Journal of Pharmaceutical and Biomedical Analysis 129 (2016) 224–228

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Preparation and characterization of nanofibrous sheets for enhanced oral dissolution of nebivolol hydrochloride

Emese Sipos[a], Zoltán István Szabó[a], Emőke Rédai[b], Péter Szabó[c,d], István Sebe[c], Romána Zelkó[c,*]

[a] Department of Pharmaceutical Industry and Management, University of Medicine and Pharmacy of Tîrgu Mureș, R-540139, Marinescu Street 38, Tirgu Mureș, Romania
[b] Department of Pharmaceutical Technology, University of Medicine and Pharmacy of Tîrgu Mureș, R-540139, Marinescu Street 38, Tirgu Mureș, Romania
[c] University Pharmacy Department of Pharmacy Administration, Semmelweis University, Hőgyes Endre Str. 7-9, H-1092, Budapest, Hungary
[d] Gedeon Richter Plc., Formulation R&D, Gyömrői Str. 19-21, H-1103 Budapest, Hungary

ARTICLE INFO

Article history:
Received 28 April 2016
Received in revised form 1 July 2016
Accepted 4 July 2016
Available online 5 July 2016

Keywords:
Nebivolol hydrochloride
Electrospinning
Nanofibers
Positron annihilation lifetime spectroscopy (PALS)
Differential scanning calorimetry (DSC)
Dissolution enhancement

ABSTRACT

Nebivolol-loaded electrospun nanofibrous sheets were prepared for the dissolution enhancement of the active with the aim of improving its oral bioavailability. Physicochemical characterization of nanofibers including differential scanning calorimetry, attenuated total reflectance Fourier transform infrared spectroscopy and positron annihilation lifetime spectroscopy were carried out in order to track the physicochemical changes related to the electrospinning process. The obtained results unanimously indicated the amorphous transition of nebivolol as a result of electrospinning, furthermore supramolecular ordering of chains of polyvinyl alcohol matrix could be revealed by positron annihilation lifetime spectroscopy. The crystalline-amorphous conversion of the active, along with the increased specific surface area of the nanofibers enabled rapid and complete dissolution. More than twice amount of active released from the fibrous sheets than from the commercial tablets. In contrast to the control tablets, the dissolution was complete and was not influenced by the pH of the applied media.

1. Introduction

Nebivolol (NEB) is a third-generation lipohilic beta(1)-selective beta-blocker used in the treatment of hypertension. In clinical studies preliminary evidence showed good efficacy and tolerability and suggested a potential for reduced mortality in patients with heart failure [1]. In addition to these advantageous properties, nebivolol protects elderly hypertensive patients from orthostatic complaints [2]. NEB has low water solubility and high membrane permeability therefore it is included in Class 2 of the Biopharmaceutical Drug Classification System. Since the drug has other drawbacks, like extensive first pass metabolism, gastric irritancy as well as longer half-life, it could be a promising candidate for fast dissolving mucoadhesive dosage forms [3], the formulation of mucoadhesive sheets could be of relevance in increasing its oral bioavailability.

Recently, several studies appeared which aimed at the improvement of the drug release with different formulation techniques, like self-nanoemulsifying drug delivery [4], nanoparticulate delivery system using carrier Eudragit® RS100 [5] or quick dissolving film using three hetero polymers [6]. Another promising way to increase the solubility of poorly soluble active substances is their incorporation into polymer nanofibers. Electrospinning is certainly one of the most popular fiber forming techniques owing to its versatility and ease of applicability. In the course of electrospinning high voltage is applied to the polymeric sample (melts, solutions or suspension) filled into a syringe equipped with a needle. If the applied voltage overcomes the surface tension of the sample droplet at the end of the needle, the droplet transforms into the so-called Taylor cone, and jets are ejected towards the target plate. The quality of the prepared fibers depends on a myriad of process parameters, and their proper selections and adjustment enable the fine tailoring of the fibers [7]. Because of the high surface area generated by electrospinning, the evaporation rate of the solvent is high, allowing for more efficient drying at ambient temperatures than that of film casting. In addition, no heat is necessary to blend ingredients during electrospinning, as they are already well blended in the liquid solution prior to spinning, making it more applicable for heat-sensitive drugs. In electrospinning, fibers of 20–2000 nm diameter are produced from a solution of a polymer, solvent and any desired additives [8].

* Corresponding author.
E-mail address: zelko.romana@pharma.semmelweis-univ.hu (R. Zelkó).

http://dx.doi.org/10.1016/j.jpba.2016.07.004
0731-7085/

The high specific-area-to-volume ratio, high porosity and the possibility of controlling the crystalline-amorphous phase transitions of the loaded drugs, make them a desirable formulation pathway to satisfy the needs of modern pharmaceutical development [9].

The primary aim of the present paper was to formulate NEB-loaded polyvinyl alcohol nanofiber sheets with electrospinning technique intended as a fast dissolving dosage form and to explore the physicochemical characteristics of the system in relation to the dissolution enhancement of the drug.

2. Materials and methods

2.1. Materials

NEB (as a hydrochloride salt) was a kind gift of Nivika Chemo Pharma PvT, India. Poly(vinyl-alcohol) (PVA) (Emprove® 18-88), hydrochloric acid (35%), and Polysorbate 60 were from Merck KGaA, Germany. Potassium dihydrogen phosphate was from Chemical (Romania). Sodium hydroxide was from Lach Ner (Czech Republic).

2.2. Preparation of drug stock solution

NEB stock solution was prepared by dissolving 0.1 g NEB hydrochloride in 20 g of water in the presence of 0.8 g Tween 60. To this solution 3 g of PVA 18-88 was added. The sample was heated for one hour at 80–90 °C until clear and homogeneous viscous solution was formed.

2.3. Preparation of nanofibrous sheets by electrospinning

Drug loaded nanofibrous sheets were prepared by electrospinning technique. The applied parameters were as follows: flow rate of 0.3 mL/h (using an Alaris GH infusion pump). The syringe was connected to a 100 Sterican injection needle through a silicon tube. Fibers were collected after a 15 cm flight on a grounded square static sheet of 625 cm^2 in area covered with an aluminum foil. Sheets with uniform diameter and thickness were obtained during 10 min continuous operation of the electrospinning apparatus. The applied voltage was 25 kV.

2.4. Morphology investigation by scanning electron microscopy (SEM)

Morphology of fibers was observed using a scanning electron microscopy (SEM) type JEOL 6380LVa (JEOL, Tokyo, Japan) after gold coating. The short parts of samples were fixed by conductive carbon adhesive tape. The applied accelerating high voltage and working distance were 10–20 kV and 10–12 mm. The average diameters of fibers were measured using 50 different randomly selected individual filaments.

2.5. Positron annihilation lifetime spectroscopy (PALS)

Positron annihilation lifetime spectroscopy (PALS) is a unique method since it is exceptionally sensitive to the free volume. PALS is capable for the characterization of various samples of different consistency, such as solids, semisolids and liquids. The number and extent of the lifetime values are characteristic for different samples. Polymers which are widely used in pharmaceutics have 3–5 lifetime values in the range of 0.1–3 ns. Most of the pharmaceutically relevant materials have 2–4 lifetime values, and generally, the greatest lifetime value relates to the lifetime of *ortho*-positronium (o-Ps). In polymers the formed o-Ps particles tend to be trapped in free volume holes and their annihilation is not governed by their intrinsic lifetime but by the electron density in the holes. Their lifetime is associated with the size of the free volume around them [10]:

$$\tau = \frac{1}{2}\left[1 - \frac{R}{R+\Delta R} + \frac{1}{2\pi}\sin\left(\frac{2\pi R}{R+\Delta R}\right)\right]^{-1} \quad (1)$$

where τ is the positronium lifetime, R the radius of the free volume hole, and ΔR a constant. As a very crude guess, we can say that a longer lifetime indicates a larger hole.

For positron lifetime measurements, a positron source made of carrier-free ^{22}NaCl was used. Its activity was around 10^6 Bq. Lifetime spectra were measured with a coincidence system based on BaF_2/XP2020Q detectors and Ortec® electronics (Oak Ridge, Tennessee, US). Every spectrum was recorded in 4096 (memory offset: 512) channels of an analyzer card for 3700 s and each contained about 1.5×10^6 coincidence events. Three parallel spectra were measured at each concentration to increase reliability. After summarizing the parallels, spectra were evaluated by the RESOLUTION computer code; the indicated errors are the deviations of the lifetime parameters obtained. Three lifetime components were found in all the samples [11,12].

2.6. ATR-FTIR spectroscopic examinations

ATR-FTIR spectra were collected on Jasco FT/IR-4200 spectrophotometer between 4000 and 400 cm^{-1} with an ATR PRO470-H single reflection accessory (Jasco) equipped with flat pressure tip. The spectral measurements were performed in absorbance mode. 50 scans at a resolution of 2 cm^{-1} were co-added by the FT-IR software (Spectra Manager-II, Jasco).

2.7. Differential scanning calorimetry (DSC)

Thermograms of NEB hydrochloride, PVA and nanofiber with and without drug were recorded on a Shimadzu Thermo Analyzer DSC 60 equipment. All samples were accurately weighted into aluminum pans and scanned at a heating rate of 5 °C/min over a temperature range of 30–300 °C.

2.8. Determination of drug content of NEB loaded microfibers

Drug content of the nanofibers were determined using a Dionex Ultimate 3000 system (Dionex, Olten, Switzerland) according to the liquid chromatographic (LC) method described in our earlier studies [13]. Fibers produced from six consecutive runs were averaged by folding and six samples were cut (~15 mg nanofiber), dissolved in 10 mL 0.1 N hydrochloric acid and assayed for drug content on the basis of a calibration curve.

2.9. Small-volume comparative dissolution testing

Small volume, comparative dissolution studies were performed in an in-house assembled dissolution setup, which consisted of 2.7 cm i.d × 11.5 cm glass tubes immersed in a water bath maintained at 37 ± 1 °C, using an Erweka ET 1500I immersion thermostat (Erweka GmbH, Heusenstamm, Germany). Stirring was achieved at 200 rpm by 10 mm × 2 mm teflon-coated stir bars, using JK SMS HS magnetic stirrers (JKI, Shanghai, China), placed under the water bath. Dissolution tests were performed in two different media: 30 mL solution of hydrochloric acid of pH 1.0 (Ph. Eur. 8) and 30 mL phosphate buffer pH 6.8 (Ph. Eur. 8). Samples of 0.7 mL were taken at predefined intervals (5, 10, 15, 20, 30, 45, 60 min), filtered and assayed using our liquid chromatographic method described earlier. Dissolution tests were run on quarter-tablets (commercial NEB 5 mg tablets; nominal concentration of a quarter-tablet: 1.25 mg