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SOLID DISPERSION INDUCED CO-CRYSTALLIZATION FOR ENHANCED DISSOLUTION RATE OF NEBIVOLOL HYDROCHLORIDE: DEVELOPMENT OF RAPIDLY DISINTEGRATING TABLETS

Ola A. Elfahl*, Ebtessam A. Essa and Gamal M. El Maghraby

Department of Pharmaceutical Technology, College of Pharmacy, Tanta University, Tanta, Egypt.

***Corresponding Author: Ola A. Elfahl**
Department of Pharmaceutical Technology, College of Pharmacy, Tanta University, Tanta, Egypt.

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ABSTRACT

Nebivolol hydrochloride is an antihypertensive agent which is classified as class II drug. Its oral bioavailability is variable due to its poor dissolution and pre-systemic metabolism. The aim of this work was to enhance the dissolution rate of nebivolol hydrochloride both in saliva pH (pH 6.8) and in gastric conditions (pH 1.2). This was done with the goal of developing fast disintegrating tablets with subsequent rapid dissolution. This was achieved by co-grinding of the drug with citric and/or tartaric acid before loading these mixtures in poloxamer by solid dispersion. The resulting dispersion was adsorbed on the solid surface of aerosil. The prepared systems were characterized by Fourier transform infrared spectroscopy, differential scanning calorimetry and X-ray diffraction in addition to dissolution testing. Co-grinding and solid dispersion developed new crystalline structure of co-crystals type. The developed co-crystals liberated nebivolol at significantly higher dissolution rate. Optimum formulations were prepared as rapidly disintegrating tablets. The developed tablets underwent rapid disintegration with subsequent rapid dissolution of nebivolol. This was recorded irrespective to the pH of the dissolution medium. The developed tablets are thus suitable for intra-oral administration and can liberate most of the drug in the mouth for absorption through the buccal cavity. The formulations can also maintain rapid dissolution rate even after swallowing.

KEYWORDS: Nebivolol, solid dispersion, poloxamer 407, citric acid, fast disintegrating tablet.

INTRODUCTION

Nebivolol hydrochloride is an antihypertensive agent which exerts its function by blocking the β1-adrenergic receptor. It has been approved for this purpose in 2007.[1,2] The main advantage of this drug depends on its long-acting characteristics which results from the selective β1-adrenergic blockage in addition to increasing the bioavailability of nitric oxide bioavailability through the L-arginine-nitric oxide pathway.[1,3,4] The solubility and dissolution characteristics of nebivolol hydrochloride categorized the drug as class II according to Bio-pharmaceutical Classification System.[4-7] This together with the first pass metabolism are responsible for the low and variable oral bioavailability which is only 12%.[4,6] The bioavailability can be increased by increasing its solubility and reducing first pass metabolism.[1]

Researchers employed many strategies to overcome the poor dissolution characteristics of drugs. These studies included particle size reduction to reach the micro or nanoscale.[8,9] Changinging the crystalline structure which can be achieved by co-processing with hydrophilic polymers to produce amorphousization, eutectic mixture formation or co-crystallization is another possibility.[10-12] Salt formation and complexation showed promising potential.[13,14] Many trials have been conducted to improve nebivolol hydrochloride oral bioavailability. These attempts utilized complexation with β-cyclodextrin, development of solid lipid nanoparticles and nanofibers or formulation of mucoadesive system. Liquid formulation such as microemulsion was also tried.[2,3,7,15-17] Of these strategies solid dispersion technique is the simplest method for enhancing the dissolution rate of drugs. The benefit will be maximized if the developed system can be formulated as fast disintegrating tablets.

Accordingly, the objective of this work was to enhance the dissolution rate of nebivolol with the goal of developing fast disintegrating tablets which liberate the drug at high rate.

MATERIALS AND METHODS

Materials

Nebivolol hydrochloride was obtained as gift sample from Marcryl Pharmaceutical industries (Eygpt). Methanol and acetonitrile (HPLC grade) were obtained from Fisher Scientific UK, Loughborough, Leics UK. Triethylamine (pharmaceutical grade) was purchased from Alpha Chemika (India). Poloxamer 407, aerosil 200 (BET surface area 200 ±25m^2/g), crosscarmellose sodium, crosspovidone, mannitol, avecil and magnesium stearate were donated by Sigma pharmaceutical company (Quesna, Egypt). Potassium dihydrogen phosphate, potassium hydroxide, citric acid and tartaric acid were obtained from IsoChem (Cairo, Egypt). Water was filtered through Millipore 0.45µm filter before used.

Chromatography

Quantification of nebivolol was achieved using a high pressure liquid chromatographic system obtained from Agilent technologies (1260 infinity, DE, Waldbronn, Germany). A mixture of 0.025M of potassium dihydrogen phosphate buffer (adjusted to pH 3.5), acetonitrile and triethyamine (40:60:0.25). This was used as the mobile phase and was pumped at 1ml/minute into a reversed phase BDS Hypersil, C18 column with an internal diameter of 150 mm×4.6mm and an average particle size of 5µm (Thermo scientific, USA). The samples (30µl) were automatically injected using a TCC 1260 autosampler and the drug content in the effluent was measured at 282 nm, using a variable wavelength UV detector (VWD 1260). The area under the peaks of the eluted drug was estimated using Agilent OpenLAB ChemStation software. The HPLC method was validated with respect to linearity, selectivity, precision and lower limit of detection (LOD) and lower limit of quantification (LOQ).

Preparation of tested formulations

Table 1 presents the composition of the tested formulations. The formulations were prepared with either dry co-grinding or solid dispersion techniques. The later was employed for formulations containing poloxamer which is waxy in nature. Dry co-grinding involved geometric mixing of the solid powders. The mixtures were subjected to co-grinding in the mortar for 15 minutes. Solid dispersions were prepared by fusion method. This was achieved by melting poloxamer and adding nebivolol which was previously co-ground with citric acid and/or tartaric acid (if any). The resulting mixture was cooled while mixing until complete solidification. For formulations containing aerosil, the resulting solid dispersion was co-ground with aerosil to provide flowable powder.

Fourier Transform Infrared (FTIR) spectroscopy

FTIR was employed to monitor any interaction between the drug and the formulation additives. The studies utilized FTIR spectrophotometer (Bruker Tensor 27, Germany). The samples were dry mixed with potassium bromide (spectroscopic grade) and the mixtures were compressed into disks which were mounted in the sample holder. The prepared samples were subjected to FTIR scanning from 4000 to 400 cm^{-1}. The FTIR data was collected and analyzed using Opus IR, FT-IR spectroscopy Software.

Table 1: The compositions of the tested formulations presented as molar ratios.

Formula	Drug	Poloxamer 407	Citric acid	Tartaric acid	Aerosil 200	Preparation method
F1	1	-	1	-	-	Co-grinding
F2	1	-	2	-	-	Co-grinding
F3	1	-	4	-	-	Co-grinding
F4	1	1	-	-	-	Solid Dispersion
F5	1	2	-	-	-	Solid Dispersion
F6	1	2	-	-	1	Solid Dispersion
F7	1	1	1	-	-	Solid Dispersion
F8	1	1	2	-	-	Solid Dispersion
F9	1	1	4	-	-	Solid Dispersion
F10	1	1	-	1	-	Solid Dispersion
F11	1	1	1	1	-	Solid Dispersion
F12	1	1	4	-	1.5	Solid Dispersion
F13	1	1	1	1	0.5	Solid Dispersion

Differential Scanning Calorimetry (DSC)

The thermal behavior of the nebivolol was monitored before and after processing. This utilized a differential scanning calorimeter (DSC 60) obtained from Shimadzu (Kyoto, Japan). Samples equivalent to 3-4 mg of the drug were loaded into aluminium pans. The pans were crimped and mounted in the sample holder in the equipment with empty pan serving as the reference pan. The thermal behavior was monitored while heating the samples at a rate of 10 ^{o}C/minutes to cover the temperature range of 30-400 ^{o}C. This was conducted under nitrogen gas which was pumped continuously at a rate of 20 ml/min. The collected data were analyzed using the TA-60 WS thermal analysis software.

Powder X-ray Diffraction (PXRD)

The X-ray diffraction patterns of the unprocessed nebivolol, the excipients and the resulting formulations were recorded using a GNR APD 2000 pro-X-Ray diffractometer (1.54056 A°) (Agrate Conturbia, Italy). Detection of the diffraction peaks was achieved by a position sensitive detector (super-speed VA° NTEC-1 detector). The samples were scanned at a step size of

0.03° to cover a range of 3 to 65°. The data were collected at ambient temperature.

Dissolution studies

The dissolution studies utilized the USP dissolution apparatus (type II); obtained from Copley (Copley Scientific Dis 6000, Nottingham, United Kingdom). The dissolution medium comprised 850 ml of potassium phosphate buffer (pH 6.8) was maintained at 37 ± 1 °C with the paddle being adjusted to 50 rpm. This medium was used for the first 5 minutes after loading the formulation, at the end of which the pH of the medium was adjusted to 1.2 using 29 ml 5N HCl. This design was employed to mimic the in vivo conditions in which the rapid disintegration will take place in the buccal cavity (pH 6.8) with subsequent swallowing to reach the stomach (pH 1.2). The study evaluated samples equivalent to 20 mg of nebivolol which were loaded into the dissolution vessels and the equipment was operated. Aliquots of the dissolution medium (3ml) were collected periodically (5, 10, 15, 30, 45 and 60 min) and immediately filtered using a 0.45µm Millipore filter before determination of the drug content using HPLC. Drug analysis was conducted after neutralization of the collected samples. The dissolution medium was replenished with fresh medium after each sample to maintain a constant volume. The cumulative amount of drug dissolved was plotted as a function of time to construct the dissolution profiles which were utilized to calculate the dissolution parameters which were used to compare between different formulations. The dissolution parameters included the amount of drug dissolved in the first 5 and 10 minutes (Q5 and Q10, respectively) and the overall dissolution efficiency (DE). DE was computed from the area under the dissolution profile expressed as a percentage relative to the corresponding area assuming 100% dissolution in the same time.[18] Statistical comparison between dissolution profiles employed the similarity factor test which was computed using the following equation.

$$F2 = 50.log\ \{[1+\frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2]^{-0.5}\}.100$$

"Where n is the number of data points, Rt is amount of drug dissolved at time t in case of the control formulation (%) and Tt is amount of drug dissolved (%) from the test formulation at the same time".

Preparation of rapidly disintegrating tablets

The formulation exhibiting the fastest drug release was used to develop rapidly disintegrating tablets with subsequent rapid dissolution of nebivolol. The composition of the prepared tablets is presented in Table 2. Nebivolol was mixed geometrically with other additives, before compression into tablets using a single punch tablet machine (Royal Artist, Kapadia Industrial Estate, BLDG, Mumbai, India). The compression process utilized 12 mm punch with the compression force being adjusted to produce tablets having a hardness of 4–5 Kp.

Table 2: The compositions of the tested tablets presented as weight ratios. Formulation details are presented in Table 1.

Ingredients	Control tablet	F9 Tablet	F10 Tablet	F11 Tablet	F12 Tablet	F13 Tablet
Unprocessed drug	20	-	-	-	-	-
Solid dispersion	-	120	60	80	150	90
Cross povidone	25	25	25	25	25	25
Cross carmellose	25	25	25	25	25	25
Mannitol	50	50	50	50	50	50
Magnesium carbonate	5	5	5	5	5	5
Avicel	375	275	335	315	245	305

The weight of solid dispersion formulation is equivalent to the dose of the drug (20 mg).

Evaluation of the prepared rapidly disintegrating tablets

Uniformity of weight

Samples of the prepared tablets (20 tablets for each formulation) were randomly selected. The weight of each tablet was recorded, the average weight was calculated for each formulation and the deviation of each tablet weight from the average weight was computed. "The tablets are considered acceptable if not more than two tablets are outside the limit (±5% deviation from the average) and none differs by more than twice the limit".

Drug content

The drug content of each of 10 tablets of a randomly selection of 30 tablets was determined. The tablet was powdered and dispersed in methanol to dissolve the drug with aid of sonication. The clear supernatant was suitably diluted with phosphate buffer before determination of the drug content using the HPLC. "The tablets were considered acceptable if they complied with the USP specification in which requires that the nebivolol content of each of at least nine tablets be in the range of 85–115% of the labeled amount. The content of the 10th tablet should not be outside the range of 75-125%. Deviation from these criteria requires quantification of the individual content of the remaining 20 tablets which should be within the limit. The tablets can also fail if any tablet was <75% or >125%".

Disintegration test

This test was conducted using a Copley Scientific disintegration tester (Model: NE4-COP, Nottingham, UK). The test utilized 6 tablets which were loaded in the equipment before recording the time required for tablet disintegration. This was conducted in distilled water which was maintained at 37°C.

Hardness test

The test was conducted on randomly selected sample (10 tablets). The hardness of each tablet was determined using Erweka hardness tester.

Wetting time

This test was conducted according to the published method.[19,20] Distilled water (6ml) was poured into a Petri dish and a filter paper was mounted into it. The tablets were placed on this filter paper. Fine allura red powder was sprinkled on the surface of each tablet. "The time required for a red color to develop on the tablet surface was recorded as the wetting time".

RESULTS AND DISCUSSION

FTIR spectroscopy

The FTIR spectra of nebivolol, poloxamer 407, citric acid, tartaric acid, aerosil and tested formulation are shown in Figures 1 and 2. The FTIR spectrum of pure nebivolol showed the characteristic absorption bands which were seen at 3384 cm^{-1} for N-H stretching, at 3217 cm^{-1} for O–H stretching vibrations, at 2965, 2925 and 2850 cm^{-1} for the C– H stretching vibrations, at 1623 and 1547 cm^{-1} for aryl substituted C=C stretching, at 1494 for NH bending, at 1076 cm^{-1} for the C–N stretching, at 1215 for alcoholic C-O stretching and at 1140 cm^{-1} for the cyclic ether C–O stretching. This spectrum is similar to that published for nebivolol.[21,22] The FTIR spectrum of poloxamer 407 showed the characteristic broad absorption band of the hydroxyl groups at 3485 cm^{-1} and revealed the aliphatic C-H stretching vibration being as two absorption bands at 2971 and 2887 cm^{-1}. The absorption band corresponding to C-O stretching was recorded at 1113 cm^{-1}. This agrees with the published spectrum for poloxamer.[23] The FTIR spectra of citric acid showed the peaks of the principle function groups. The alcoholic OH, central and terminal carboxylic OH groups were recorded as three absorption bands at 3497, 3450, 3293 cm^{-1}, respectively. The existence of resonance in case of carboxylic OH group explains the existence of its absorption bands at lower wave number compared to the alcoholic OH group. The carboxylic C=O was revealed as two absorption bands at 1751 and 1707 cm^{-1}. The dimerization of citric acid was evidenced by recording six intense bands at 3497, 3293, 1751, 1707, 1176 and 1142 cm^{-1}. The recorded spectrum and assignment of peaks correlate with the published data on citric acid.[24,25]

The FTIR spectrum of tartaric acid revealed the hydroxyl groups as bi-forked peak at 3416 cm^{-1}. The carbonyl group was seen at 1741 cm^{-1} with the C-O-C bending vibrations being noticed at 1449, 1393 cm^{-1}. The peaks corresponding to C-O stretching vibrations were detected at 1283 and 1251 cm^{-1}. These findings are in agreement with the published spectrum.[26]

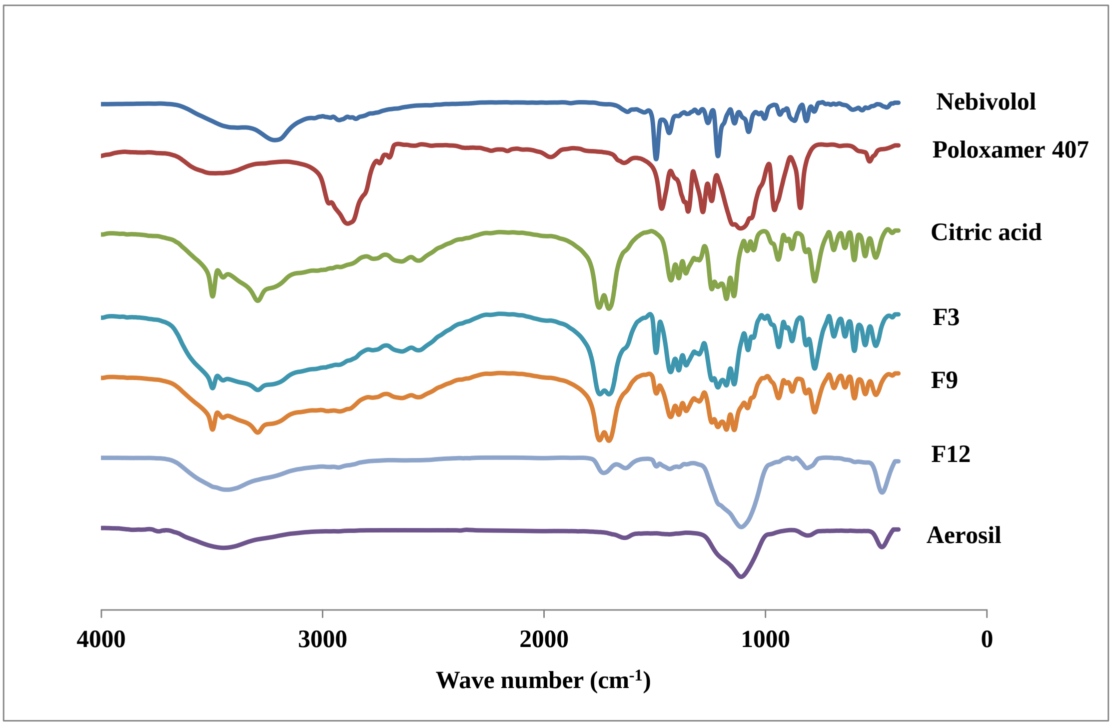

Figure 1: FTIR spectra of nebivolol, poloxamer 407, citric acid, F3, F9, F12 and aerosil 200. Formulation details are in Table 1.

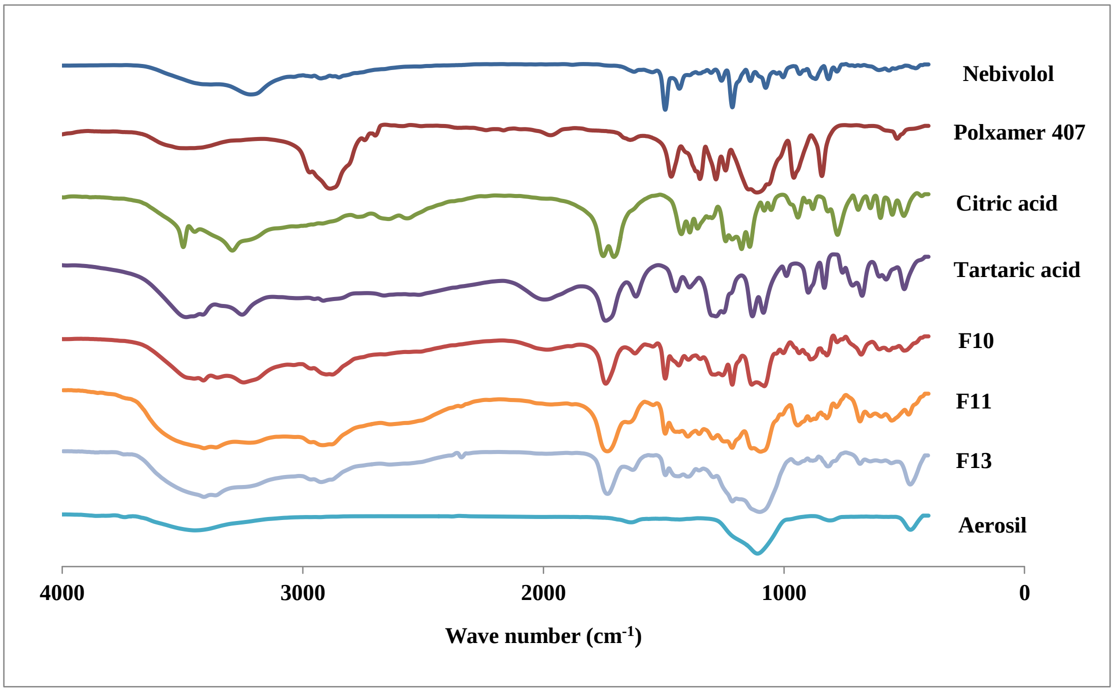

Figure 2: FTIR spectra of nebivolol, poloxamer 407, citric acid, tartaric acid, F10, F11, F13 and aerosil 200. Formulation details are in Table 1.

The FTIR spectrum of aerosil 200 correlated with its functional groups and showed the specific absorption band at 3448 cm^{-1} for the OH group originating from the hydrogen bonding between silica oxygen and water of crystallization or moisture. The peak of H-O-H bending vibration was noticed at around 1637cm^{-1}. The absorption band corresponding to SiO symmetric and asymmetric stretching and SiO bending vibrations were seen at 1110, 810 and 474 cm^{-1}, respectively. This spectrum is similar to that published in other studies.[27,28]

Co-grinding of nebivolol with citric acid produced a powdered mixture showing FTIR spectrum which is not identical to the summation of the spectra of both compounds. The difference was manifested as broadening of the peaks corresponding to the carboxylic C=O groups of citric acid which were recorded at 1751 and 1707 cm^{-1}. The peak corresponding to the N-H bending vibration of the drug (at 1494 cm^{-1}) was also broadened (Figure 1). These changes indicate the existence of hydrogen bonding between citric acid and nebivolol. Incorporating this mixture in poloxamer by fusion method produced solid dispersion system having an FTIR spectrum similar to that recorded with the co-ground mixture but the magnitude of broadening of the absorption band corresponding to N-H bending of nebivolol was greater. This suggests a contribution of poloxamer to the hydrogen bonding process (Figure 1). Adsorbing this solid dispersion on the surface of aerosil intensified the hydrogen bonding process with the peaks corresponding to the carboxylic C=O groups of citric acid showing marked broadening which result in fusion of the 2 absorption band into one broad band at 1731 cm^{-1}. The peak corresponding to the N-H bending of the drug was broadened further. These findings reflect the contribution of the silica to the hydrogen bonding.

Grinding the drug with tartaric acid and formulating the product as solid dispersion with poloxamer resulted in a change in the FTIR of the mixture with the clear change being seen as sharpening of the band corresponding to the carbonyl group of tartaric acid. This effect may be explained on the base that the drug and poloxamer competed with the intermolecular hydrogen bonding of the acid. This was associated with shifting of the peak of OH of tartaric acid (Figure 2). These effects suggest the existence of hydrogen bonding between the components of the mixture. Addition of citric acid to this mixture strengthened the evidence of hydrogen bonding as indicated from the greater broadening of the absorption bands of hydrogen bonding groups. The surprising finding is the fusion of the carbonyl groups of the acids into one absorption band. Adsorption of this formula on aerosil retained the hydrogen bonding capacity (Figure 2).

Differential Scanning Calorimetry (DSC)

Figures 3 and 4 show representative DSC thermograms of nebivolol, excipients and the prepared formulations. The thermodynamic parameters are presented in Table 3. The thermal behavior of pure unprocessed nebivolol was characterized by produced a thermogram with a distinct endothermic peak at 228.8 °C. This peak is attributed to the melting transition of the drug. In addition to the main endothermic peak the thermogram showed broad endotherm in the range of 284-310 °C corresponding to the decomposition of the drug. These results correlate with the published data on nebivolol.[7,16] For poloxamer, the melting transition was shown at 53.8 °C as sharp endothermic peak which agrees with the published work on the same surfactant.[23] The melting transition of citric acid was noticed at 156 °C with its decomposition being revealed as broad endotherm at 209.3 °C (Figure 3), agreeing with the reported thermal behavior for citric acid.[25,29] The thermogram of tartaric acid DSC showed an asymmetric endothermic peak at 215.7 °C which can be attributed to the melting of the acid. The asymmetry and broadness of the transition can be attributed to the existence as DL mixture. In addition, an endotherm was noticed at 76 °C for the adsorbed moisture (Figure 4). This is in agreement with the published literature.[29] The amorphous nature of pure aerosil was reflected by the absence of any thermal events in its thermogram correlating with the specification of the material and agreeing with the published work.[27]

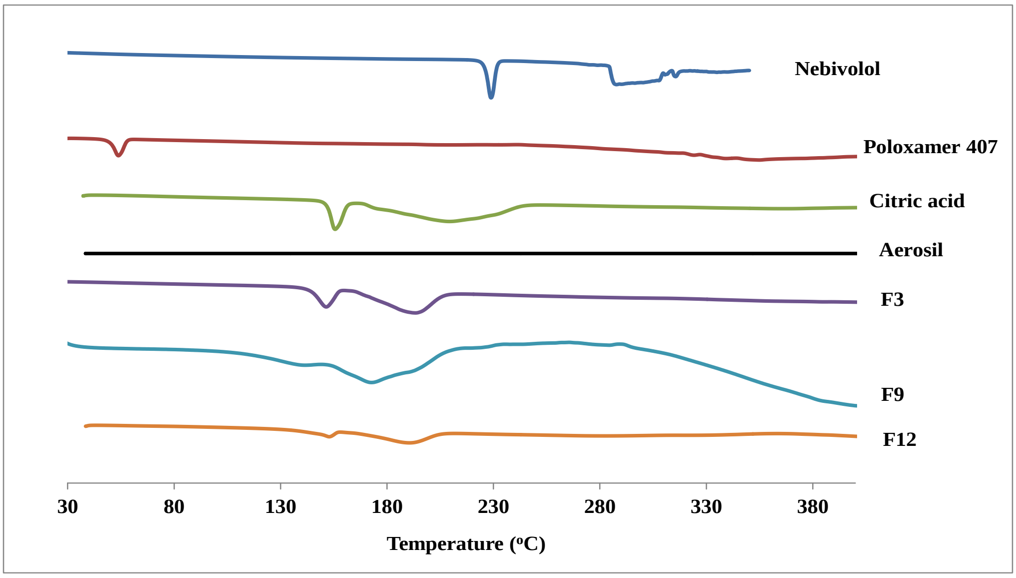

Figure 3: DSC thermograms of nebivolol, poloxamer 407, citric acid, F3, F9, F12 and aerosil 200. Formulation details are in Table 1.

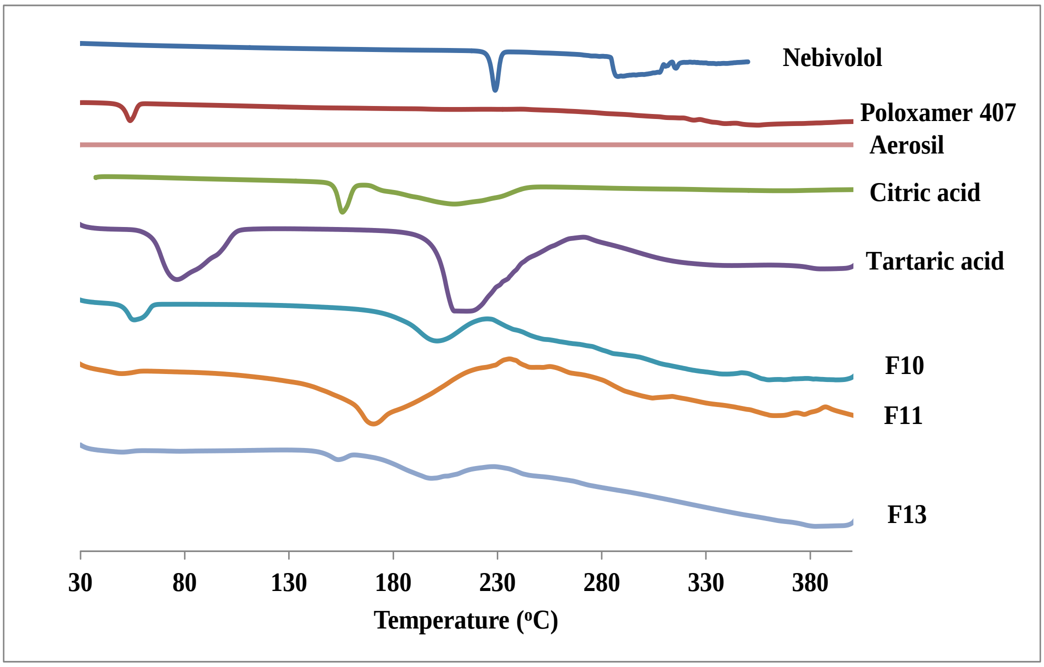

Figure 4: DSC thermograms of nebivolol, poloxamer 407, citric acid, tartaric acid, F10, F11, F13 and aerosil 200. Formulation details are in Table 1.

Table 3: The thermodynamic parameters of nebivolol, poloxamer 407, citric acid, tartaric acid and the tested formulations.

	Onset (^{o}C)	Endset (^{o}C)	T_m(^{o}C)
Nebivolol	224.5 284.6	234.4 310.3	228.8 287.5
Poloxamer 407	50	59	53.8
Citric acid	151 170.6	162 244.5	156 209.3
Tartaric acid	64.4 200.7	103.9 242.7	76.3 215.7
F3	144.2 164.4	158.2 209.8	151.7 193.5
F9	130.8 154.7	149.5 208.4	141.6 172.6
F10	49.8 182	64.7 211.4	55.9 201.1
F11	156.7	196.6	170.6
F12	150.4 168	157.9 206	152.9 190.7
F13	145.3 174.6	159.7 218.9	153.6 198.4

Co-grinding of nebivolol with citric acid produced a powdered mixture in which showed a compromised thermal behavior. The thermogram of this mixture showed the disappearance of the endothermic peak of nebivolol and revealed two endothermic peaks at 151.7 ^{o}C and 193.5 ^{o}C. Those peaks were even different from those of citric acid. This behavior suggests the development of new crystalline species but this suggestion requires confirmation by X-ray diffraction (see below). Incorporation of the co-ground mixture in melted poloxamer resulted in a solid dispersion with further modification of the thermal to reveal asymmetric peak with two apexes at 141.6 and 172.6 ^{o}C. Adsorption of this system on aerosil produced a system with thermal pattern similar to that of the co-ground drug/citric acid mixture (Figure 3 and Table 3).

Grinding the drug with tartaric acid and formulating the product as solid dispersion with poloxamer showed a thermogram characterized by disappearance of main endothermic peak of nebivolol and appearance of new endothermic peaks at 55.9 ^{o}C and 201.1 ^{o}C (Figure 4 and Table 3). Grinding nebivolol, tartaric acid and citric acid then adding this mixture on poloxamer showed a new solid dispersion which has a new asymmetric endothermic peak recorded at 170.6 ^{o}C. Adsorption the previous solid dispersion on aerosil surface releaved a thermogram with new endothermic peaks which were seen at 153.6 and 198.4 ^{o}C. These changes again suggest the development of new crystalline material which requires confirmation by X-ray diffraction.

Powder X-ray diffraction (PXRD)

The recorded X-ray difractograms of nebivolol, poloxamer, citric acid, tartaric acid and tested formulations are shown in Figures 5 and 6. The characteristic diffraction peaks are presented in Table 4. The diffractogram of unprocessed drug revealed its crystalline nature which was reflected from the recorded distinct diffraction peaks at 2θ values of 11.58, 13.02, 14.45, 16.01, 18.12, 18.8, 19.86, 20.06, 21.02, 22.69, 24.68, 25.31^o. This pattern is similar to that reported for nebivolol in literature.[6] The X-ray diffraction pattern of unprocessed poloxamer showed two main diffraction peaks at 2θ values of 19.49 and 23.61^o, correlating with the published data for the same polymer.[23] The crystallinity of citric acid was indicated from its X-ray diffractogram which revealed distinct diffraction peaks at 2θ values 14.36, 16.68, 18.19, 19.66,23.93, 24.1, 25.07, 26.19, 28.08, 29.03, 31.44, 33.85, 35.23, 37.56, 41.19, 43.46, 51.78, 58.08^o, simulating the published pattern.[29,30] The diffractogram of tartaric acid showed diffraction peaks at 2θ values 11.88, 12.72, 19.35, 20.19, 21.12, 23.55, 29.45, 30.43, 31.4, 32.33, 33.62, 37.68, 38.16, 38.58, 41.49, 42.88, 47.99, 48.62, 51.1, 53.12, 54.66^o, agreeing with the published work on the same material.[29]

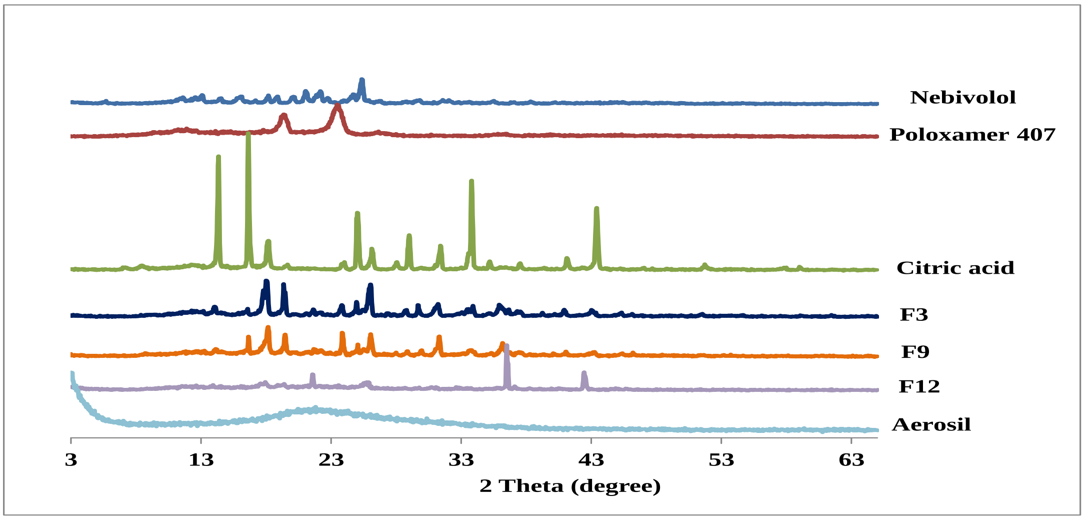

Figure 5: X-ray diffraction pattern of nebivolol, poloxamer 407, citric acid, F3, F9, F12 and aerosil 200. Formulation details are in Table 1.

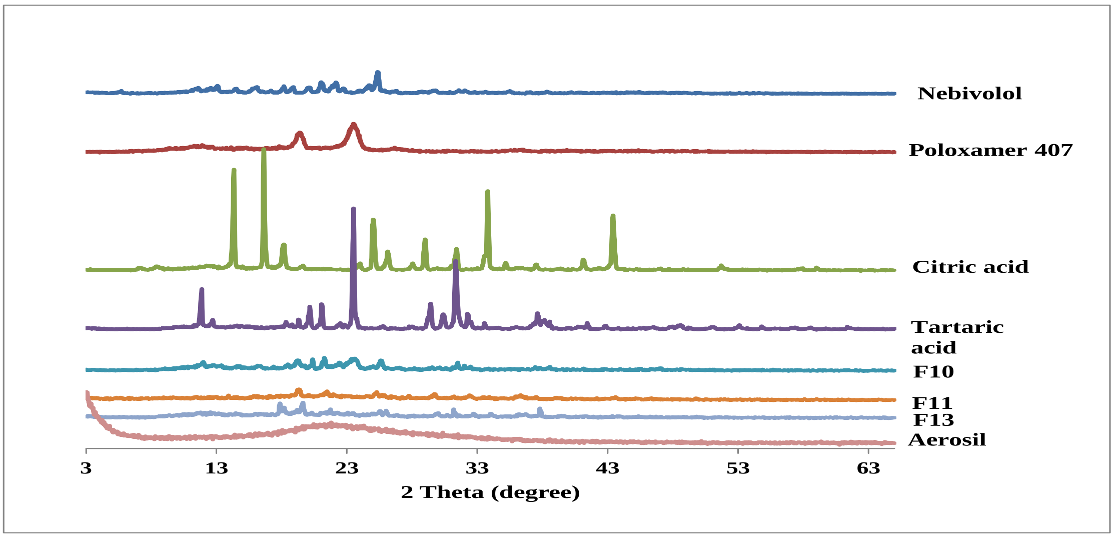

Figure 6: X-ray diffraction pattern of nebivolol, poloxamer 407, citric acid, tartaric acid, F10, F11, F13 and aerosil 200. Formulation details are in Table 1.

Table 4: The characteristic diffraction peaks of nebivolol, poloxamer 407, citric acid, tartaric acid and the tested formulations.

	2 Theta(degree)
Nebivolol	11.58, 13.02, 14.45, 16.01, 18.12, 18.8,19.86, 20.06, 21.02, 22.69, 24.68, 25.31
Poloxamer 407	19.49, 23.61
Citric acid	14.36, 16.68, 18.19, 19.66,23.93, 24.1, 25.07, 26.19, 28.08, 29.03, 31.44, 33.85, 35.23, 37.56, 41.19, 43.46, 51.78, 58.08
Tartaric acid	11.88, 12.72, 19.35, 20.19, 21.12, 23.55, 29.45, 30.43, 31.4, 32.33, 33.62, 37.68, 38.16, 38.58, 41.49, 42.88, 47.99, 48.62, 51.1, 53.12, 54.66
F3	13.95, 16.41, 17.92, 19.28, 21.52, 23.7, 24.85, 25.88, 28.84, 29.6, 31.08, 33.4, 35.88, 37.42, 39.14, 40.81, 42.94, 45.17, 51.39
F9	14.13, 18.13, 19.45, 21.71, 23.88, 25.52, 26.04, 28.85, 29.94, 31.3, 33.69, 36.19, 41.05, 43.21, 46.24
F10	11.88, 19.14, 20.28, 21.18, 25.52, 31.41
F11	21.38,25.21,29.66
F12	17.36, 21.66, 25.9, 36.6, 42.6
F13	17.88, 19.61, 21.7, 25.54, 26.01, 31.23, 37.83

Co-grinding of nebivolol with citric acid produced a powdered mixture showing a diffractogram different from that of the individual components with new peaks appearing at 2θ values of 13.95, 16.41, 17.92, 19.28, 21.52, 23.7, 24.85, 25.88, 28.84, 29.6, 31.08, 33.4, 35.88, 37.42, 39.14, 40.81, 42.94, 45.17, 51.39° (Table 4). Addition this mixture to the melted poloxamer resulted in a solid dispersion which has a different X-ray pattern characterized by appearance of new peaks being noticed at 2θ values 14.13, 18.13, 19.45, 21.71, 23.88, 25.52, 26.04, 28.85, 29.94, 31.3, 33.69, 36.19, 41.05, 43.21, 46.24°. Peaks of excess citric acid were seen at 16.64, 25.04, 31.3° but with lower intensity. Adsorption of this system on aerosil resulted a new product with new peaks recorded at 2 θ values 17.36, 21.66, 25.69, 36.6, 42.6° (Figure 5 and Table 4). This confirms the development of new crystalline species probably of co-crystal type.

Grinding the drug with tartaric acid and formulating the product as solid dispersion with poloxamer showed a diffractogram characterized by appearance of new peaks at 2θ values 11.88, 19.14, 20.28, 21.18, 25.52, 31.41° (Figure 6 and Table 4). It is important to note that the peaks corresponding to poloxamer existed in the diffractogram. Co-grinding of nebivolol with tartaric acid and citric acid then adding this mixture on poloxamer showed a new solid dispersion with different crystalline structure as indicated from the appearance of new peaks which were recorded at 2θ values 21.38, 25.21, 29.66°. The peak corresponding to poloxamer (at 19.49°) was also noticed but with lower intensity. Adsorption the previous solid dispersion on aerosil surface releaved a diffractogram pattern characterized by new peaks which were seen at 2θ values 17.88, 19.61, 21.7, 25.54, 26.01, 31.23, 37.83 ° (Figure 6 and table 4). These findings confirm the development of new crystalline species for nebivolol with tartaric and/or citric acid with the presence of poloxamer and aerosil 200 contributing to the crystalline structure of the product. The recorded changes can be taken as an evidence for co-crystallization. Similar changes have been considered diagnostic for co-crystal formation between other drugs with citric acid, tartaric acid or aerosil.[27,30,31]

Dissolution studies

Figure 7 shows the dissolution profiles of unprocessed nebivolol and the tested formulations. These profiles were used to calculate the amount of drug dissolved in the first 5 min (Q5), 10 min (Q10) in addition to the dissolution efficiency. The calculated dissolution parameters are presented in Table 5. The dissolution profile of pure unprocessed nebivolol revealed slow dissolution at the saliva pH (pH 6.8) and in gastric conditions (pH 1.2) (Figure 7). The drug liberated only 2.5 % of the dose in the first 5 minutes which was monitored in simulated saliva. The amount of drug released reached only 3.6 % 5 minutes after shifting to the gastric conditions with the total amount dissolved in 60 minutes being just 8.7%. The overall dissolution efficiency of unprocessed nebivolol was only 5.5% (Table 5). These values were recorded for such alkaline drug even after monitoring its dissolution in the acidic environment. This can be attributed to the hydrophobic nature of nebivolol. This dissolution pattern is similar to the dissolution behavior for the same drug which was recorded by other investigators.[16,21] Co-grinding of nebivolol with citric acid in different ratio (1:1, 1:2, 1:4) resulted in a statistically significant ($P < 0.05$) increase in the dissolution rate of the drug compared with the unprocessed nebivolol but the amount liberated was lower than that required for rapidly dissolving formulation (Figure 7 and Table 5). For example formulation containing the highest amount of citric acid (1:4 weight ratio, F3) was able to liberate 21.5% and 40.8% of the drug in the first 5 and 10 minutes, respectively with the overall dissolution efficiency reaching 51.6%. Incorporation of those co-ground mixtures in melted poloxamer in the same different ratios formed solid dispersions which significantly increased dissolution rate at least 4 fold more than co-ground mixtures. The enhanced dissolution was reflected in the Q5 and Q10 values which were increased to reach 67.6% and 78.2%, respectively with the dissolution efficiency reaching 70.7% after incorporation of F3 in poloxamer (F9). Adsorption of F9 on aerosil resulted in a new solid dispersion system which dramatically increased % drug dissolved. This effect was seen on both Q5 and Q10 which were reached to 69.9% and 87% with overall dissolution efficiency 79.7%. Successful improving of dissolution was seen for similar hydrophobic basic drugs after solid dispersion formation in presence of acidifiers as citric acid.[32]

Preparation of solid dispersions of nebivolol with poloxamer alone in ratio 1:1/ 1:2 increased dissolution in acidic medium (Q10) and (Q60) but % drug released in first 5 minutes (Q5) (alkaline medium) was not significantly affected ($p > 0.05$). The dissolution enhancement was higher in ratio 1:2 (F5) in which Q10 reach 66.9 with DE 71%. Adsorption of F5 on aerosil developed a new solid dispersion which has dissolution pattern similar to F5. Solid dispersions of poloxamer 407 with other hydrophobic drugs showed the same pattern of dissolution enhancement.[33] These results reflected the need for both the pH modifier in addition to the change in crystalline structure which was imparted after solid dispersion formation.

Co-grinding of nebivolol with tartaric acid then adding this mixture on poloxamer forming a new solid dispersion (F10) that liberated 55% of the labeled amount of nebivolol in the first 5 minutes, 73.2% in the first 10 min and up to 89.2% in 60 minutes with dissolution efficiency 73.5%. Other reports showed successful dissolution enhancement for different basic drugs after solid dispersion formation in presence of acidifiers as tartaric acid.[34] Co-grinding citric acid with tartaric acid and nebivolol then adding co-ground mixture on melted poloxamer resulted in a solid dispersion (F11) which also increased dissolution in both Q5 and Q10 to reach 59.4% and 66% respectively.

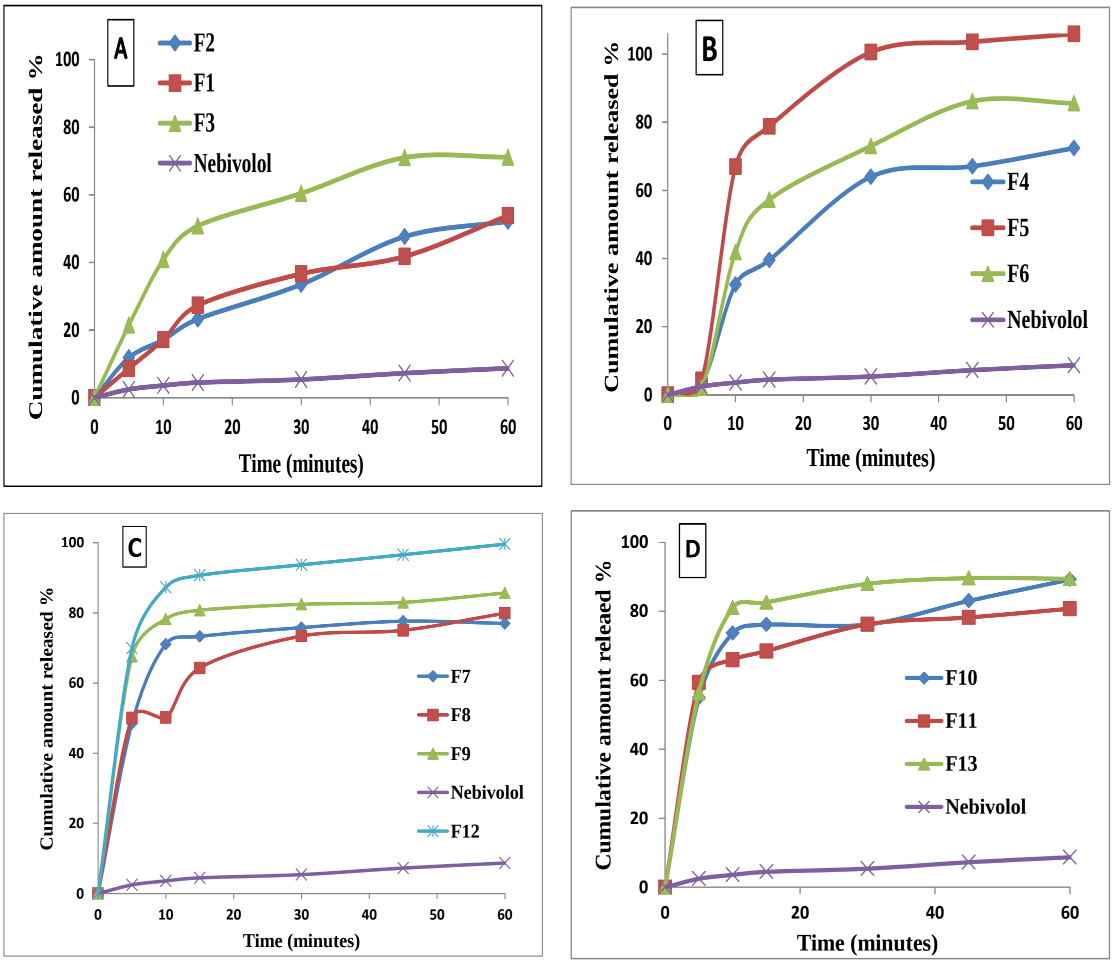

Figure 7: The dissolution profiles of unprocessed nebivolol, the tested formulations. Formulation details are in Table 1.

Dissolution efficiency for this formula was calculated to give 60.8%. Adsorption of this solid dispersion on aerosil surface has a dissolution behavior that was not been dramatically different from F11. Q5, Q10 and Q60 were recorded as 56.3%, 81% and 89.4%. DE was 80.2%.

The similarity factor test was used for comparing between nebivolol dissolution profiles which were recorded with various formulations. This comparison reflected the superiority of all processed formulation over the unprocessed drug.

Table 5: The amount of drug released after 5 minutes (Q5), 10 minutes (Q10), 60 minutes (Q60) and the dissolution efficiency of nebivolol prepared in different formulation.

Formula	Q5min (%)	Q10min (%)	Q60min (%)	Dissolution efficiency (%)
Nebivolol	2.5 (1)	3.6(0.74)	8.7(0.83)	5.5 (0.57)
F1	8.6 (1.8)	17.2 (4.5)	53.9(2.3)	28.8 (2.3)
F2	11.8 (2.6)	17.1 (1.9)	52.1 (3.1)	31.3 (2.4)
F3	21.5 (1.8)	40.8 (5)	71.1 (5.1)	51.6 (4.7)
F4	3.1 (1.9)	32.4 (4)	72.4 (2.7)	42.9 (1.9)
F5	4.3 (0.64)	66.9 (5.8)	105.9 (9.9)	71 (6.2)
F6	2 (0.47)	41.9 (3.3)	85.5 (2.8)	58.3 (2.4)
F7	48.4 (4)	71 (0.9)	77 (4.4)	64.8 (4.1)
F8	50 (8.5)	50.2 (10.4)	80 (4.3)	63.6 (4.6)
F9	67.6 (5)	78.2 (4.6)	85.6 (5.7)	70.7 (2.4)
F10	54.9 (6.6)	73.6 (4.7)	89.2(10.2)	73.5(5.3)
F11	59.4 (8.5)	66 (3.2)	80.7 (1.1)	60.8 (2.1)
F12	69.9 (6)	87 (3.9)	99.6 (2.8)	79.9(1.4)
F13	56.3 (0.37)	81 (4)	89.4 (4.3)	80.2(2.8)

Values between brackets are S.D. (n = 3).

Characterization of fast disintegrating tablets

The weight variation results reflected that the prepared tablets were of uniform weight with the recorded deviation from the average weight being in the range of 0.01-1.7%. Also the tablets were acceptable with respect to their drug content in which the recovered drug was within the specified range. The average tablet hardness for the test tablets was 4.5±0.4kp. This hardness value is suitable for rapidly disintegrating tablets. The prepared tablets showed fast disintegration time values which were in the range of 10-25 seconds for the prepared formulations. The wetting time values were 60±30, 40±20, 56±2, 50±10, 70±20 and 90±30 seconds for the control, F9, F10, F11, F12 and F13 tablets, respectively. Presence of high concentration of superdisintegrants is responsible for rapid disintegration and short wetting time.

To investigate the effect of compression and processing on the dissolution rate of the drug, the dissolution of drug from the prepared tablets was monitored in comparison to its dissolution from the corresponding formulation. The comparison was achieved using the calculated dissolution parameters. A control tablet containing the unprocessed drug was prepared and evaluated. The dissolution profiles of nebivolol from the prepared tablets are shown in Figure 8. These profiles reflect rapid dissolution pattern of nebivolol from the test tablet which included the drug in the form of solid dispersion. The calculated Q5 values were 20.8%, 55.6%, 40%, 53.2%, 53.2% and 57.8% for the control, F9, F10, F11, F12 and F13 tablets, respectively. The calculated Q10 values were 49.8%, 74.6%, 61%, 72.6%, 67.4% and 73.8% for the control, F9, F10, F11, F12 and F13 tablets, respectively. The overall dissolution efficiency values were 52.5%, 67%, 60.7%, 67.5%, 65.2% and 67.8% for the control, F9, F10, F11, F12 and F13 tablets, respectively (Table 6). Interestingly, the dissolution behavior of the control tablet was better than the corresponding unprocessed powder of nebivolol. This can be explained on the base that mixing of the hydrophobic drug with the hydrophilic tablet excipients resulted in adsorption of the drug particles on the large surface area of these excipients. This was subsequently able to enhance the dispersability of the drug within the dissolution medium. Similar behavior was recorded with other hydrophobic drugs.[35,36]

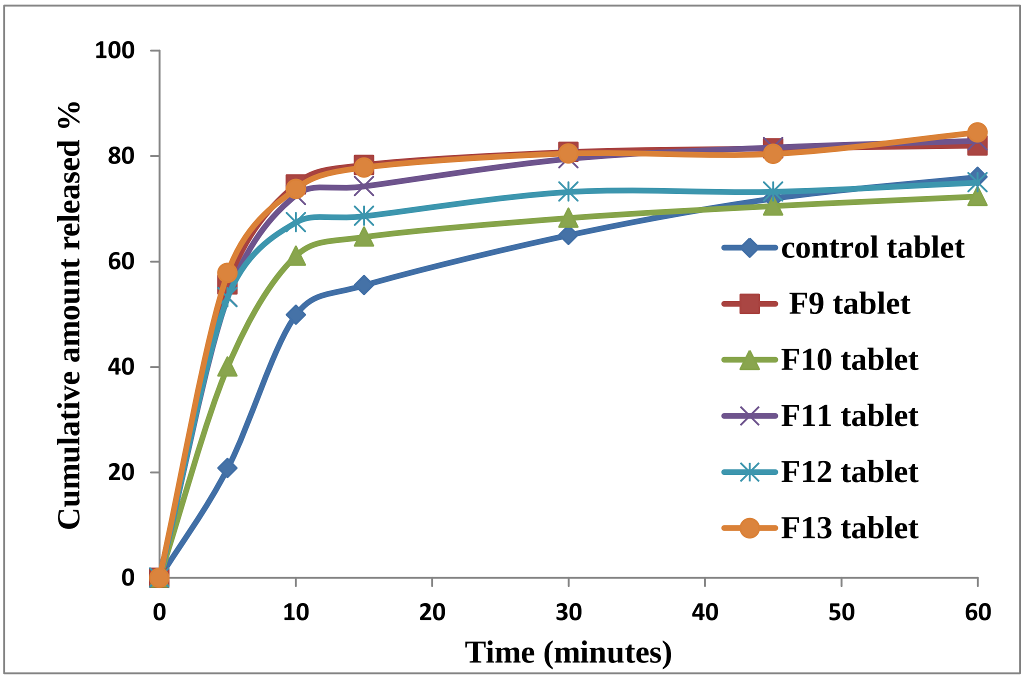

Figure 8: The dissolution profiles of fast disintegrating tablets. The details of tablet formulations are in Table 2.

Table 6: The amount of drug released after 5 minutes (Q5), 10 minutes (Q10), 60 minutes (Q60) and the dissolution efficiency of nebivolol prepared in different tablets.

Tablet	Q5min (%)	Q10min (%)	Q60min (%)	Dissolution efficiency (%)
Control Tablet	20.8 (3.4)	49.8 (7.6)	76 (7.9)	52.7 (4.5)
F9T	55.6 (6.8)	74.6 (5.6)	82 (4.6)	67 (4)
F10 T	40 (5.6)	61 (2.3)	72.3 (1.6)	60.7 (2)
F11 T	53.2 (5.2)	72.6 (5.4)	82.9 (9)	67.5 (6.4)
F12 T	53.2 (4.4)	67.4 (3.1)	75 (2.7)	65.2 (1.9)
F13 T	57.8 (6.3)	73.8 (4.9)	84.5 (6.5)	67.8 (4.7)

Values between brackets are S.D. (n = 3).

CONCLUSION

Solid dispersion of nebivolol with poloxamer in presence of acidifiers as citric acid or/and tartaric acid developed new crystalline structure of co-crystal type. The developed co-crystals liberated the drug at significantly high rate irrespective to the pH of the dissolution medium. The specification of the developed co-crystals was further enhanced after dispersion on the surface of aerosil 200. The developed co-crystals were successfully developed as rapidly disintegrating tablets with subsequent fast dissolution of nebivolol even at the salivary pH.

REFERENCES

1. Narkhede RS, Gujar KN, Gambhire VM. Design and evaluation of self‑nanoemulsifying drug delivery systems for nebivolol Hydrochloride. Asian J Pharm, 2014; 8(3): 200-209.
2. Ustundag-Okur N, Yurdasiper A, Gundogdu E, Gokc EH. Modification of solid lipid nanoparticles loaded with nebivolol hydrochloride for improvement of oral bioavailability in treatment of hypertension: polyethylene glycol versus chitosan oligosaccharide lactate. J Microencapsul, 2016; 33(1): 30-42.
3. Kadam VB, Bamane GS, Raut GS. Solubility Enhancement of Nebivolol Hydrochloride using β-CD Complexation Technique. Int. J. Curr. Pharm. Sci, 2014; 1(1): 6-12.
4. Thadkala K, Sailu C, Aukunuru J. Formulation, optimization and evaluation of oral nanosuspension tablets of nebivolol hydrochloride for enhancement of dissoluton rate. Der Pharmacia Lettre, 2015; 7(3): 71-84.
5. Shirsand SB, Wadageri GV, Raju SA, Kolli G, Reddy PVP. Design and Evaluation of Mucoadhesive Bilayer Buccal Tablets of Nebivolol. RGUHS J Pharm Sci, 2013; 3(1): 40-47.
6. Khutle NM, Vijaya C. Formulation Studies on Novel Self-Solidifying Self-Nanoemulsifying Drug Delivery Systems of Nebivolol Hydrochloride. Pharmaceutical Nanotechnology, 2014; 2(2): 87-100.
7. Sipos E, Szabó ZI, Rédai E, Szabó P, Sebe I, Zelkó R. Preparation and characterization of nanofibrous sheets for enhancedoral dissolution of nebivolol hydrochloride. J Pharm Biomed Anal, 2016; 129: 224-228.
8. Vogt M, Kunath K, Dressman JB. Dissolution enhancement of fenofibrate micronization, cogrinding and spray-drying: comparison with commercial preparations. Eur J Pharm Biopharm, 2008; 68(2): 283-88.
9. Arunkumar N, Deecaraman M, Rani C, Mohanraj KP, Kumar KV. Preparation and solid state characterization of atorvastatin nanosuspensions for enhanced solubility and dissolution. Int J PharmTech Res, 2009; 1(4): 1725-30.
10. Leuner C, Dressman J. Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm Biopharm, 2000; 50(1): 47-60.
11. Gupta MK, Goldman D, Bogner RH, Tseng YC. Enhanced drug dissolution and bulk properties of solid dispersions granulated with a surface adsorbent. Pharm Dev Technol, 2001; 6(4): 563-72.
12. Arafa MF, El-Gizawy SA, Osman MA, El Maghraby GM. Xylitol as a potential co-crystal co-former for enhancing dissolution rate of felodipine: preparation and evaluation of sublingual tablets. Pharm Dev Technol, 2016; 23(5): 454-463.
13. Serajuddin ATM. Salt Formation to Improve Drug Solubility. Adv Drug Deliv Rev, 2007; 59: 603-616.
14. Upadhye SB, Kulkarni SJ, Majumdar S, Avery MA, Gul W, ElSohly MA, Repka MA. Preparation and characterization of inclusion complexes of a hemisuccinate ester prodrug of delta9-tetrahydrocannabinol with modified beta-cyclodextrins. AAPS PharmSciTech, 2010; 11(2): 509-17.
15. Sonali L, Hardik N, Hitesh D, Dharmik M. Development and characterization of fast dissolving muchoadehsive microsphere of nebivolol hydrochloride using modified methacrylate polymer. Int. J. Pharm. Sci, 2013; 3(1): 136-141.
16. Shah I, Bhatt S, Yadav A. Enhancement of Solubility and Dissolution of Nebivolol By Solid Dispersion Technique. Int J Pharm Pharm Sci, 2014; 6(7): 566-571.
17. Nemichand SK, Laxman SD. Solubility Enhancement of Nebivolol by Micro Emulsion Technique. J Young pharm, 2016; 8(4): 356-367.
18. Khan KA. The concept of dissolution efficiency. J Pharm Pharmacol, 1975; 27(1): 48-9.
19. Jain CP, Naruka PS. Formulation and evaluation of fast dissolving tablets of valsartan. Int J Pharm Pharm Sci, 2009; 1(1): 219-26.
20. El Maghraby GM, Elsergany RN. Fast disintegrating tablets of nisoldipine for intra-oral administration. Pharm Dev Technol, 2014; 19(6): 641-50.
21. Avachat A, Raut V. Solubility and dissolution enhancement of Nebivolol hydrochloride using hydrophilic carriers. Asian J Pharm Sci, 2012; 7(5): 337-345.
22. Vijayanand P, Patil JS, Reddy MV. Formulation and comparative pharmacokinetic evaluation of orodispersible tablets and films of nebivolol hydrochloride. J. Pharm. Investig, 2014; 45(2): 237-247.
23. Sultan AA, El- Gizawy SA, Osman MA, El Maghraby GM. Self dispersing mixed micelles forming systems for enhancing dissolution and intestinal permeability of hydrochlorothiazide. Colloids Surf B Biointerfaces, 2017; 149: 206-216.
24. Bichara LC, Lanús HE, Ferrer EG, Gramajo MB, Brandán SA. Vibrational Study and Force Field of the Citric Acid Dimer Based on the SQM Methodology. Adv Phys Chem, 2011; 2011: 1-10.

25. Lin LH, Hsu PC, Lin SY. Theophylline–citric acid co-crystals easily induced by DSC–FTIR microspectroscopy or different storage conditions. Asian J Pharm Sci, 2013; 8(1): 19-27.
26. Sodanapalli R, Nair R, Bachala T. Preparation and Pharmaceutical Characterization of Supra molecular Complex of Isoniazid with L (+) Tartaric acid. J Biomed Sci and Res, 2011; 3(2): 397-402.
27. El-Gizawy SA, Osman MA, Arafa MF, El Maghraby GM. Aerosil as a novel co-crystal co-former for improving the dissoluteion rate of hydrochlorothiazide. Int J Pharm, 2015; 478(2): 773-778.
28. Fayed NF, Osman MA, El Maghraby GM. Enhancement of dissolution rate and intestinal stability of candesartan cilexitil. J App Pharm Sci, 2016; 6(2): 102-111.
29. Ali AM, Ali AA, Maghrabi IA. Clozapine-carboxylic acid plasticized co-amorphous dispersions: Preparation, characterization and solution stability evaluation. Acta Pharm, 2015; 65(2): 133-46.
30. Mukaida M, Watanabe Y, Sugano K, Terada K. Identification and physicochemical characterization of caffine-citric acid co crystal polymorphs. Eur J Pharm Sci, 2015; 79: 61-66.
31. Xuejuan S, Jing G, Ziling L, Jianjun Z, Yuan G. Enhanced dissolution of famotidine by cocrystal formation with tartaric acid and maleic acid. JCPU, 2013; 44(2): 124-129.
32. Shim JB, Lee JK, Jo H, Hwang JH, Jeong SM, Jo JI, Lee D, Yuk SH, Khang G. Effect of Acidifier on the Dissolution Property of a Solid Dispersion of Raloxifene HCl. MACROMOL RES, 2013; 21(1): 42-48.
33. Medarevic DP, Kachrimanis K, Mitric M, Djuris J, Djuric Z, Ibric S. Dissolution rate enhancement and physicochemical characterization of carbamazepine-poloxamer solid dispersions. Pharm Dev Technol, 2015; 21(3): 268-76.
34. Kojo Y, Matsunaga S, Suzuki H, Taniguchi C, Kawabata Y, Wada K, Yamauchi Y, Seto Y, Sato H, Onoue S. Improved Dissolution of Dipyridamole with the Combination of pH-Modifier and Solid Dispersion Technology. Chem Pharm Bull, 2017; 65(5): 426-431.
35. Essa EA, Elmarakby AO, Donia AMA, El Maghraby GM. Controlled precipitation for enhanced dissolution rate of flurbiprofen: development of rapidly disintegrating tablets. Drug Dev Ind Pharm, 2017; 43(9): 1430-1439.
36. Arafa MF, El-Gizawy SA, Osman MA, El Maghraby GM. Sucralose as co-crystal co-former for hydrochlorothiazide: development of oral disintegrating tablets: Drug Dev Ind Pharm, 2016; 42(8): 1225-33.