INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

FORMULATION AND INVITRO/ INVIVO EVALUATION OF DRIED NANOSUSPENSIONS OF NEBIVOLOL

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ARTICLE INFO

Article history

Received 08/03/2017

Available online 31/03/2017

Keywords

Nebivolol,
Oral Nanosuspension,
Tween80,
Tween 20,
Soluplus,
PEG 200,
PEG 400,
Methanol and Water.

ABSTRACT

In the present study, an attempt was made to prepare oral Nanosuspension of Nebivolol (a beta1 blocker), in order increase drug solubility and to overcome bioavailability problems, to reduce dose dependent side effects and frequency of administration. Nanosuspension containing the drug was prepared by precipitation method using combinations of polymers (such as tween 80, tween 20, soluplus, PEG 200, PEG 400 and methanol) in to 12 formulations $F_1$to F_{12}. Subsequent drying was done by spray drying method to form dried nanosuspensions. The Nanosuspensions were evaluated for particle size, zeta potential, drug content uniformity, *in-vitro* drug release, short-term stability, and drug- excipient interactions (FTIR). IR spectroscopic studies indicated that there are no drug-excipient interactions. The formulation F_{10} containing Tween 80, Tween 20, Soluplus, PEG 200, PG, Methanol and water were found to be promising, which showed 100% drug release within 15 min when compared to the optimised formulation (Bystolic) which showed only 91.95% drug release for 20mins. SEM (scanning Electron Microscopy) analysis showed average particle size to be at 300.3nm and it also shown desired zetapotential value. Short-term stability studies indicated stability with respect to drug content and dissolution. Dried Nanosuspension of nebivolol can be prepared by precipitation method and spray drying was done. Among all the formulations, F10 was found to be promising with 100% drug release in 15mins. Short-term stability studies of the promising formulation indicated that there are no significant changes in dissolution parameter values after 3 months.

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*Please cite this article in press as **S Raja Shekhar** et al. Formulation and Invitro/Invivo Evaluation of Dried Nanosuspensions of Nebivolol. Indo American Journal of Pharmaceutical Research.2017:7(03).*

INTRODUCTION

Nanosuspensions are colloidal dispersions of nanosized drug particles stabilized by surfactants. They can also be defined as a biphasic system consisting of pure drug particles dispersed in an aqueous vehicle in which the diameter of the suspended particle is less than 1µm in size. Reduction of drug particles to nanometer range leads to an enhanced dissolution rate not only because of increased surface area but also because of saturation solubility. More than 40 percent of the drugs coming from High-throughput screening are poorly soluble in water. Obviously poorly water-soluble drugs show many problems in formulating them in conventional dosage forms. One of the critical problems associated with poorly soluble drugs is too low bioavailability and or erratic absorption. Nanosuspensions are promising strategy for the efficient delivery of hydrophobic drugs.

Types of manufacturing Methods

For the preparation of nanosuspensions, mostly two methods namely "Bottom up technology" and "Top down technology" are used. Bottom up technology is an assembling method to form nanoparticles like precipitation, microemulsion, melt emulsification method and top down technology involves the disintegration of larger particles into nanoparticles, examples of which are high-pressure homogenization and milling methods. Following are the list of manufacturing methods of nanosuspensions: [1, 2]

1. Precipitation.
2. Lipid Emulsion/Microemulsion Template.
3. Melt emulsification method.
4. High Pressure Homogenization.
5. Milling Techniques.
6. Media milling.
7. Dry Cogrinding.
8. Microprecipitation – High-Pressure Homogenization (Nanoedge).
9. Nanojet Technology.
10. Supercritical Fluid Methods.

Application of Nanosuspensions:

Nanosuspensions have various applications. Some of which are Bioavailability Enhancement, Ocular Administration, Pulmonary administration. Targeted drug delivery etca

MATERIALS AND METHODS

Materials:

Nebivolol drug was procured by Spectrum labs, Hyderabad as a gift sample. PEG 4000, soluplus and Mithanol was procured form Nihar chemicals Ltd. Tween 20, Tween 80 and water was procured from SD labs and all other excipients used were of analytical grade.

Methods:

Pre-formulation studies:

The overall objective of the pre-formulation is to generate information useful to the formulator in developing stable and bio available dosage forms which can be mass produced.

Organoleptic properties:

The colour, odour and taste of the drug were recorded using descriptive terminology and found to be white to off-white crystalline powder, tasteless and odourless.

Melting Point:

The temperature at which the first particle of the substance completely melts is regarded as melting point of the substance. The temperature at which the first particle starts to melt and last particle completely melts is regarded as the range of melting point. Melting point of the drug was determined by capillary tube method and found to be 156-160^{o}C.

Solubility studies:

Solubility of nebivolol was carried out in different solvents like- soluble in octanol, slightly soluble in methanol, very slightly soluble in water. Saturated solutions were prepared by adding excess drug to the vehicles and shaking on the shaker for 48 hr. at 25°C under constant vibration. Filtered samples (1ml) were diluted appropriately with 0.1N Hcl buffer and solubility of nebivolol was determined spectrophotometrically at 282nm.[3].

Drug-Excipient Interactions Studies:

There is always possibility of drug excipient interaction in any formulation due to their intimate contact. The technique employed in this study is IR spectroscopy. IR spectroscopy is one of the most powerful analytical techniques, which offers possibility of chemical identification. The IR spectra of Nebivolol, PEG-200, Tween 80, PEG-400, Tween 20, PG, Soluplus Methanol and Water formulations (F_1 to F_{12}) were obtained by KBr pellet method.(Perkin-Elmer series 1615 FTIR Spectrometer). [4, 5]

Determination of absorption maximum (λmax):

Accurately weighed 100mg of nebivolol was dissolved in 0.1N Hcl buffer taken in a clean 100ml volumetric flask. The volume was made up to 100ml with the same which will give stock solution-I with concentration 1000µg/ml. From the stock solution-I, 5ml was pipette out in 50ml volumetric flask. The volume was made up to 50ml using 0.1N Hcl buffer to obtain stock solution-II with a concentration 100µg/ml. From stock solution-II, 1ml was pipette out in 10ml volumetric flask. The volume was made up to 10ml using 0.1N Hcl buffer to get a concentration of 10µg/ml. This solution was then scanned at 200-400nm in UV-Visible double beam spectrophotometer to attain the absorption maximum (λ-max).

Preparation of Calibration Curve

10 mg of nebivolol was dissolved in 10 ml of pH 0.1N Hcl by slight shaking (1000 mcg/ml). 1 ml of this solution was taken and made up to 20 ml with 0.1NHcl, which gives 40 mcg/ ml concentration (stock solution). From the stock solution, concentrations of 4, 8, 12, 16 and 10µg/ml in 0.1NHcl were prepared. The absorbance of diluted solutions was measured at 282 nm and a standard plot was drawn using the data obtained.

Method of Preparation of Nanosuspension:

Nano suspension precipitation method has been employed to prepare oral Nanosuspension of nebivolol using tween 80, PEG 200, methanol, propylene glycol as polymers.

Procedure:

All the ingredients including drug, polymer and excipients were weighed accurately according to the batch formula (Table-1). The required amount of polymer (carrier) and stabilizer were accurately weighed and added to required measure of H_2O in a beaker. The drug is dissolved in solvent (methanol) and added to the above mixture in a drop wise manner using a syringe while on slow stirring on magnetic stirrer for 1h and then sonication for another 1h.

Table 1: Composition of Nanosuspension of Nebivolol.

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
NEBIVOLOL(mg)	25	25	25	25	25	25	25	25	25	25	25	25
TWEEN 20(ml)						2	2		2	1	1	1
SOLUPLUS(mg)			1.5	1.5						1.5	1.5	1.5
TWEEN 80(ml)	1	1		1	2			2		1	1	0.5
PEG 200(ml)	1		1	1	2	2		3	3	1		0.5
PEG 400(ml)		1					2				1	0.5
PROPYLENE GLYCOL(ml)	2	2	2	2	2	2	2	2	2	2	2	2
METHANOL(ml)	5	5	5	5	5	5	5	5	5	5	5	5
PEPPERMINT Oil(ml)	1	1	1	1	1	1	1	1	1	1	1	1
WATER(ml) qs to 20ml	qs	qs	qs	qs	qs	qs	qs	qs	qs	Qs	qs	qs

Evaluation parameters of Nanosuspension Nebivolol:

The nanosuspension was evaluated for various parameters:

Drug content uniformity

10ml of each formulation was taken and dissolved in 10ml isotonic solution and kept overnight. 10 mg (similar as in formulation) of drug was taken and dilution was made to 10µg/ml. The dilutions were filtered and analyzed using UV for their content uniformity. The absorbance of the formulations were read using one cm cell in a UV-Vis spectrophotometer. The instrument was set at 282 nm. The drug content in each formulation was calculated based on the absorbance values of known standard solutions.[6]

Entrapment efficacy:

Entrapment efficacy was calculated by following formula:

%Entrapment efficiency= Drug content *100/Drug added in each formulation

%Transmittance:

%Transmittance was measured by U.V spectroscopy at a wavelength of 245nm.A graph for %particle range vs. formulations was plotted.

pH measurement

The pH values were measured at 25 ^{o}C using a pH digital meter at 20 ± 1^{o}C. The formulation was brought in contact with the electrode of pH meter and equilibrated for 1 min. This method was done in triplicate and mean was calculated along with standard deviation.

Particle size and shape

Particle size and shape of the formulated microcapsules was determined by using Optical Microscope.

***In vitro* drug release study:**

This is carried out in USP XXIII dissolution test apparatus-II (Electrolab TDT-06N), employing paddle stirrer at 50 rpm and 200 ml of pH 0.1N HCl buffer as dissolution medium. The release study is performed at 37 ±0.5 ^{o}C. The disk is placed at the bottom of the dissolution vessel. Samples of 5 ml are withdrawn at predetermined time intervals and replaced with fresh medium. The samples were filtered through 0.22 µm membrane filter disc (Millipore Corporation) and analyzed for Nebivolol after appropriate dilution by measuring the absorbance at 282 nm.

The results of *in vitro* release profiles obtained for the formulations were fitted into four models of data treatment as follows:

1. Cumulative percent drug released versus time (zero order kinetic model).
2. Log cumulative percent drug remaining versus time (first- order kinetic model).

Whenever a new solid dosage form is developed or produced, it is necessary to ensure that drug dissolution occurs in an appropriate manner. Drug dissolution from solid dosage forms has been described by kinetic models in which the dissolved amount of drug (Q) is a function of the test time, t or Q=f(t). Some analytical definitions of the Q(t) function are commonly used, such as Zero order, First order, Higuchi and Korsmeyer–Peppas models.

Mechanism of Drug Release:

To find out the drug release mechanism due to swelling (upon hydration) along with gradual erosion of the matrix, first 60% drug release data can be fitted in Korsmeyer–Peppas model which is often used to describe the drug release behaviour from polymeric systems when the mechanism is not well-known or when more than one type of release phenomena is involved.

$$\mathbf{Log\ (M_t / M_\infty) = Log\ K_{KP} + n\ Log\ t}$$

Where,

M_t = is the amount of drug release at time t,

M_∞ = is the amount of drug release after infinite time,

K_{KP} = is a release rate constant incorporating structural and geometrical characteristics of Tablet.

n = is the release exponent indicative of the mechanism of drug release.

Zeta potential: The zeta Potential is defined as the difference in potential between the surface of the tightly bound layer (shear plane) and the electro-neutral region of the solution. [7 , 8]

Table 2: Zeta potential for colloids in water and their stability.

Zeta Potential [mV]	Stability behaviour of the colloid
0 to ±5	Rapid coagulation or flocculation
from ±10 to ±30	Incipient instability
from ±30 to ±40	Moderate stability
from ±40 to ±60	Good stability
more than ±61	Excellent stability

Stability studies:

Short- term stability studies were performed at a temperature of 40 ± 2^{O} C / 75 ± 5% RH over a period of three months (90 days) on the promising Nanosuspension of Nebivolol (formulations F_1 to F_{15}). Sufficient number of Nanosuspension (15) were packed in amber colored rubber Stoppard vials and kept in stability chamber maintained at 40± 2^{O} C / 75± 5% RH. Samples were taken at one month interval for drug content estimation. At the end of three month period, dissolution test was also performed to determine the drug release profiles. [9-11]

Spray drying of Nanosuspension:

Spray drying was carried out to get the dry nano size powder. An optimised batch of aqueous nanosuspension was transferred into nano size powder by a lab spray dryer LU-222 lab ultima. Spray dried powder was directly collected after the process. In this process, the spray dryer was set to the conditions given in following table.

Table 3: Spray dryer Parameter.

Inlet Temperature	105-110^{o}C
Outlet Temperature	100^{o}C
Cool Temperature	40^{o}C
Aspirator flow rate	45 nm^{3}/hr
Feed pump flow rate	3 ml/min
Cycle time	70 min

***In vivo* studies of nebivolol**

Animal preparation

Twelve New Zealand white rabbits of either sex rabbits were (weighing 2-3 kg) selected for this study, all the animals were healthy during the period of the experiment. Animals were maintained at room temperature 25^{0}C, RH 45% and 12h alternate light and dark cycle with 100 % fresh air exchange in animal rooms, uninterrupted power and water supply and rabbits were fed with standard diet and water ad libitum. The protocol of animal study was approved by the institutional animal ethics committee.

Study design:

The rabbits were fasted overnight before administration of the formulations the rabbits were randomly divided into two groups each group contains six animals. The group A rabbits were received optimized formulation contain nebivolol nanosuspensions in a dose of 25mg/kg then group b receives pure nebivolol 25mg/kg. Animals are treated with equivalent to animal body weight. Blood samples for pharmacokinetic analysis were obtained at different time intervals 0, 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, 12.00, 16.00 & 24.00h after dosing. Blood samples were collected in heparinised tubes and were centrifuged for 10min at 3,000 rpm at room temperature.

Determination of nebivolol in Rabbit plasma by HPLC method:

Determination of nebivolol by high performance liquid chromatography using a RP-C18 chromatographic column, PhenomenexKinetex (150 mm × 4.6 mm with i.d of 0.5 mm.) and mobile phase consist of 0.005M ammonium acetate solution, acetonitrile and triethylamine in the ratio 60:40:0.1(v/v) and pH 3.0 was adjusted with orthophosphoric acid. Detection was carried out at 269nm at a flow rate of 1.5 ml/min. The retention time of Amlodipine and Nebivolol was 3.911 and 5.818 min, respectively [12].

Preparation of Plasma Samples for HPLC Analysis

Rabbit plasma (0.5 ml) samples were prepared for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was re suspended with 1 ml of acetonitrile by vortexing for 1 min. After centrifugation (5000 – 6000 rpm for 10 min), the acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. Samples were reconstituted in 200 µ1 of 70 % of acetonitrile and 30% water was injected for HPLC analysis.

Pharmacokinetic data analysis

The pharmacokinetic parameters, peak plasma concentrations (C_{max}) and time to reach peak concentration (t_{max}) were directly obtained from concentration time data. In the present study, AUC_{0-t} refers to the AUC from 0 to 24 hrs, which was determined by linear trapezoidal rule and $AUC_{0-\alpha}$ refers to the AUC from time at zero hours to infinity.

The $AUC_{0-\alpha}$ was calculated using the formula AUC_{0-t} + [C_{last}/K] where C_{last} is the concentration in µg/ml at the last time point and K is the elimination rate constant.

Various pharmacokinetic parameters like area under the curve [AUC], elimination half life (t½). Volume of distribution (V_d), total clearance (Cl_T) and mean residence time for each subject using a non compartmental pharmacokinetic programme. The pharmacokinetic parameters were performed by a non compartmental analysis using Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA). All values are expressed as the mean ±SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Difference with $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Calibration curve:

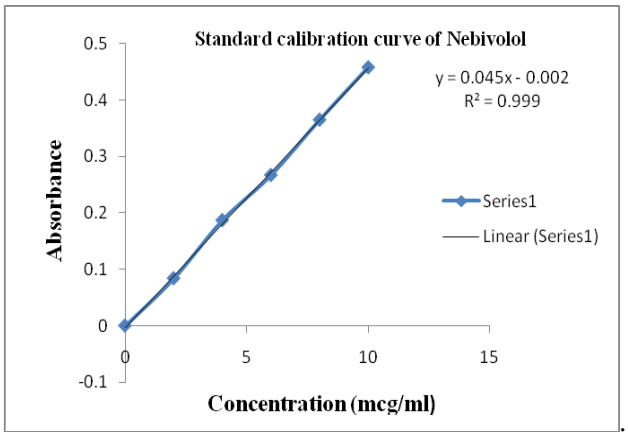

Figure 1: Standard calibration curve of Nebivolol (1.2ph buffer).

DISCUSSION

The regression value was closer to 1 indicating the method obeyed Beer-lambert's law.

Drug excipient compatibility:

Drug and excipient compatibility was confirmed by comparing spectra of FT-IR analysis of pure drug with that of various excipients used in the formulation.

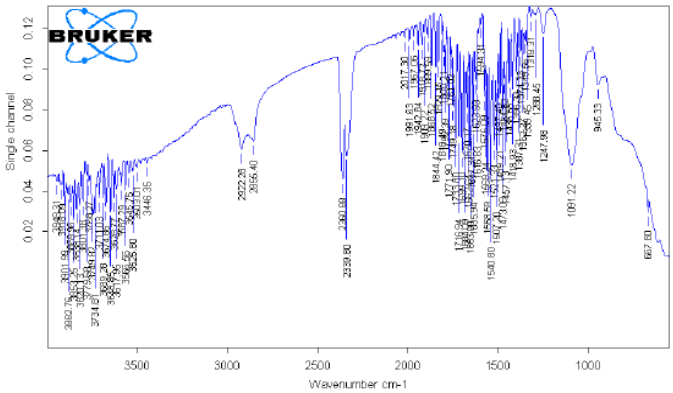

Figure 2: FTIR Spectra of Tween 80.

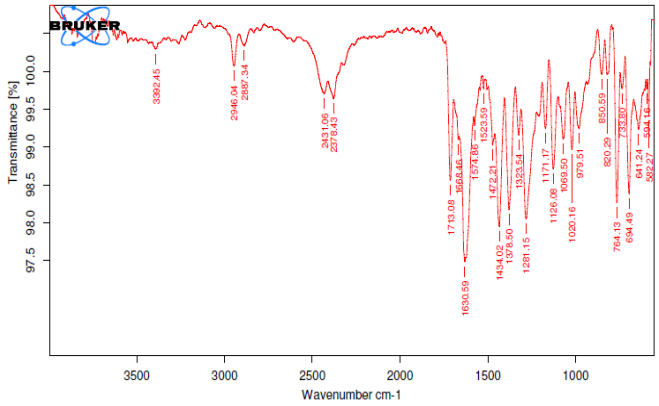

Figure 3: FTIR spectra of Soluplus.

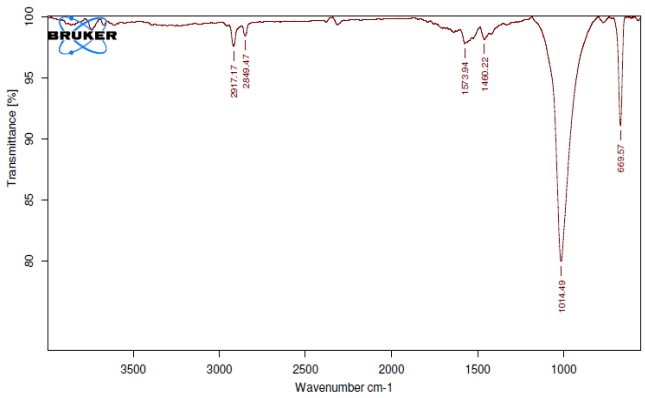

Figure 4: FTIR spectra of PEG 200.

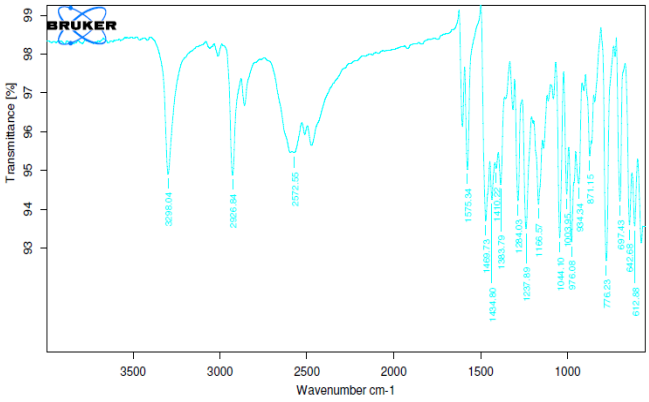

Figure 5: FTIR spectra of PEG 400.

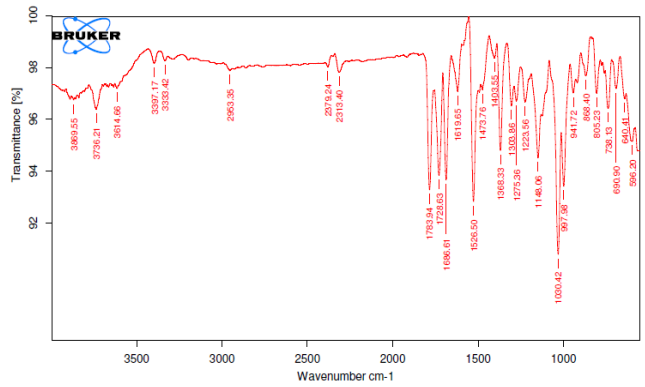

Figure 6: FTIR spectra of optimized formula.

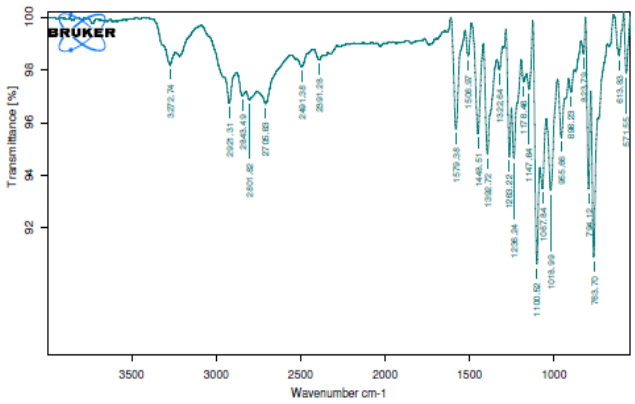

Figure 7: FTIR spectra of Nebivolol (pure drug).

Characteristic peaks In FT-IR spectra of Nebivolol:

Table 4: FTIR Interpretaion of Nebivolol.

S. No	Functional group	Wave number (cm^{-1})
1	N-H stretching	3404.51
2	O-H stretching	3244.41
3	C-H stretch	1357.94
4	C-N stretch	1215.21

DISCUSSION

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and excipients were studied. The characteristic absorption peaks of were obtained and they were in official limits (±100 cm^{-1}) the drug is compatible with excepients.

Drug content:

The drug content of the formulated Nanosuspension was found in the range of 93.86 to 99.87 respectively.

Table 5: Drug content for different Formulated Nanosuspension.

Formulation code	Mean % drug content* ± S.D (CV)
F1	90.72±1.76 (1.08)
F2	92.87±0.36 (0.80)
F3	93.27±0.98 (0.76)
F4	96.94±1.06 (1.36)
F5	95.55±0.89 (0.93)
F6	93.96±1.02(1.10)
F7	98.45±1.67 (1.01)
F8	96.56±0.67 (0.69)
F9	97.26±1.22 (1.33)
F10	99.87±0.84 (0.88)
F11	92.27±0.76 (0.81)
F12	97.58±1.34 (1.40)

Entrapment efficacy:

The entrapment efficacy of the formulated Nanosuspension was found to be in the range of 60.4%-98.57% respectively.

%Transmittance measurement:

UV-Visible spectrum of pure Nanosuspension was recorded in range of 200-400 nm.

Zeta Potential:

The measurement itself is a particle electrophoresis, the particle velocity is determined via the doppler shift of the laser light scattered by the moving particles. The field strength applied was 20 V/cm. The electrophoretic mobility was converted to the zeta potential in mV using the Helmholtz-Smoluchowski equation. At standard measuring conditions (room temperature of 25 °C, water) this equation can be simplified to the multiplication of the measured electrophoretic mobility (µm/cm per V/cm) by a factor of 12.8, yielding the ZP in mV.

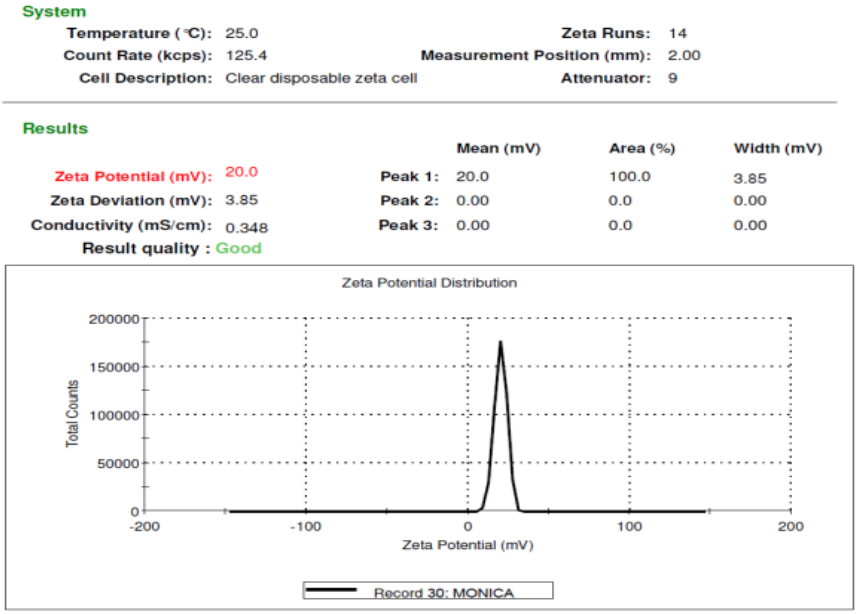

Figure 8: ZetaPotential for F_{10} formulation.

DISCUSSION

The Zeta potential for the optimised formulation was found to be 20mv. When compared to the standard zeta potential values the optimised F_{10} formulation was stable.

Particle size

The optimized batch (f10) had a average particle size of 300.3nm with 0.218 poly dispersivty index which indicate the particles are in uniform distribution. The particle size distributionpattern of the optimized nanosuspension formulation is given in figure 9.

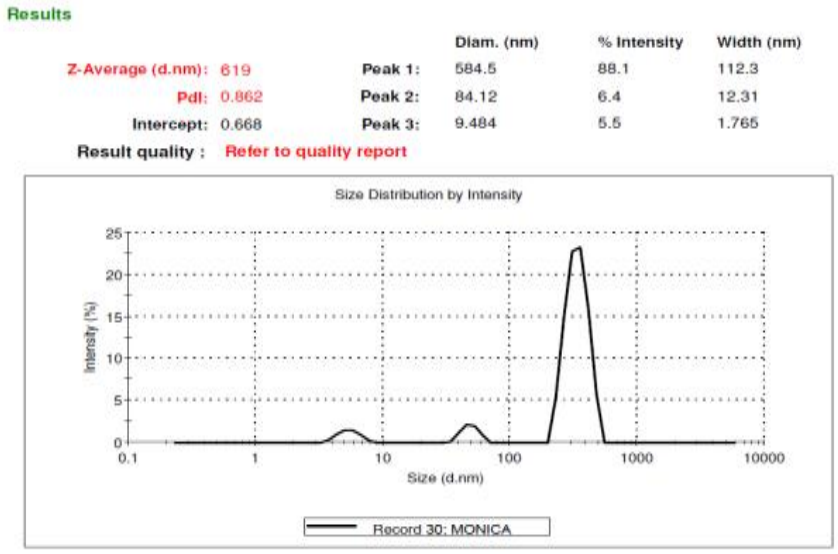

Figure 9: particle size graph for optimized formulation F_{10}.

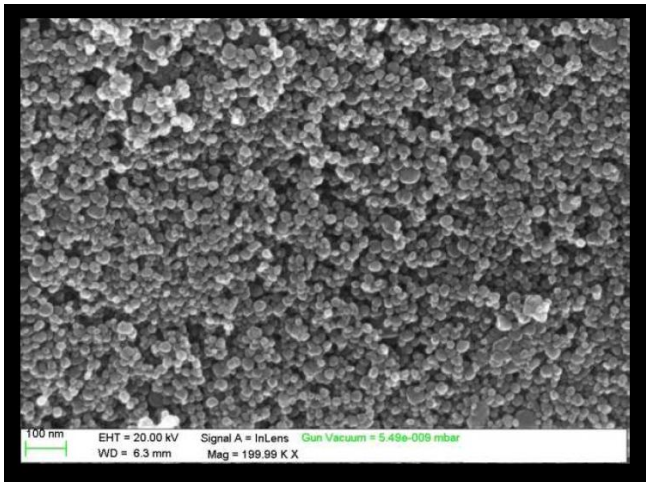

Figure 10: SEM Photograph For F10 formulation.

Dissolution results:

Table 6: in vitro drug release data of formulations F_1 to F_{12}.

Time (min)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0	0	0	0	0	0	0
5	45.78	48.9	65.72	55.02	51.96	67.24	70.03	73.36	77.09	93.24	62.66	64.02
10	50.42	58.08	70.03	62.66	53.34	68.08	71.84	76.04	86.66	94.8	65.72	77.09
15	53.34	61.01	74.09	67.24	64.02	73.36	74.09	87.12	99.36	100.88	70.03	87.12
20	55.02	62.66	81.01	82.54	71.84	79.48	87.12	91.72	100.88		71.84	90.02
25	56.54	70.03	88.66	84.6	77.09	87.12	93.24	93.24			82.54	93.24
30	65.72	71.84	97.82	90.2	85.06	88.66	97.82	103.94			91.72	99.36
35	67.24	82.54	99.36	96.03	93.24	97.82	102.42				94.08	
40	76.4	94.08		99.36	97.82	99.36					97.82	
45	84.6	96.03			99.36							
50	91.72											

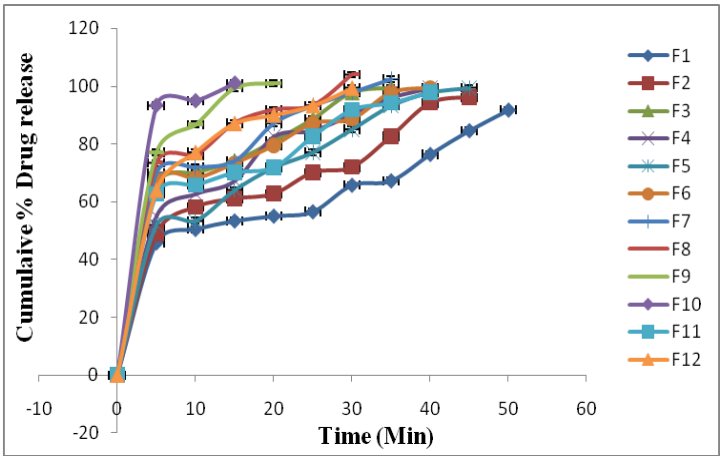

Figure 11: in vitro drug release data of formulations F1 to F12.

Drug release kinetics:
Zero order:

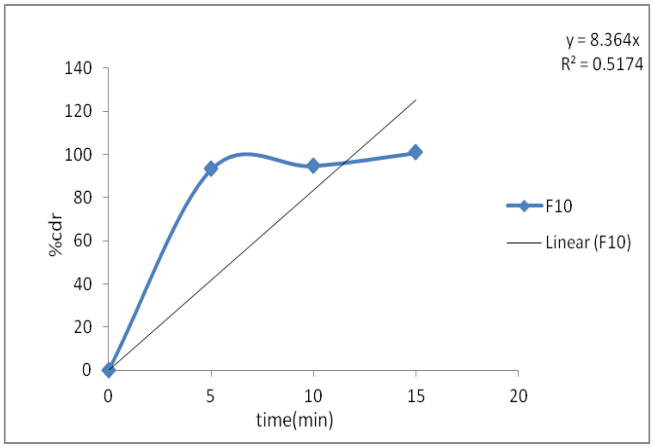

Figure 12: Cumulative percent drug released vs time plots (zero order) of formulation F10.

First order:

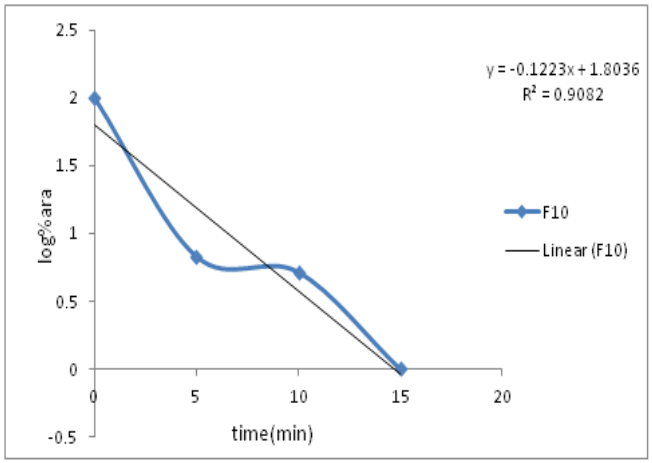

Figure 13: Log cumulative percent drug released vs time plot (First order) of formulation F10.

Table 7: Kinetic data of the formulations:

Order of kinetics	Zero order	First order
Regression	0.517	0.908

DISCUSSION:

The drug release from the Nanosuspension was explained by the using mathematical model equations such as zero order, first order, and equation methods. Based on the regression values it was concluded that the optimized formulation F10 follows first order kinetics.

Drug content stability data:

Table 8: Drug content data of stability formulation (F_{10}).

Sl. No.	Trial No.	1st day (%)	30th day (%)	60th day (%)	90th day (%)
1.	I	95.89	95.41	95.29	94.30
2.	II	96.56	96.63	96.40	95.89
3.	III	97.23	95.47	95.10	94.63
4.	Mean (X)	95.56	95.83	95.60	94.94
5.	S.D	±0.67	±0.68	±0.70	±0.84

Table 9: Statistical analysis of drug content data for the stability formulation (F_{10}).

Sl. No.	Trial	1st Day A	90th Day B	A – B
1.	I	5.89	94.30	1.59
2.	II	96.56	95.89	0.67
3.	III	97.23	94.63	.60
4.	Mean (X)	5.56	94.94	.62

't' = 2.94 ($p < 0.05$).

Table 10: *In vitro* drug release data of the stability formulation (F10).

Sl. No.	Time (min)	Cumulative* % drug released* ± S.D at 40± 1° C			
		1st day	30th day	60th day	90th day
1	0	0	0	0	0
2.	5	83.24±0.60	82.08±0.56	81.89±0.68	81.45±0.68
3.	10	95.8±1.00	93.42±0.36	92.80±1.06	95.02±0.47
4.	15	100.88±0.88	99.20±0.64	100.95±0.44	99.89±0.59

*Average of three determinations.

Table 11: *In-vitro* dissolution study of Innovator.

S. No	Time (min)	Absorbance	Concentration (µg/ml)	Amount (mg/ml)	Cumulative Drug Release	Cumulative % Drug Release
1	0	0	0	0	0	0
2	10	0.0695	1.9879	0.0198	17.89	89.45
3	20	0.0714	2.0412	0.0204	18.39	91.95
4	30	0.0746	2.1308	0.0213	19.21	96.09
5	45	0.0753	2.1504	0.0215	19.41	97.07

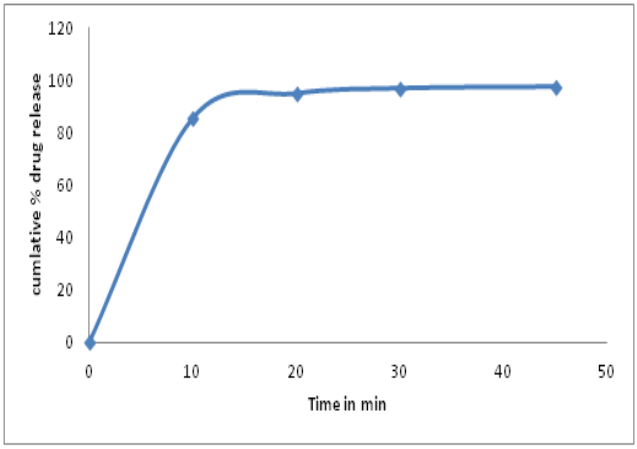

Figure 14: *In-vitro* dissolution study of Innovator.

Comparison profile for F10 formulation and marketed formulation:
The optimized formulation F10 dissolution profile was compared with the marketed formulation Bystolic.

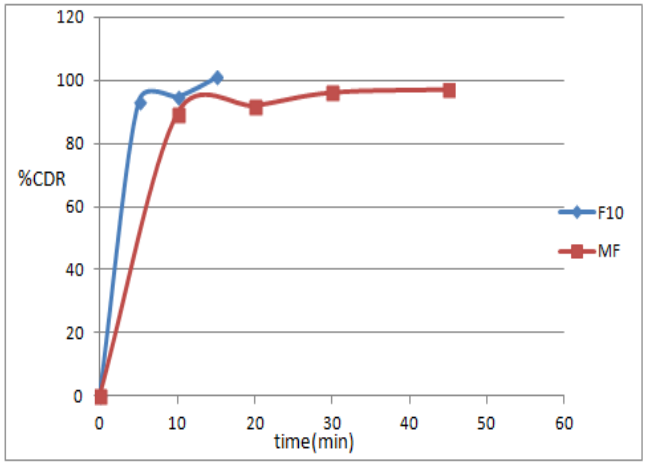

Figure 15: Dissolution comparison of optimized formulation F10 & Marketed Formulation.

Discussion:

On comparing the best Optimized formula i.e.,F10 with conventional formulation, it was clearly observed that the %drug release was i.e.,100.88% within 15mins by best formulation, whereas it is 91.95% within 20 mins for the conventional formulation. So, the % of drug release was more in F10 Nanosuspension than the conventional tablet.

Pharmacokinetic study:

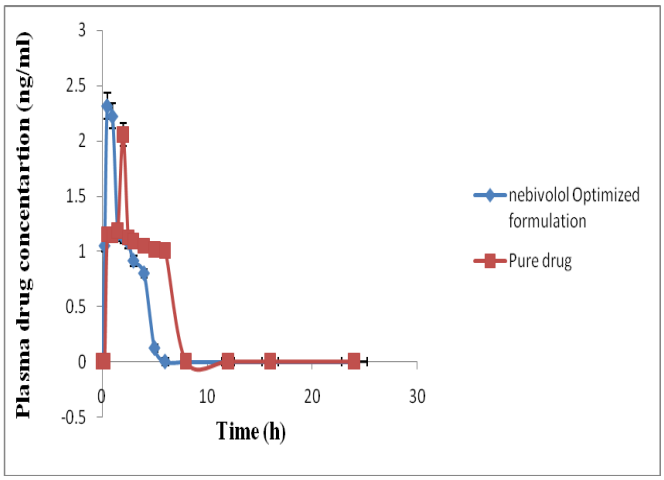

Figure 16: Plasma concentrations at different time intervals of nebivolol Optimized formulation and pure drug.

Table 12: Comparison of pharmacokinetic parameters of nebivolol optimized formulation film and Pure drug (mean ± SD, n = 6).

Parameters	optimized formulation	innovator
Cmax(ng/ml)	2.225±0.4	2.054±0.2
AUC0-t(ng hr/ml)	22.06±0.44	17.95±0.26
AUC0–∞(ng hr/ml)	25.14±0.14	19.04±0.12
Tmax(h)	1.00±0.5	2.00±0.1
t1/2(h)	1.253 ± 0.519	2.664 ± 0.01
Kel (hr-1)	1.336 ± 0.11	1.196 ± 0.33

Pharmacokinetic parameters comparison for nebivolol

The bioavailability optimized formulation and Pure drug parameters for the both test and reference standard are summarized in Table 12. Mean time to reach peak drug concentration (T_{max}) was 1.00±0.5h and 2.00±0.1h for the optimized and commercial formulations, respectively, while mean maximum drug concentration (C_{max}) was 2.225±0.4ng/ml and 2.054±0.2ng/ml, respectively. The statistical comparison of $AUC_{0-\infty}$ and AUC_{0-t} indicated significant difference between the two optimized formulation and pure drug and there was a significant difference for the C_{max} and T_{max} was observed in this study. As the prepared nano suspensions were exhibited immediate release higher bioavailability when compared with pure drug.

CONCLUSIONS

Oral Nanosuspension of nebivolol can be prepared by precipitation method using Tween20, Tween80, PEG 200, PEG 400, soluplus, methanol and water. IR spectroscopic studies indicated that there are no drug-excepient interactions. All the designed formulations of Nanosuspension displayed first order release kinetics and drug release. Among all the formulations F10 containing Tween 20, Tween 80 , soluplus, PEG 200 and methanol, F10 were found to be promising, which showed formulation F10 is 100.88%, of drug released respectively with in 10 mins. Among all formulations (F1-F12) of Nanosuspensions, the F10 was showed best drug released compared to remaining formulations. Short-term stability studies of the promising formulations indicated that there are no significant changes in dissolution parameter values after 3 months at 40 ± 2^{o} C / 75 ±5% RH. From the invivo study as the prepared nano suspensions were exhibited immediate release higher bioavailability when compared with pure drug.

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