WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 7, 1596-1613. Research Article ISSN 2277– 7105

FORMULATION AND EVALUATION OF NEBIVOLOL HYDROCHLORIDE SUBLINGUAL TABLETS BY USING *IN SITU* MICROCRYSTALS

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Article Received on
03 May 2020,

Revised on 24 May 2020,
Accepted on 14 June 2020,

DOI: 10.20959/wjpr20207-17797

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ABSTRACT

The present study was aimed to formulate and evaluate Nebivolol hydrochloride sublingual tablets using *in situ* microcrystals to enhance the dissolution rate. It is a third generation β adrenergic blocker mainly used for hypertension treatment and belongs to BCS class-II drug. It's a very potent drug but the problem associated with this is a low solubility of drug leads to low dissolution and variable bioavailability. By changing 3 variables each two levels, eight formulations were prepared with full factorial design. *In situ* microcrystals were formulated by using poloxamer and Rhamnolipid as stabilizing agents by solvent change method. FTIR, DSC, SEM, and XRD studies were carried out for investigation of interaction with excipients, particle morphology, and crystallinity of drug respectively. Formulation M7 was considered as optimal showing the lesser particle size (2.57µm) and enhanced *in vitro* dissolution (84.33%) in comparison with an untreated drug. Sublingual tablets were formulated to enhance the poor oral bioavailability of drug due to extensive first-pass metabolism by CYP P450 2D6. Tablets were prepared by incorporating *in situ* microcrystals along with a different concentration of superdisintegrant (SSG, crospovidone) by direct compression method. Formulation F7 was considered as optimal with disintegration time up to 13.66 seconds. *In vitro* dissolution and *in vitro* permeation were found to be 98.57% and 82.99% respectively within 30 minutes. Thus, it can be concluded that the sublingual tablet containing Nebivolol hydrochloride *in situ* microcrystals seems to be a promising formulation for safe and effective delivery via sublingual route for the treatment of hypertension providing faster action.

KEYWORDS: Sublingual tablet, *In situ* Microcrystallization method, Nebivolol hydrochloride, Poloxamer 188, Rhamnolipid.

INTRODUCTION

Poor solubility is one of the crucial challenges in drug development. An estimated 40% of drugs fall under BCS Class 2 (low solubility and high permeability) or class 4 (low solubility and low permeability). The drug's insufficient dissolution rate is the rate-limiting factor in oral bioavailability for poor water soluble API. Therefore various techniques are approached to enhance bioavailability and solubility. Solubility enhancement techniques have been divided into two types i.e. Physical and chemical modification. The physical modification comprises of particle size reduction (micronization and nanosuspension), solid solution, solid dispersion, and cryogenic method. The chemical modification is inclusive of pH adjustment, complexation, and use of buffer, derivatization, and salt formation. One way to enhance the dissolution rate is to minimize particle size, increasing the total surface area.[1]

To address the issue of dissolution and bioavailability, the use of drug powders containing micronized drug particles in many types of pharmaceutical dosage forms has been increased. Micronization refers to the "size reduction technique where the particle size distribution is less than 10μ". Generally used techniques for the production of micronized drug particles involve mechanical communication, spray drying, and supercritical fluid (SCF) technology. The characteristics of the resulting drug product cannot be controlled using these above techniques and external processing conditions like mechanical force, temperature, and pressure are required. Hence, a newer technique called *in situ* micronization is developed to overcome the limitations associated with the other techniques.

In situ micronization is a novel technique in which micron-sized crystals are obtained while their processing themselves without the need for any further particle size reduction. It is one of the easiest methods to produce microcrystal which is a single-step process with common equipment.[2] The type of stabilizing agent in this method has a significant impact on the morphology of particle and thus on its flow. Stabilizing agents possess a high affinity towards the newly formed hydrophobic surface that stabilizes microcrystals sterically against the growth of the crystals by forming a protective layer around microcrystals which results in microcrystals with low adhesivity, lesser agglomeration and low electrostatic behavior that would improve the flow of powder. In addition to this, it also enhances wetting properties and dissolution rate. [1-3]

Nebivolol Hydrochloride is a new third-generation β adrenergic blocker mainly used in the treatment of hypertension. It produces unique nitric oxide-mediated vasodilatory effects. The drug is associated with increased insulin sensitivity, decreased serum lipid, and no increase in new-onset diabetes.[4] It belongs to BCS class II drug with low solubility. Water solubility is 0.0403 mg/ml.[5] *In situ* micronization is used to enhance solubility and dissolution rate and faster absorption of the drug into the systemic circulation. The drug also undergoes extensive first-pass hepatic metabolism and has an oral bioavailability of 12% in extensive metabolizers, since the sublingual route bypasses the first-pass effect, its bioavailability can be increased.[6,7] The sublingual tablet is beneficial for the condition like Dysphagia, and patients who are mentally retarded, uncooperative, nauseated.[8] The molecular weight of the drug is 405.4 which making it a suitable candidate for sublingual route administration.

MATERIALS AND METHOD

Nebivolol hydrochloride was obtained as a gift sample from Unichem Laboratories Limited, Goa. Poloxamer 188 was provided as a gift sample by Venus Ethoxyether's Pvt. Ltd. Goa. Rhamnolipid was bought from AGAE Technologies, Corvallis. Crospovidone was procured from S. D. Fine Chemicals, Mumbai, India. Sodium starch glycolate was procured from Himedia Lab Pvt. Ltd, Mumbai. All other chemicals were of analytical grade.

Preparation of Nebivolol Hydrochloride *in situ* microcrystals by solvent change method

In this technique, microcrystals were prepared by the solvent change method by instantaneously mixing two liquids in the presence of a stabilizing agent (Poloxamer 188 and Rhamnolipid). The process was conducted in an ice bath. Nebivolol Hydrochloride (0.5gm or 1gm) was dissolved in 40 ml of methanol (as solvent) and stabilizing agent (0.05gm or 0.1gm) in 100 ml water (as a nonsolvent) in the first step. Microfine dispersion was created spontaneously batch-wise mixing of the non-solvent which was quickly poured into drug solution under stirring at 26,000 rpm using ultra-homogenizer. The mixture was allowed to mix for 15 min in an ice bath. Crystals were filtered using Whatman filter paper no 1 and dried at 45°C in the oven. By changing 3 variables each at two levels, eight different formulations were prepared with a full factorial design.[9-11] (Table No 1)

Table No 1: Different studied process variables, their levels in the factorial design, and formulation of *in situ* microcrystals of Nebivolol hydrochloride.

Independent variables	Level	
	I	II
Drug concentration (X1)	0.5 gm	1 gm
Stabilizing type (X2)	Rhamnolipids	Poloxamer 188
Stabilizing concentration (X3)	0.05 gm/100 ml water	0.1 gm/100 ml water
Dependent variables (Response) Y1=*In vitro* dissolution rate (%) Y2=Particle size (µm)		

Formulation Code	Drug Concentration	Stabilizing Type	Stabilizing Concentration
M1	1	Rhamnolipids	0.1
M2	1	Rhamnolipids	0.05
M3	0.5	Rhamnolipids	0.1
M4	0.5	Rhamnolipids	0.05
M5	1	Poloxamer 188	0.1
M6	1	Poloxamer 188	0.05
M7	0.5	Poloxamer 188	0.1
M8	0.5	Poloxamer 188	0.05

Design of experiment (DOE)

Full factorial two-level design set up using Design expert version 12 was utilized for the design of the experiment. Three input (independent) factors i.e. the concentration of a drug, Stabilizing type, and Stabilizing concentration with two levels were chosen to study the effect on response i.e. particle size and *in vitro* dissolution.

Particle Size Determination and SEM morphology[9,11]

The eyepiece micrometer was calibrated by using a standard stage micrometer at 45X to determine the particle size of pure drug. Suspension of the sample was prepared using propylene glycol and mounted on a slide and placed on the mechanical stage. The size of the particle was estimated with the help of an eyepiece micrometer.[9]

The particle size measurement was performed with the help of a nanotrac instrument. The formulation with lesser particle size was considered as optimized formulation. Scanning electron micrographs of pure drug and optimized microcrystals were taken using a scanning electron microscope (model-JSM 6360) operating at 15 kV. The specimens were mounted on metal stub with double-sided adhesive tape and coated under vacuum with gold in an argon atmosphere before observation.[11]

X-Ray Powder Diffraction[9]

The cavity of the metal sample holder of the x-ray diffractometer was filled with the ground sample powder and then smoothed with a spatula. X-ray diffraction pattern of Nebivolol Hydrochloride and optimized formulation were obtained using an x-ray diffractometer (Brucker, model D2 Phaser) at 40 kV, 30 mA over a range of 10-90°2θ, using K alpha radiation of a wavelength of 1.54A°.[9]

FT-IR Spectroscopy[10]

All the FT-IR spectra were recorded using an IR spectrophotometer (SHIMADZU) over a scanning range of 4000-500cm^{-1}. Samples were prepared by mixing with KBr powder and compressing into a disk by hydraulic pressure.

Differential Scanning Calorimetry[10]

The DSC test of pure Nebivolol Hydrochloride and physical mixture (drug and stabilizer) were developed in the DSC-60 thermogram. The samples were prepared in the ration 1:1 (drug: stabilizer) and were sealed in an aluminum pan before analysis. Under a nitrogen atmosphere, an empty pan was kept as a reference. A heating rate of 10°C/min was employed. The scanning temperature range was 25-300°C.

Drug Content

The equivalent weight of prepared microcrystals containing 20 mg of the drug was taken and dissolved in 10 ml of methanol followed by volume made up to 100 ml with 6.8 pH phosphate buffer. From stock solution 1 ml was diluted to 10 ml to get a concentration of 20 µg/ml and absorbance of the solution was measured at 281 nm using UV Spectrophotometer.

***In vitro* Dissolution Study**

All the microcrystals and pure drug were subjected to a dissolution profile using the USP type II dissolution apparatus which was maintained at 37 ±0.5°C for 30 min at 50 rpm. The dissolution medium used was pH 6.8 phosphate buffer. At specified intervals, the aliquot sample was extracted from 250 ml, filtered and analyzed at λmax 281 nm using a UV spectrophotometer and maintained sink condition.

Preparation of sublingual tablet of Nebivolol Hydrochloride *in situ* microcrystals[12,13]

Sublingual tablets containing optimized microcrystals equivalent to 5 mg of the drug were prepared by the direct compression method. Microcrystals equivalent to 5 mg of Nebivolol

Hydrochloride was weighed and mixed with super-disintegrants (crospovidone, sodium starch glycolate). Other ingredients such as mannitol and microcrystalline cellulose (AvicelPH-102) used as diluents, Stevia as a sweetener. All excipients were blended and passed through sieve 60# and then powder blends of batches F1-F8 were tested for powder characteristics. Using remik mini press I unit, the powder blend was then compressed into tablets using the direct compression technique using a 6 mm flat punch.

Table No 2: Composition of the sublingual tablet batches (F1 to F8).

Ingredients(mg)	Formulation Code							
	F1	F2	F3	F4	F5	F6	F7	F8
Nebivolol HCl	5	6*	6*	6*	6*	6*	6*	6*
Sodium starch glycolate	-	-	2.5	5	7.5	-	-	-
Crospovidone	7.5	-	-	-	-	2.5	5	7.5
Mannitol	46.5	53	50.5	48	45.5	50.5	48	45.5
Microcrystalline cellulose	40	40	40	40	40	40	40	40
Stevia	1	1	1	1	1	1	1	1
Total Weight	100	100	100	100	100	100	100	100

**Microcrystals equivalent to 5 mg of Nebivolol Hydrochloride.*

Evaluation of pre-compressed blend

Pre-compression parameters for powder blend like bulk density, tapped density, compressibility index, Hausner's ratio, and angle of repose were determined.[14]

Evaluation of *in situ* micronized Nebivolol Hydrochloride Sublingual Tablet[15-18]

All the sublingual tablets of Nebivolol hydrochloride were subjected to Pharmacopoeial tests. Tablets thickness and hardness were determined using vernier caliper and Monsanto hardness tester respectively. Friability, disintegration time, wetting time, and *in vitro* dissolution were also performed as per Indian Pharmacopoeial specifications.

Drug Content

Five tablets were selected randomly, weighted, and crushed. The drug was extracted in 6.8 pH phosphate buffer. The absorbance of the solution was measured against the blank at λ_{max} 281 nm using UV Spectrophotometer.

Wetting Time[15]

The tablets were placed in the middle of two layers of absorbent paper mounted into a Petri dish. After the paper was thoroughly wetted with distilled water, Excess water was drained.

The time it took for the water to pass through the entire tablet from the wetted absorbent paper was then monitored using a stopwatch.

In vitro disintegration Time

In vitro disintegration study of Nebivolol Hydrochloride tablets was carried out by using a disintegration tester. The tablets were placed in the disintegration tube which was placed in a pH 6.8 phosphate buffer maintained at 37ºC ±0.5°C. The time needed for complete disintegration that is without leaving any residue on the screen is reported as disintegration time.

In vitro dissolution rate

In USP dissolution test apparatus the *in vitro* release of drug from the formulated tablets were conducted using 250 ml of pH 6.8 phosphate buffer as dissolution medium maintained at 37.0± 0.5°C and stirring rate of 50 rpm. 5 ml samples were withdrawn from the dissolution medium followed by filtrations and analyzed at different time intervals of 5 min. All samples were analyzed at λ_{max} 281 nm using UV Spectrophotometer, by maintaining the sink condition.

In vitro Permeation Study of Sublingual Tablet[12]

The *in vitro* permeation of the Nebivolol hydrochloride Sublingual tablets were carried out in an altered Franz Diffusion cell at 37±0.5ºC. Dialysis membrane-150 (cut off molwt 12000-14000 Dalton) was used to verify the permeation of drug. The dialysis membrane was mounted between the donor and receptor compartment. The sublingual tablet was placed with membrane facing core, and the compartments were clamped together. The donor and receptor compartment was filled with 1 ml and 40 ml of phosphate buffer (pH6.8) respectively followed by the hydrodynamics in the compartment was maintained by stirring with a magnetic bead at uniform slow speed. 1 ml sample was extracted at pre-determined time intervals and analyzed for drug content using a UV Spectrophotometer at 281 nm.

Stability studies of optimized formulation[16]

A short term stability study was carried out for optimized formulation according to ICH guidelines at 40±2°C and RH of 75±5% and at room temperature at 25± 2°C, and 60±5% RH for 1 month. After the interval of 15 and 30 days, tablets were examined for hardness, friability, drug content, disintegration time, wetting time, and *in vitro* dissolution profile.

RESULTS ANDDISCUSSION

Micronization of Nebivolol hydrochloride

It is well known that Microcrystallization affects the crystal surface causes an increase in system energy. Stabilizers that have an affinity to the crystal surface were used in this method covers the particle of drug and prevent the growth of micronized particles through steric hindrance as well as enhanced their water solubility.

SEM morphology and Particle size

The untreated Nebivolol hydrochloride and optimized microcrystal formulation (M7) was observed in Figure 1 using electron microscopy (1000 X). The micrographs showed that microcrystals were cube-shaped and about 2.511 µm to 5.591µm in size while the untreated drug is about 14.5 µm and crystals were irregular shaped. The particle size of the formulations M1-M8 was given in Table No 3.

Statistical analysis of the particle size of microcrystals produced by different formulations by ANOVA test showed in Figure 2 concluded that the drug concentration and stabilizing concentration had a significant effect ($P<0.05$) on the particle size while the type of stabilizing agent does not have a significant effect on the particle size ($P>0.05$). Statistical analysis showed that increasing drug concentration from 0.5 to 1 had a synergistic effect on particle size while changing Rhamnolipid to Poloxamer 188 and increasing the percentage of stabilizing concentration from 0.05 to 0.1 had an antagonistic effect on particle size.

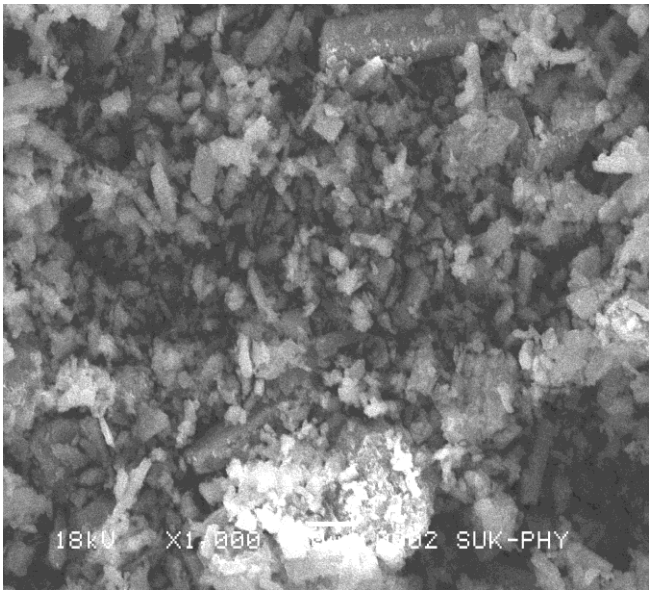

Figure 1(A): Scanning electron micrographs of untreated Nebivolol hydrochloride drug,

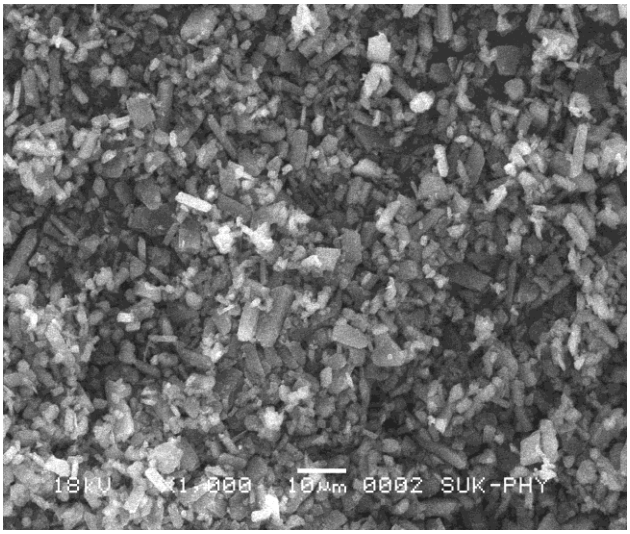

Figure 1(B): Scanning electron micrographs of optimized microcrystal formulation M7.

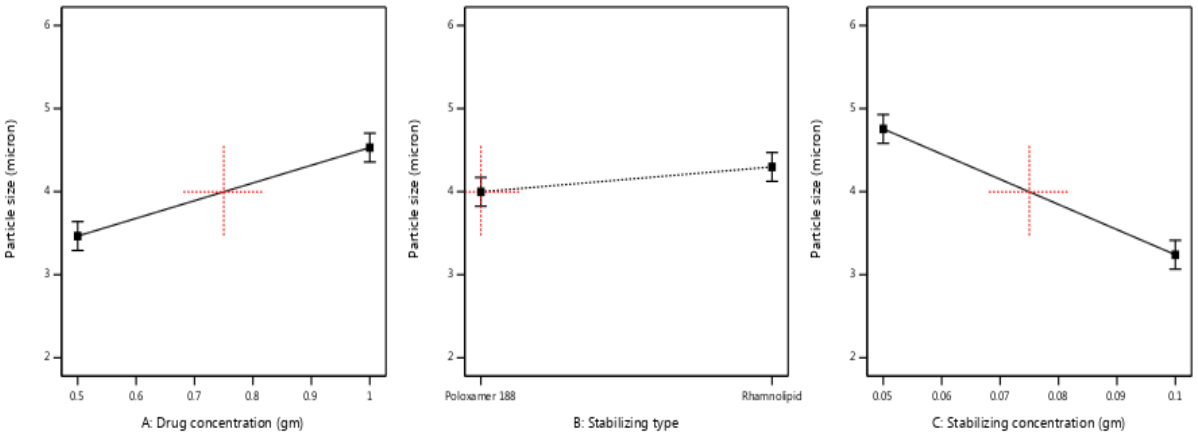

Figure 2: Effect of drug concentration, stabilizing type, and stabilizing concentration on the particle size of *in situ* microcrystals.

X-Ray Powder Diffraction

The diffraction spectrum of untreated Nebivolol Hydrochloride and optimized microcrystal formulation is shown in Figure 3. The major peaks are present in the diffractogram of the optimized formulation but have less intensity than an untreated drug. The sharp peaks are present at a diffraction angle of 2θ 21.1, 22.07, 24.76, and 25.35. Because of the peak height is influenced by crystal size and crystallinity, the reduction in peak height implies a reduction in particle size and the development of the microcrystalline form of the drug.

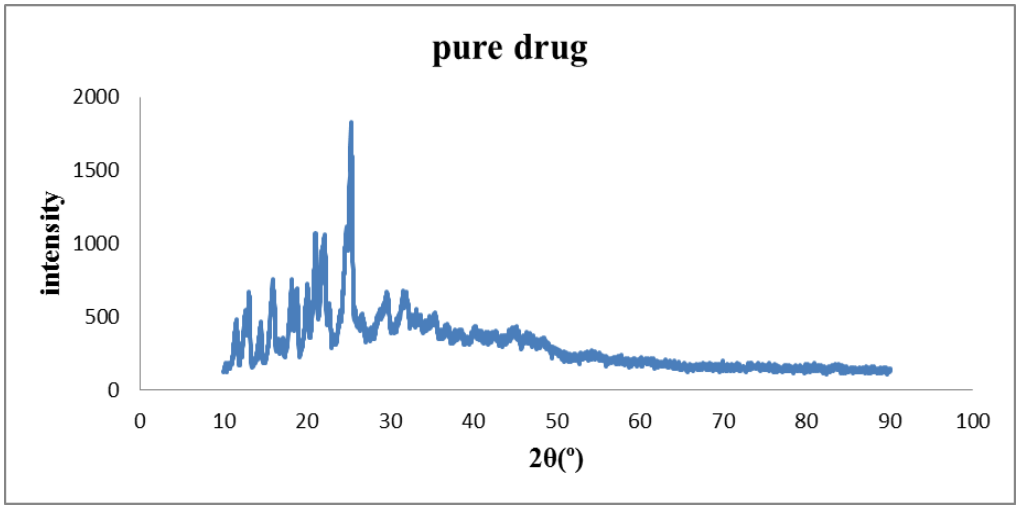

Figure 3(A): X-ray diffraction pattern of untreated Nebivolol hydrochloride.

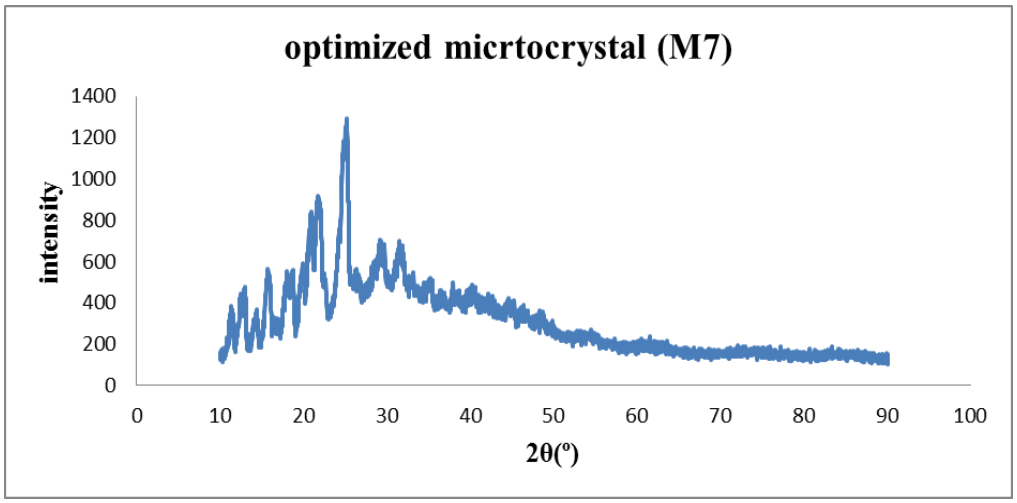

Figure 3(B): X-ray diffraction pattern of optimized microcrystal formulation (M7).

FTIR spectroscopy

The FTIR spectrum of the physical mixture did not show much deviation as compared with the pure drug sample.This indicated that there was no difference between the internal structure and conformation of this sample at the molecular level. Thus, it is inferred that Nebivolol hydrochloride is compatible with the stabilizers.

Differential scanning Calorimetry (DSC) Analysis

The pure drug exhibits a sharp endothermic peak at 227.2°C. A sharp peak of Poloxamer 188 was obtained at 57.40°C. Physical mixture (drug + poloxamer 188) showed sharp endotherms at 225.4°C and 57.77°C corresponding to melting point/transition temperature. No thermal shift for the pure drug and mixed drug-stabilizer sample was observed. This indicates that the drug is pure and compatible with the stabilizer.

Drug Content

The results obtained for the percent drug content of microcrystals in pH 6.8 was ranges from 90±0.23 to 98.3±0.24% as shown in Table No 3.

***In vitro* Dissolution Study**

As showed in Figure 4 and Table No 3, *in vitro* dissolution profile of untreated drug and microcrystals in pH 6.8 phosphate buffer were determined. According to results, the *in vitro* dissolution rate of treated samples was significant faster than the untreated sample (P<0.05).

Statistical analysis by two way ANOVA showed that drug concentration and stabilizing concentration had a significant impact on *in vitro* dissolution (P<0.05). The type of stabilizing agents had no significant impact on this parameter (P>0.05). The ANOVA test showed that increasing the drug concentration from 0.5-1% had a lowering effect on dissolution while the increasing concentration of stabilizing agents from 0.05-0.1% and changing Rhamnolipid to Poloxamer 188 had an additive effect on *in vitro* dissolution profile.

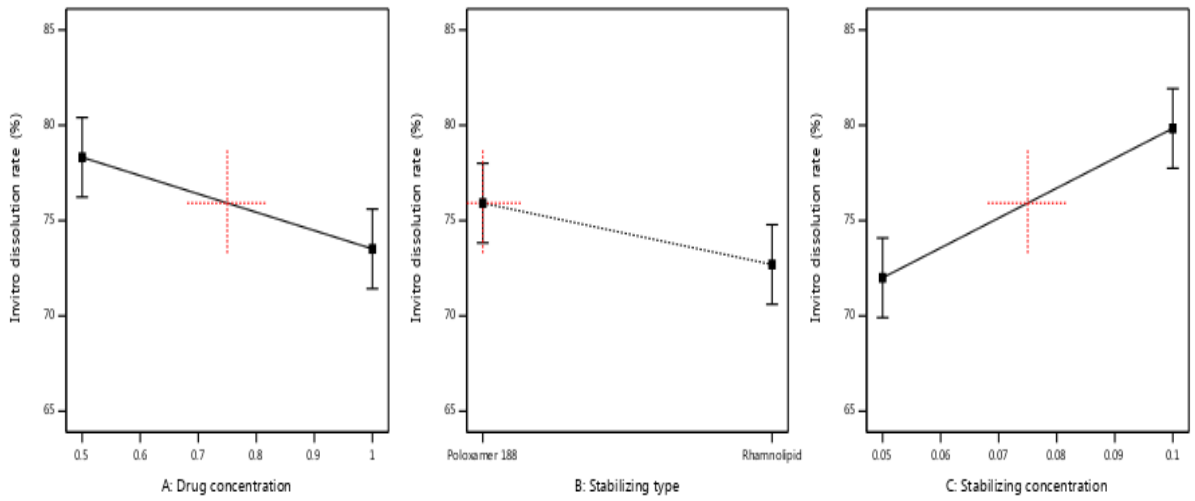

Figure 4: Effect of drug concentration, stabilizing type, and stabilizing concentration on *invitro* drug dissolution.

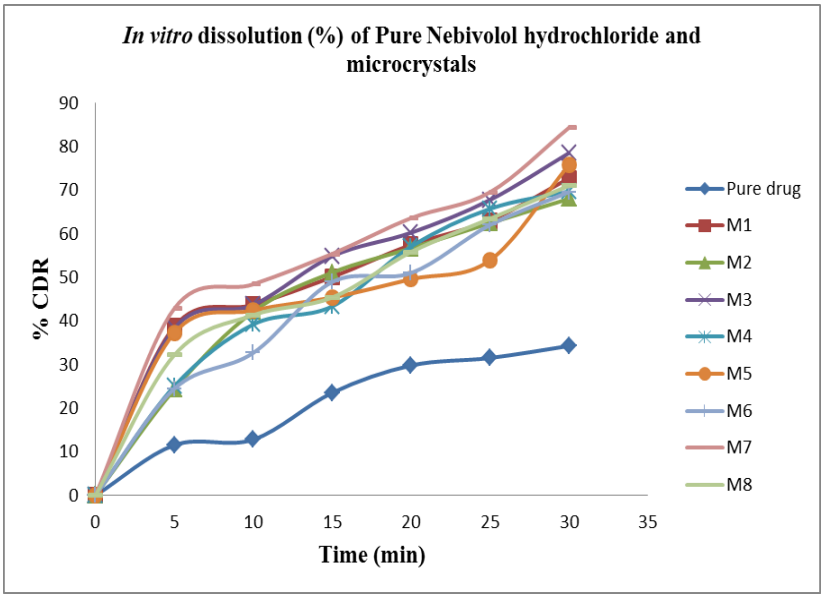

Figure 5: *In vitro* dissolution profile of the pure drug and*in situ* microcrystals formulation (M1-M8).

Table No 3: Particle size, in vitro dissolution, and drug content of untreated drug and microcrystals formulation M1-M8.

Formulation code	Particle size (µm)	In vitro dissolution (%)	Drug content (%)
Pure drug	14.5	34.28±0.78	91.5±0.23
M1	4.060	72.71±0.41	96.8±0.83
M2	5.590	68±0.54	90±0.23
M3	2.955	78.50±0.55	97.5±0.23
M4	4.590	69.70±0.67	91.5±0.41
M5	3.980	75.79±0.47	97.5±0.23
M6	5.090	69.55±0.56	97.3±0.23
M7	2.571	84.30±0.81	98.3±0.24
M7	4.350	71±0.68	97.05±0.24

Resulted data is an average of triplicates (mean ± SD).

Optimization of Nebivolol hydrochloride *in situ* microcrystals

Statistical analysis of response Y1- *In vitro* dissolution shows that the model F- value of 13.97 implies the model is significant. The value of P less than 0.05 indicates model terms are significant.

Equation of response - $Y1=74.31-2.40X_1-1.61X_2+3.1X_3$

The polynomial equation can be applied to find the conclusion after looking at the magnitude of coefficients and mathematical sign it carries. By looking above equation, it is evident that the factors, concentration of drug (X1), and Stabilizing type (X2) had an antagonistic effect

on the *in vitro* dissolution, which means that, as a concentration of drug increases, the *in vitro* dissolution decrease. The concentration of stabilizing agent (X3) had a synergistic effect on the dissolution, means as the concentration of stabilizer increased, the dissolution increased.

Statistical analysis of response Y2 -Particle size shows that the model F- value of 74.93 implies the model is significant. The value of P less than 0.05 indicates model terms are significant.

Equation of response - $Y_2=4.15+0.5325X_1+0.1500X_2-0.7575X_3$

By looking into the above equation, it concluded that the concentration of the drug (X1) and type of stabilizing agent (X2) have a significant effect on the particle size (Y2). This signified that as the concentration of drug increases, particle size was increased, and the type of stabilizing agent didn't significantly affect the particle size. The concentration of stabilizing agents (X3) has an antagonistic effect on the particle size which revealed that, as the concentration of stabilizing agents increased, particle size decreased.

"The multivariate combination and interaction of independent variables and process parameters are shown to provide quality assurance is called design space". So a numerical optimization technique (desirability function) and a graphical optimization technique (overlay plot) were used to create design space and optimize all the responses (Figure 6). The overlay plot gives regions that do not follow the requirements as grayed out, leaving a yellow color operating window.

The optimal formulation was achieved by imposing constraints on dependent response and independent variables. The constraints for the response, invitro dissolution, and particle size were set between 80%-90% and 2µm-3µm respectively. The optimal region for getting the desired response value was obtained between the range of 0.5 to 0.6 and 0.09-0.1 for X1 and X3 respectively for poloxamer 188. Hence formulation M7 was considered as an optimized formulation as it falls in the yellow portion of the overlay plot, with desirability equal to 1. The optimized formulation had a level of X1=0.5 and X2=0.1 with the predictable response as 82.23% and 2.7µm for invitro dissolution rate and particle size respectively. The observed values of invitro dissolution (84.33%) and particle size (2.57µm) were in close agreement to the model predicted values.

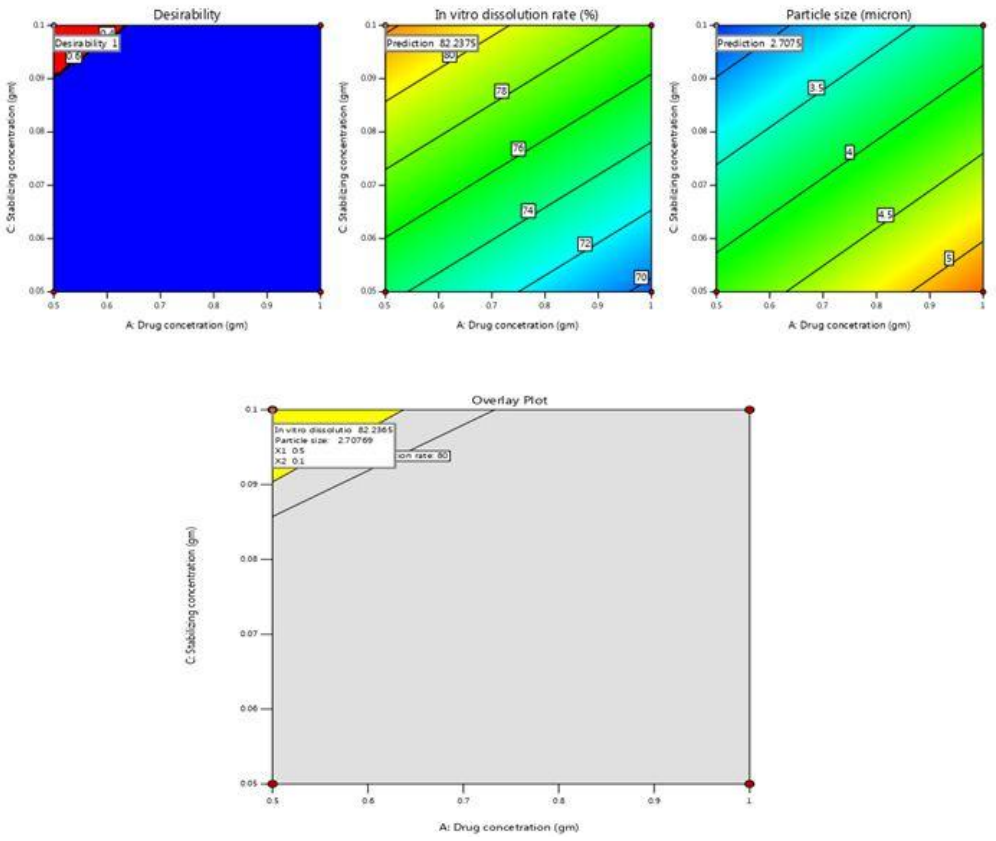

Figure 6: Optimization of *in situ* microcrystals containing poloxamer 188.

EVALUATION OF SUBLINGUAL TABLET FORMULATION

Evaluation of pre-compressed blend

The micromeritics properties of Nebivolol hydrochloride sublingual tablets blend of F1-F8 formulations for bulk density, tapped density, Hausner's ratio, compressibility index, and angle of repose were within the acceptable limit. These results revealed that Nebivolol hydrochloride sublingual tablet powder blends were having a free flowing property with a good compressibility index.

Evaluation of post-compression parameters of sublingual tablets

The tablet's thickness and diameter range were found to be 2.63±0.0288 and 6.06±0.0577 mm respectively. Tablet hardness ranged from 1.83±0.2886 to 2.83±0.2886 Kg/cm^2 for all formulations which are indicative of good mechanical strength. Percent friability ranged from 0.38±0.1400% to 0.54±0.0852% which was within the IP limit. Weight variation was found to be in the range of 98.22±0.0106 mg to 103.70±0.0130 mg which was within the acceptable limit as per IP. Percentage drug content of all formulations was found to be in range of 90.00±0.05% to 98.00±0.05% of Nebivolol hydrochloride which was within acceptable IP limit.

The wetting time and disintegration time for all the formulations were within the range of 10.33±0.57 to 21.33±0.57 seconds and 13.66±0.57 to 40.66±0.57seconds respectively. The results concluded that the wetting time and disintegration period decreased with an increase in super disintegration concentration due to its faster swelling and capillary action in the disruption of the tablet system.

In vitro dissolution profile results revealed that formulation containing crospovidone showed higher drug release as compared to the formulation containing SSG. Among all formulation, F8 containing 7.5% crospovidone showed the highest drug release of 98.57±0.46% within 30 minutes, hence considered as optimal formulation. Here it was also observed that *in vitro* drug dissolution increased with an increased in the concentration of super-disintegrating agents.

Table No 4: Post compression Parameters of sublingual tablet formulation F1-F8.

Formulation	Post compression parameters				
	Thickness (mm)	Diameter (mm)	Friability (%)	Hardness (kg/cm^2)	Wight variation (mg)
F1	2.61±0.0288	6.03±0.0577	0.48±0.0619	2.66±0.5773	98.28±0.0106
F2	2.61±0.0288	6.01±0.0288	0.52±0.1114	1.83±0.2886	102.10±0.0286
F3	2.61±0.0288	6.00±0	0.50±0.4037	2.66±0.5773	99.32±0.0251
F4	2.61±0.0288	6.00±0	0.52±0.1114	1.83±0.2886	99.55±0.0224
F5	2.61±0.0288	6.03±0.0057	0.38±0.1400	2.66±0.5773	100.10±0.0102
F6	2.63±0.0288	6.03±0.0057	0.54±0.0852	2.0±0	99.67±0.0105
F7	2.61±0.0288	6.0±0	0.48±0.0620	2.16±0.2886	103.70±0.0130
F8	2.61±0.0288	6.06±0.0577	0.49±0.0489	2.83±0.2886	101.50±0.0170

Resulted data is an average of triplicates (mean ± SD).

Table No 5: Post Compression parameters of sublingual tablet formulations F1-F8.

Formulations	*In vitro* disintegration Time (sec)	Wetting time (sec)	Drug Content (%)	*In vitro* dissolution profile (%)
F1	14.66±0.57	10.33±0.57	90.00±0.05	64.44±0.24
F2	40.66±0.57	21.33±0.57	91.60±0.17	80.79±0.48
F3	18.66±0.57	17.66±0.57	95.55±0.02	84.52±0.41
F4	17.66±0.57	15.00±0.57	93.88±0.05	92.97±0.25
F5	15.66±0.57	10.66±0.57	98.00±0.05	96.12±0.25
F6	15.66±0.57	14.66±0.57	96.00±0.13	89.12±0.25
F7	13.66±0.57	12.33±0.57	90.00±0.17	96.01±0.25
F8	13.66±0.57	10.33±0.57	93.00±0.05	98.57±0.46

Resulted data is an average of triplicates (mean ± SD).

Comparison of Dissolution profile of F1, F2, and F8 Formulation

The *in vitro* dissolution profile of F8 (optimized), F1 and F2 formulation was compared as shown in Figure 7.

These concluded that the tablet formulation F8 indicates better drug release than F1 formulation. Hence, *in situ* microcrystalline product proved to have a better drug release profile compared to the untreated drug.

It also concluded that F8 indicates better drug release as compared to F2 formulation. Hence, superdisintegrants proved to have a major role in the dissolution.

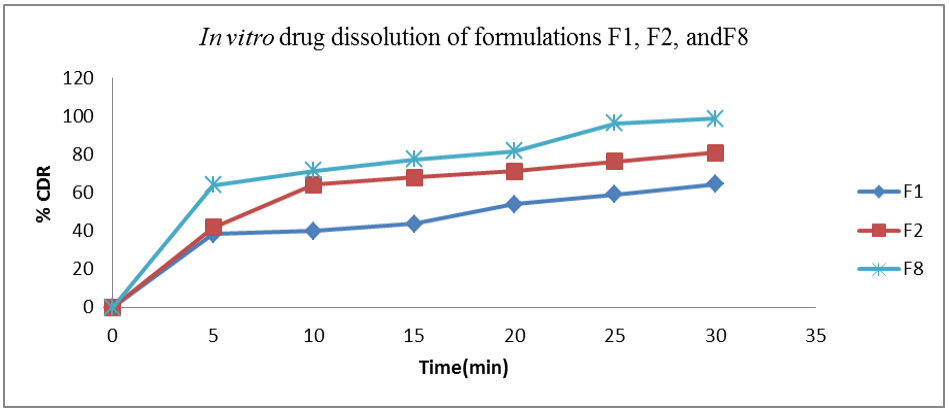

Figure 7: Comparison of *In vitro* drug dissolution (%) of sublingual tablet formulation F1, F2, and F8.

***In vitro* Permeation study**

Sublingual tablets of Nebivolol hydrochloride using *in situ* microcrystals seem to be promising for cautious and effectual delivery by a sublingual route since it showed satisfactory *in vitro* permeation of 82.99% as compared to the sublingual tablet of an untreated drug showed *in vitro* permeation of 69.73% through Franz Diffusion cell.

Stability study

Stability testes of F8 formulation showed no significant change in hardness, drug content, disintegration time, wetting time, and *in vitro* dissolution. Hence it was inferred from these results that formulation F8 was stable and retained its original properties.

CONCLUSION

This study was an attempt to formulate Nebivolol hydrochloride sublingual tablet for hypertension treatment. Based on experimental investigation it can be concluded that the

particle size of the prepared in situ microcrystals range of 2.571 µm to 5.590 µm. *In vitro* dissolution study and particle size determination concluded that the concentration of drug and a stabilizing agent has a significant effect on the particle size. The optimized formulation M7 showed the smallest particle size of 2.571µm and *in vitro* dissolution of 84.30±0.81%.

The sublingual tablets were formulated by incorporating optimized microcrystal formulation M7. Formulation F8 showed the highest *in vitro* drug dissolution of 98.57±0.4% at the end of 30 minutes and a rapid disintegration time of 13.66±0.57 seconds. Formulation F8 exhibits a good wetting time of 10.33±0.05 sounds. Hence formulation F8 containing 7.5% w/w of crospovidone was selected as the optimized formulation. Thus, it can be concluded that the formulation of a sublingual tablet of Nebivolol hydrochloride seems to be a promising formulation for safe and effective delivery via sublingual route for the treatment of hypertension providing faster action.

ACKNOWLEDGMENT

Authors are thankful to UNICHEM Laboratories Limited, Goa for providing Nebivolol hydrochloride drug as a gift sample. Also, the authors are thankful to the Principal of KLE College of Pharmacy, Belagavi for providing Rhamnolipids 90 % from AGAE Technologies, Corvallis, and Shivaji University, Kolhapur for providing laboratory facilities.

Conflict of interest

There are no conflicts of interest.

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