International Journal of Pharmaceutics 389 (2010) 186–194

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

Understanding the quality of protein loaded PLGA nanoparticles variability by Plackett–Burman design

Ziyaur Rahman[a], Ahmed S. Zidan[a,b], Muhammad J. Habib[c], Mansoor A. Khan[a,*]

[a] Division of Product Quality and Research, Center of Drug Evaluation and Research, Food and Drug Administration, MD, USA
[b] Faculty of Pharmacy, Zagazig University, Zagazig, Egypt
[c] School of Pharmacy, Howard University, Washington, DC, USA

ARTICLE INFO

Article history:
Received 26 May 2009
Received in revised form 7 November 2009
Accepted 17 December 2009
Available online 29 December 2009

Keywords:
CyA
PLGA
QbD
Plackett–Burman
Nanoparticles and dissolution efficiency

ABSTRACT

The aim of this investigation was to screen and understand the product variability due to important factors affecting the characteristics CyA-PLGA nanoparticles prepared by O/W emulsification-solvent evaporation method. Independent variables studied were cyclosporine A (CyA) (X_1), PLGA (X_2), and emulsifier concentration namely SLS (X_3), stirring rate (X_4), type of organic solvent employed (chloroform or dichloromethane, X_5) and organic to aqueous phase ratio (X_6). The nanoparticles properties considered were encapsulation efficiency (Y_1), mean particle size (Y_2), zeta potential (Y_3), burst effect (Y_4) and dissolution efficiency (Y_5). The statistical analysis of the results allowed determining the most influent factors. The nanoparticles were characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray powder diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy. The factors combination showed variability of entrapment efficiency (Y_1), mean particle size (Y_2) and zeta potential (Y_3) from 10.17% to 93.01%, 41.60 to 372.80 nm and 29.60 to 34.90 mV, respectively. Initially, nanoparticles showed burst effect followed by sustained release during the 7-day in vitro release study period. The dissolution efficiency (Y_5) varied from 52.67% to 84.11%. The nanoparticles revealed Higuchi release pattern and release occurred by coupling of diffusion and erosion. In conclusion, this study revealed the potential of QbD in understanding the effect of formulation and process variables on the characteristics on CyA-PLGA nanoparticles.

1. Introduction

Cyclosporine (CyA) is a cyclic neutral undecapeptide produced by fungus *Tolypocladium inflatum* which contains mainly D-amino acid, with a potent immunosuppressive activity that has been used to prevent allograft rejection in various organ transplantation such as kidney, liver, heart, lung and pancreas (Matzke and Luke, 1988; Lemley and Katz, 1988), in psoriasis (Costanzo et al., 2009) and atopic dermatitis (Akhavan and Rudikoff, 2008). It has been explored in the treatment of autoimmune disorders such as rheumatoid arthritis (Richardson and Emery, 1995) and Behçet's uveitis disease (Akman-Demir et al., 2008). New evidences are emerging its role in controlling ulcerative colitis (Yadav and Liu, 2009), and as a neuroprotective agent (Hatton et al., 2008).

Despite its promising pharmacological profile and great therapeutic value, the bioavailability after oral administration is low with high inter-patient variability (20–50%) (Lindholm et al., 1988; Fahr, 1993). The low oral bioavailability is due to its poor aqueous solubility (0.02 mg/ml) (Miyake et al., 2000) and furthermore, it is a substrate of p-glycoprotein (Charuk et al., 1995).

Many formulation strategies were investigated to improve solubility and bioavailability of CyA such as complexation with cyclodextrin (Matilainen et al., 2006), and particulate delivery system including microspheres (Yeung and Chaw, 2009) and liposome (Czogalla, 2009). The formulation of CyA in nanoparticles dosage has received much attention in the last few years mainly due to its ability to improve bioavailability and could be a better alternative to current delivery system. Biodegradable materials investigated for nanoparticles of CyA are chitosan (El-Shabouri, 2002) polycaprolactone (Varela et al., 2001), PLGA (Italia et al., 2007) and hydroxypropylmethyl cellulose phthalate (Wang et al., 2004). Investigators claimed 1.8-fold increase in bioavailability of CyA by chitosan based nanoparticles when compared with neoral microemulsion in Beagle dogs (El-Shabouri, 2002). Similarly, PLGA nanoparticles of CyA showed 119.2% relative bioavailability, low toxicity and prolonged release when compared with Sandimmune neoral dosage (Italia et al., 2007). PLGA based nanoparticles have distinct advantage of being FDA

* Corresponding author at: FDA/CDER/DPQR, White Oak, LS Building 64, Room 1070, 10903 New Hampshire Ave, Silver Spring, MD 20993-002, USA.
Tel.: +1 301 796 0016.
E-mail address: Mansoor.Khan@fda.hhs.gov (M.A. Khan).

0378-5173/$ – see front matter © 2010 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2009.12.040

approved excipient, and will not encounter regulatory hurdle in approval.

Quality by design (QbD) is a FDA initiative to pharmaceutical development (FDA guidance of industry, 2006). It is a deliberate design effort from product conceptualization to commercialization. The objective of QbD approach is to design a process in such a way that manufactures pharmaceuticals that consistently meet critical quality attributes. Another objective is to identify and control critical source of variability in the process, and understand the impact of formulation components and process parameters on the critical quality attributes. Thus, one of the components of QbD strategy is to understand variables and their interactions, and their impact on the critical quality attributes. A process and formulation can be understood by developing them based on multivariate analysis of designed experiments and/or historical data that identify and characterize the critical-to-quality process parameters, and also identify the root causes of variability. To understand process and formulation, many statistical designs of experiment (DOE) are used. The most commonly used (DOE) is Plackett–Burman, which is a very efficient screening design used when only main effects are of interest to be investigated (Plackett and Burman, 1946).

The focus of this study was to design nanoparticles by QbD approach and evaluate the effects of different formulation and processing parameters on the characteristics CyA-PLGA nanoparticles. A Plackett–Burman screening experimental design was used to identify critical parameters that influence nanoparticles characteristics including entrapment efficiency, particle size, zeta potential, burst release and dissolution efficiency.

2. Materials and methods

Cyclosporine (Purity 99%) was purchased from Poli Industria Chemica S.P.A. (Rozzano, Milano, Italy). Poly(lactide-co-glycolide) (PLGA, lactide:glycolide = 50:50, inherent viscosity: 0.58 dL/g in hexafluoroisopropanol, Mw ≈ 31,000 Da) was purchased from Lactel International Absorbable Polymers (Pelham, AL, USA). Dichloromethane and chloroform (HPLC grade) was obtained from Fisher Scientific Co. (Norcross, GA, USA). Sodium lauryl sulphate and sodium azide was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents used were analytical or HPLC grade.

2.1. Design of experiment

Traditional development of pharmaceutical formulation is based on time and energy consuming approach of changing one variable at a time while keeping other variables constant. Use of experimental design (DOE) technique allows testing of large number of variables simultaneously in a few experimental run. Screening design are the most powerful DOE techniques that determine the most critical factors in the pharmaceutical development. Most common screening design is Plackett–Burman (PB) design that screens large number of factors and identify critical one in a minimal number of run with good degree of accuracy. Generally, number of run needed to investigate the main effects are equal to 2^n or multiple of 4 in PB designs instead of 2 as in the case of full factorial design (Plackett and Burman, 1946). PB screening design with 12 experiments was constructed using software JMP version 7.0.1 (SAS, NC, USA). The linear equation of the model is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + \cdots + b_nX_n$$

where Y is the response, b_0 is the constant and $b_1, b_2 \ldots b_n$ are the coefficient of factor $X_1, X_2 \ldots X_n$ (representing the effect of each factor ordered within −1, +1).

Table 1
Experimental factors and their level.

Factor	Factor significance	Level (−1)	Level (+)
X_1	Drug (mg)	50	100
X_2	Polymer (mg)	200	400
X_3	Emulsifier concentration (%)	0.05	0.10
X_4	Stirring rate (rpm)	600	900
X_5	Type of organic solvent	Dichloromethane	Chloroform
X_6	Organic to aqueous phase ratio	1:10	1:20

Independent process and formulation variables selected were drug (X_1), polymer (X_2), and surfactant concentration (X_3), stirring rate (X_4), type of solvent (X_5) and organic to aqueous phase ratio (X_6). The parameter level selection was based on preliminary study and on literature. Parameter studied in preliminary investigation was homogenization time and mechanical stirrer speed. Homogenization time did not have a significant impact on particle size and was kept constant for all the experiments. Mechanical stirrer speeds have impact on nanoparticle size and entrapment and included in the design. Solvents are selected based on the report of Italia et al. who reported effect of solvent on particle size (Italia et al., 2007). Similarly, level of drug, polymer, surfactant level and external volume are selected based on the literature (Shi et al., 2009). We could not conduct study all the variables. That is why we selected the ones that we thought are critical. The two levels of independent factors for the screening design and experiment domain of each variable were summarized in Tables 1 and 2. The dependent variables were encapsulation efficiency (Y_1), particle size (Y_2), zeta potential (Y_3), burst release (Y_4) and dissolution efficiency (DE) (Y_5).

2.2. Preparation of CyA-PLGA nanoparticles

CyA-PLGA nanoparticles were prepared according to emulsification-solvent evaporation technique (Kawashima et al., 1999). Briefly, CyA and PLGA were codissolved in 10 ml of organic solvent (dichloromethane or chloroform). Sodium lauryl sulphate solution (0.05%, w/v or 0.10%, w/v) was prepared in deionized water. Drug and polymer solution was added drop-wise to surfactant solution to make organic to aqueous phase ratio of 1:10 or 1:20 while stirring at 300 rpm and homogenizing by probe type homogenizer PowerGen 125 (Fisher Scientific, PA, USA) and continued homogenization for 10 min at 6000 rpm after complete addition of organic phase into aqueous phase. The nanoparticles formation and subsequent hardening was effected as a result of solvent evaporation by mechanical stirring at 600 rpm or 900 rpm at room temperature. The nanoparticles were retrieved from the aqueous solution by centrifugation at 49,500 × g (RC-5C, Sorwall Instruments/Thermo Scientific, MA, USA) for 30 min. The obtained nanoparticles were washed twice with 20 ml of deionized water, frozen at −80 °C and freeze dried in Freeze Dry/Shell Freeze System (Labconco Corp., MI, USA) at −10 °C for 48 h. The dried particles were stored in fridge until further study.

2.3. Drug entrapment efficiency

Five milligrams of freeze-dried nanoparticles were dissolved in 5 ml of chloroform, sonicated for 5 min and vortexed. Hundred microliters of solution was diluted with 900 μl of the mobile phase for CyA quantization using a Hewlett–Packard (HP) HPLC instrument (Agilent technologies, CA, USA) that consist of a quaternary HP 1050 pump, HP 1050 autosampler, and 1050 HP UV detector set at a wavelength of 203 nm and column compartment thermostated at 70 °C. The HPLC stationary phase was composed of a C8, 4.6 mm × 250 mm (3.5 μm packing) reverse phase chromatography Zorbax SB-C8 column and a C8, 4.6 mm × 12.5 mm (5 μm packing) Zorbax SB-C8 reliance guard col-