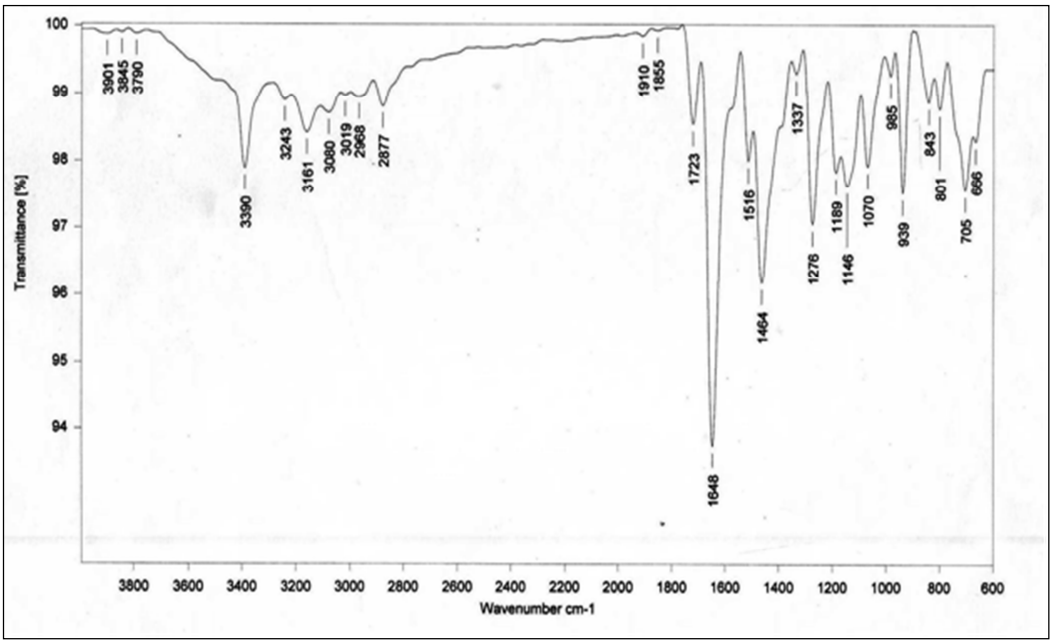


Fig: 4 IR Spectroscopy study of drug +polymer (Eudragit RL 100)

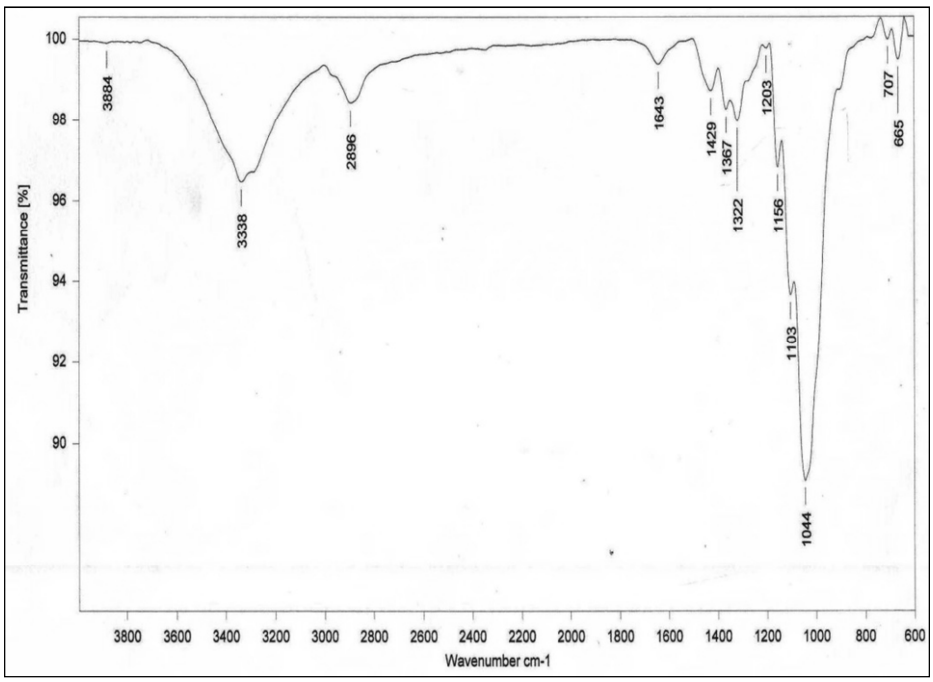


Fig: 5 IR Spectroscopy study of Physical mixture

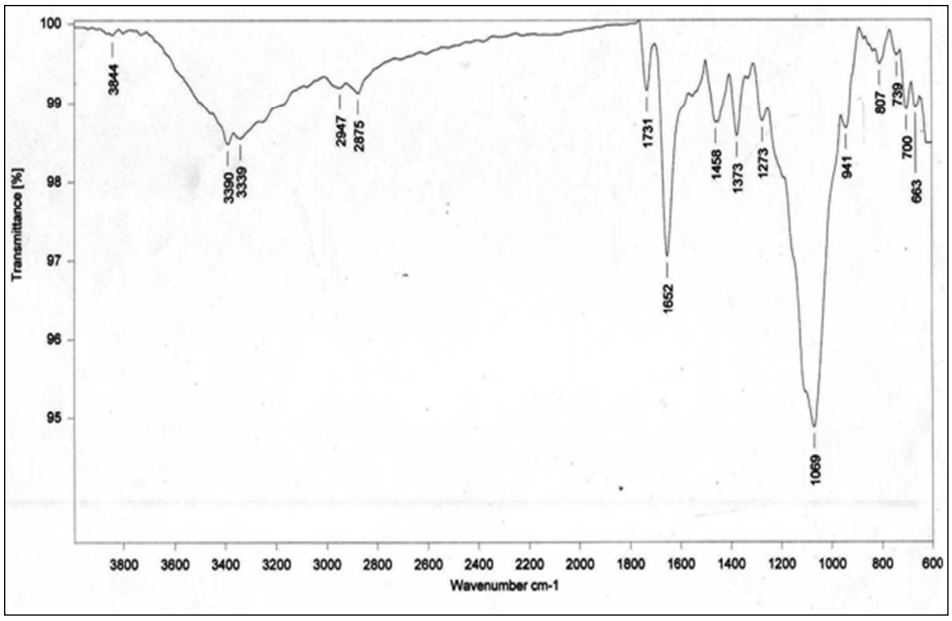


Fig: 5 IR Spectroscopy study of optimized nanosuspension formulation (F6)

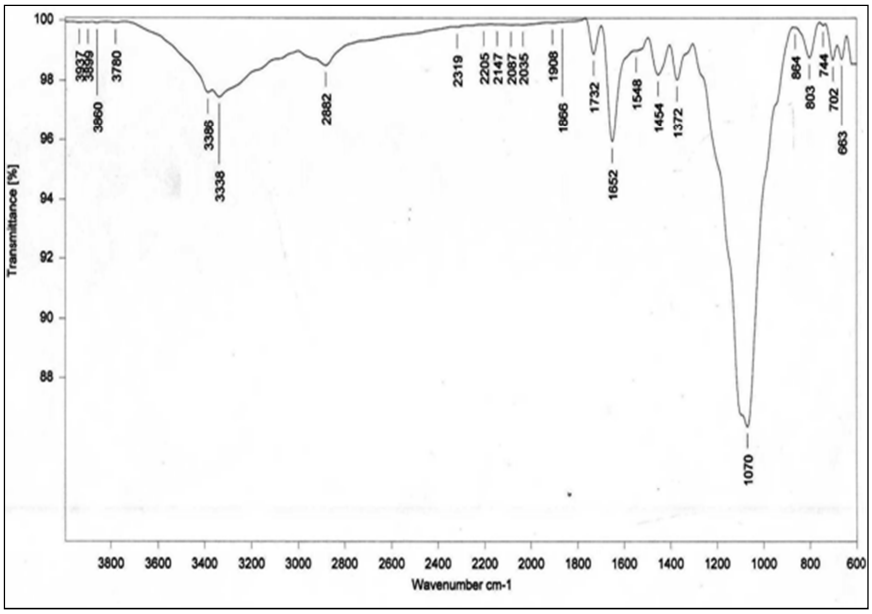
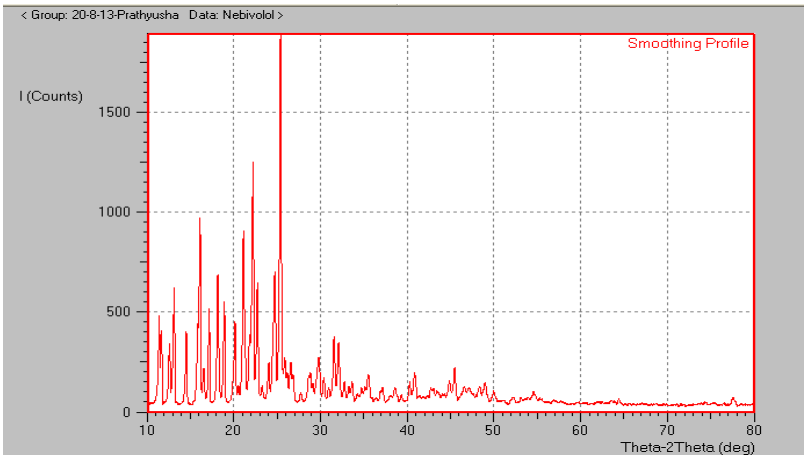


Fig: 6 IR Spectroscopy study of optimized nanosuspension tablet (F6)



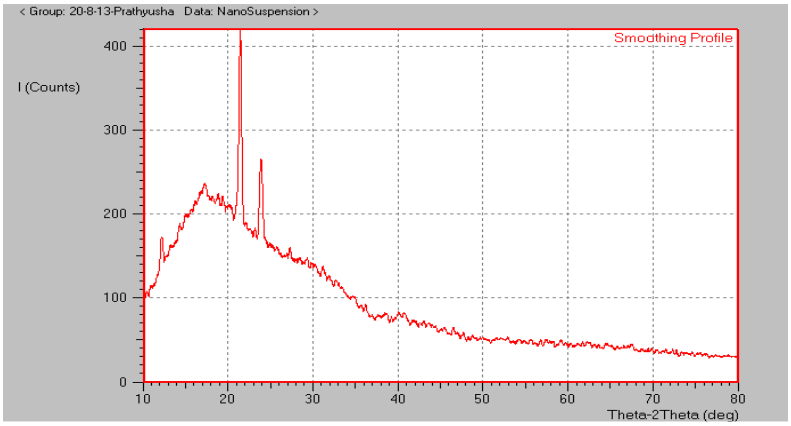


Fig: 7 XRPD of pure drug and optimized nanosuspension (F6)

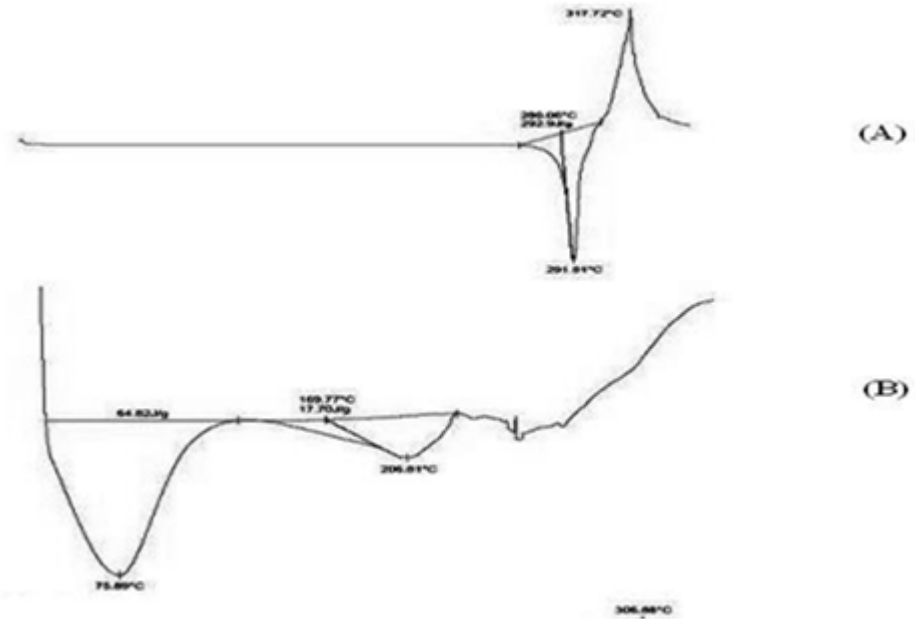


Fig: 8 DSC of pure drug (A), Optimized formulation of nanosuspension tablet (B)

CONCLUSION

From the present study it can be concluded that nanosuspension tablets changes the properties of poorly soluble drugs like nebivolo hydrochloride and increases the wetting property and surface area of the drug particle and indirectly inccresaes the dissolution and oral bioavailability of drugs. The nanosuspension tablets shown higher dissolution rate compare to the innovator product and pure drug. The present study is proven that nanosuspension tablets are promising alteranative technigue for improving dissolution rate and bioavailability of drugs.

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