European Journal of Pharmaceutics and Biopharmaceutics xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Effect of amphiphilic graft co-polymer-carrier on physical stability of bosentan nanocomposite: Assessment of solubility, dissolution and bioavailability

Prakash N. Kendre [a,b,*], Pravin D. Chaudhari [c]

[a] SRES' Sanjivani College of Pharmaceutical Education & Research Kopargaon, Ahmednagar, Maharashtra 423 603, India
[b] J.N.T. University, Hyderabad, 500 085, India
[c] PES' Modern College of Pharmacy, Yamunanagar, Sector 21, Nigdi, Pune, Maharashtra 411 044, India

ARTICLE INFO

Article history:
Received 9 December 2016
Revised 17 March 2017
Accepted in revised form 23 June 2017
Available online xxxx

Keywords:
Graft co-polymer
Soluplus®
Nanocomposite
Bosentan
Solubility and bioavailability

ABSTRACT

Bosentan is a dual endothelin receptor antagonist used in the treatment of pulmonary arterial hypertension (PAH). But the solubility and bioavailability of this drug are poor, which has restricted the design and development of dosage forms for efficient and successful therapy. The present study was carried out to develop nanocomposites using an amphiphilic graft co-polymer (Soluplus®) as a carrier to enhance the solubility and bioavailability of bosentan. The graft co-polymer-based nanocomposite formulation was prepared using the single-emulsion technique. The nanocomposite was characterised in terms of particle size analysis, solubility, percentage entrapment efficiency, drug-loading capacity, surface morphology, drug content, in vitro dissolution, stability and bioavailability. FT-IR study revealed that there was no interaction between the drug and Soluplus®. DSC analysis of the nanocomposite formulation confirmed that the bosentan was completely encapsulated within a Soluplus®. XRD analysis showed that the drug was converted to an amorphous form irreversibly. SEM images showed that the particles were of size 96–129 μm and had slightly smooth to rough textured surface. TEM analysis indicated that the diameters of the prepared bosentan nanocomposite after dispersion in distilled water were 13.69–96.78 nm. Statistically significant increases in the solubility, dissolution and bioavailability of the drug were observed. It was confirmed that the use of a graft co-polymer carrier-based nanocomposite formulation is a good approach for efficient delivery of bosentan, the solubility and bioavailability being increased manifold.

1. Introduction

Recent developments in combinatorial chemistry, high-throughput screening (HTS), biology and genetics have led to the synthesis of many drug candidates, but the need to address practical problems has also increased proportionately. The development of new dosage forms of most of the available drugs has been restricted due to their poor solubility, which is the rate-limiting step for bioavailability [1]. The use of a scientific framework for classifying drugs on the basis of their solubility and permeability has become popular; such frameworks are referred to as biopharmaceutical classification systems [2].

It has been proved that the problem of poor bioavailability does not arise with any drug candidate with solubility greater than 10 mg/ml at the physiological pH of 7. In contrast, there are bioavailability problems with drug candidates with aqueous solubility less than 1 mg/ml. This indicates the prime importance of solubility in relation to bioavailability [3].

Poor solubility of drugs has been addressed through the development of new drug delivery approaches such as reducing the particle size of the drug, micronisation [4], inclusion complexation [5,6], salt formation [7], pH adjustment [8], co-solvency [9–11], the liquisolid technique [12], supercritical fluid technology [13] and the use of self-emulsifying micro-emulsion systems [14].

Bosentan is a dual endothelin receptor antagonist used in the treatment of pulmonary arterial hypertension (PAH). It has an elimination half-life of 5 h. Because of the poor solubility of bosentan, it is classified as a BCS Class II. It is insoluble in water and in aqueous buffer solutions with pH values in the range 1–5. The solubility of bosentan increases to 0.43 mg/ml at pH 7.5. It is freely soluble in dichloromethane, acetonitrile and chloroform and is soluble in ethanol.

* Corresponding author at: Sanjivani College of Pharmaceutical Education & Research Kopargaon, Ahmednagar, Maharashtra 423 603, India.
E-mail address: prakashkendre@gmail.com (P.N. Kendre).

http://dx.doi.org/10.1016/j.ejpb.2017.06.024
0939-6411/

The present study involved the development of a Soluplus® (polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol graft co-polymer)-based nanocomposite formulation using the single-emulsion technique, followed by lyophilisation, which results in a stable, free-flowing, powdered nanocomposite containing bosentan. Soluplus® is an amphiphilic graft co-polymer specially designed and manufactured by BASF Corporation to enhance the solubility of poorly soluble drugs. Because of its bifunctional behaviour, it is expected to serve as an excellent matrix for dissolving drugs in aqueous media [15,16]. Characterisation of the nanocomposite formulation developed was carried out in terms of particle size, solubility, percent entrapment efficiency, drug-loading capacity, surface morphology, drug content, in vitro dissolution, stability and bioavailability.

2. Materials and methods

2.1. Materials

Bosentan was obtained as a gift sample from Dr. Reddy's Laboratories Pvt. Ltd., Hyderabad, India. Soluplus® was gifted by BASF Corporation, Mumbai, Maharashtra, India. All other excipients and reagents were obtained from the Central Chemical Store of Sanjivani College of Pharmaceutical Education and Research, Kopargaon, and they were of analytical grade.

2.2. Methods

2.2.1. Preparation of nanocomposites of bosentan

A nanoemulsion of bosentan and Soluplus® was prepared using the single-emulsion technique. Two phases (organic and aqueous phases) were prepared, and a surfactant was added. In the first step, Soluplus® was dissolved in 5 ml of dichloromethane. Mixing was carried out until a clear, homogenous mixture was formed. In the second step, the drug was dissolved completely in the same solvent, 5 ml of dichloromethane. The two phases were mixed and stirred until a uniform mixture, the organic phase, was formed. The drug-to-Soluplus® ratio was maintained at 1:1, 1:2, 1:3, 1:4 and 1:5 (five batches were prepared as BNC1 to BNC5 respectively). An aqueous phase was prepared by adding 0.05% TWEEN® 80 in 20 ml of distilled water. The aqueous phase was kept in an ice bath, and the organic phase was added drop-wise (approximately 0.1–0.3 ml/minute) to the aqueous phase using a syringe pump. A probe sonicator (PKS-750, PCi Analytics, Mumbai, India) was used to homogenise the two phases effectively. The resultant mixture was stirred continuously for 24 h to remove the dichloromethane, and the remaining part of the mixture was lyophilised at −40 °C for 12 h. The lyophilised mass was ground to obtain a free-flowing powder, which was sifted through a 100 μm sieve and kept in a desiccator until further use. Physical mixtures of bosentan and Soluplus® (Bose-PM) were prepared by gentle mixing in a mortar and pestle. Mixtures were prepared with the same ratios used to prepare the bosentan–Soluplus® nanocomposites (Bose-NC) [17].

2.2.2. Solubility study

A phase solubility study was conducted to predict the maximum concentration of Soluplus® that will help increase the solubility of bosentan. The shaking flask method of Higuchi and Connors was used to determine the solubility [18]. An excess amount of the drug was placed in 25 ml volumetric flasks containing 1–5% w/v Soluplus® solutions. These samples were sonicated for 20 min and placed in a thermo-stable orbital flask shaker at 37 ± 0.5 °C for 48 h (Dolphine, Nashik). The samples were centrifuged at 12,000 rpm for 20 min, and the supernatants were filtered through Whatman filter paper (0.45 μm pore size). The concentrations of bosentan (in mg/ml) were determined using a UV spectrophotometer (1650 PC, Shimadzu, Japan) at 272 nm.

In order to obtain a suitable concentration of Soluplus® for solubilisation of bosentan, ΔG°tr values were calculated using the Gibbs–Helmholtz equation [19a]:

$$\Delta G\ tr = 2.303RT\log\left(\frac{S0}{Ss}\right), \quad (1)$$

where S_0/Ss is the ratio of the molar solubilities of bosentan before and after treatment with Soluplus®. R and T are the gas constant (8.31 J K^{-1}) and temperature (in degrees Kelvin), respectively. The negative Gibbs free-energy value obtained using above equation indicates better solubilisation. The solubilities of the plain drug, physical mixtures and nanocomposite were determined using shake flask method as described in earlier section. Excess amounts of the plain drug, physical mixtures and nanocomposite were added to water and buffered solvents of different pH values (1.2 and 6.8) to determine the solubilities. The results were converted to units of mg/ml, and ANOVA (analysis of variance) was used to check for statistically significant differences among the samples [20].

2.2.3. Fourier Transform Infrared Spectroscopy (FT-IR)

The interaction between bosentan and Soluplus® was studied using an FT-IR spectrophotometer (Shimadzu, Japan). Spectra were recorded in the range 4000–400 cm^{-1}. All the samples were prepared using the KBr pellet technique, and the spectra were recorded at a resolution of 0.15 cm^{-1} and a scanning speed of 20 scan/s.

2.2.4. Differential scanning Calorimetry (DSC)

The thermal properties of the drug and the nanocomposite were determined using a differential scanning calorimeter (Mettler, Switzerland). Samples were heated at a rate of 10 °C/min from ambient temperature to their melting points. The study was carried out at R.C. Patel College of Pharmacy, Shirpur, India.

2.2.5. X-ray diffraction (XRD)

Various XRD spectra/patterns were recorded using a Philips PW3710 analytical X-ray diffractometer to confirm the change in the basic form of the drug (crystalline or amorphous) after formulation of the final dosage form. Samples (2 mg) were heated in hermetically sealed aluminium pans from 40 °C to 300 °C at a constant rate of 10 °C/minute. To maintain an inert atmosphere, nitrogen was purged at a flow rate of 20 ml/min.

2.2.6. Surface morphological study by scanning Electron microscopy (SEM)

The surface properties were observed and evaluated using SEM. The changes in the surface morphology of the resultant solid dispersion after encapsulation into Soluplus® were studied using this higher-resolution scanning electron microscopy. The samples were covered with double-sided sticking tape and placed on aluminium stubs. They were sealed and coated with gold ions (200 A°) under low pressure for 5 min using a sputtering device.

2.2.7. Transmission Electron microscopy

After dilution of bosentan nanocomposite more than 200 times in simulated GI fluid, the physical appearance was checked using TEM. Very little smear of sample was mounted on a copper grid which previously coated with carbon and analysed.

2.2.8. Particle size distribution and zeta potential analysis

The size of the nanocomposite particles dispersed in the aqueous media was evaluated using a Malvern Zetasizer (Zetasizer

Please cite this article in press as: P.N. Kendre, P.D. Chaudhari, Effect of amphiphilic graft co-polymer-carrier on physical stability of bosentan nanocomposite: Assessment of solubility, dissolution and bioavailability, Eur. J. Pharm. Biopharm. (2017), http://dx.doi.org/10.1016/j.ejpb.2017.06.024