Development and Evaluation of Paclitaxel Nanoparticles Using a Quality-by-Design Approach

FIRAT YERLIKAYA,[1] AYSEGUL OZGEN,[2] IMRAN VURAL,[1] OLGUN GUVEN,[3] ERGUN KARAAGAOGLU,[4] MANSOOR A. KHAN,[5] YILMAZ CAPAN[1]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey
[2]Division of Physical Chemistry, Department of Chemistry, Faculty of Science, Hacettepe University, Ankara, Turkey
[3]Division of Polymer Chemistry, Department of Chemistry, Faculty of Science, Hacettepe University, Ankara, Turkey
[4]Department of Biostatistics, Faculty of Medicine, Hacettepe University, Ankara, Turkey
[5]Division of Product Quality Research, Office of Testing and Research, OPS, CDER, FDA, Silver Spring, Maryland

Received 6 May 2013; revised 26 June 2013; accepted 10 July 2013

Published online 5 August 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23686

ABSTRACT: The aims of this study were to develop and characterize paclitaxel nanoparticles, to identify and control critical sources of variability in the process, and to understand the impact of formulation and process parameters on the critical quality attributes (CQAs) using a quality-by-design (QbD) approach. For this, a risk assessment study was performed with various formulation and process parameters to determine their impact on CQAs of nanoparticles, which were determined to be average particle size, zeta potential, and encapsulation efficiency. Potential risk factors were identified using an Ishikawa diagram and screened by Plackett–Burman design and finally nanoparticles were optimized using Box–Behnken design. The optimized formulation was further characterized by Fourier transform infrared spectroscopy, X-ray diffractometry, differential scanning calorimetry, scanning electron microscopy, atomic force microscopy, and gas chromatography. It was observed that paclitaxel transformed from crystalline state to amorphous state while totally encapsulating into the nanoparticles. The nanoparticles were spherical, smooth, and homogenous with no dichloromethane residue. *In vitro* cytotoxicity test showed that the developed nanoparticles are more efficient than free paclitaxel in terms of antitumor activity (more than 25%). In conclusion, this study demonstrated that understanding formulation and process parameters with the philosophy of QbD is useful for the optimization of complex drug delivery systems. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3748–3761, 2013

Keywords: paclitaxel; quality by design; design of experiments; Plackett–Burman; Box–Behnken; formulation; multivariate analysis; mathematical model; nanoparticles; polymeric drug delivery systems

INTRODUCTION

Quality-by-design (QbD) has been promoted by the United States Food and Drug Administration (US FDA) as a way to enhance pharmaceutical development through design efforts from product conceptualization to commercialization.[1] The International Conference on Harmonisation defines QbD as[2] : "... *is a systematic approach to pharmaceutical development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.*" QbD is a new paradigm that emphasizes product and process understanding gained through product development and during the lifecycle of a product. Since the US FDA's Process Analytical Technology (PAT) guideline was released, many studies have been published.[3–13]

In a QbD study, one major objective is to develop a detailed process and product understanding, which can be reached through well-established design of experiment tools. Conventional experimental approaches have many disadvantages. Changing a single experimental factor at a time and keeping other factors constant leads to more experiments than are feasible, especially if many variables are of concern. In addition, this eliminates the possibility of evaluating factor interactions. Statistical experimental designs (DoE) provide more accurate results with fewer runs compared with the conventional approaches. In addition, optimization of product and process is possible by DoE based on advantages such as extrapolation of data and plotting of the results.[14] There are many experimental designs in the literature that can be applied to reduce the number of studies while obtaining more useful data. If the aim is to screen a large number of variables, first-order models such as the Plackett–Burman design can be used. If the goal is to mathematically estimate a response or to precisely optimize a process, second-order models such as the Box–Behnken design are preferred.[3,15–20]

Nanoparticles are considered to be effective carriers for delivery of anticancer drugs. Because of their potential to alter the tissue distribution of drugs, nanoparticles are expected to enhance pharmacokinetics and reduce side effects. Nanoparticles can be used for passive tumor targeting as they accumulate in certain solid tumors by the enhanced permeability and retention (EPR) effect as a result of their submicron particle sizes.[21] In pharmaceutical nanotechnology, poly(lactide-co-glycolic acid) (PLGA) is a widely used polymeric vehicle for controlled release of hydrophilic and hydrophobic drugs. It is approved for human use by the US FDA, and several

Correspondence to: Yilmaz Capan (Telephone/Fax: +90-3123052080; E-mail: ycapan@hacettepe.edu.tr)

The views presented in this article do not necessarily reflect those of the United States Food and Drug Administration.

Journal of Pharmaceutical Sciences, Vol. 102, 3748–3761 (2013)

PLGA-based formulations have received worldwide marketing approval.[22] The degradation products of PLGA are nontoxic, thus making it a biodegradable polymer.[23]

Paclitaxel is a promising drug for solid tumors and is approved by the US FDA for the treatment of metastatic ovarian cancer, breast carcinoma, and non-small cell lung cancer. Paclitaxel is a diterpenoid pseudoalkaloid and was isolated from the bark of Pacific Yew (*Taxus brevifolia*). Paclitaxel has very low water solubility (log $P = 3.96$) and poor intestinal permeability, and is therefore a Class IV drug in the biopharmaceutical classification system. Its molecular weight is relatively high (853.9 Da), it is a P-glycoprotein substrate, and has high affinity for metabolizing enzymes.[24] As a result, the drug shows poor bioavailability (less than 10%) when administered orally.[25] There are several investigational and US FDA approved paclitaxel products such as Taxol® (Bristol-Myers Squibb, New York City, NY, USA), Abraxane® (Celgene, Summit, NJ, USA), Nanoxel™ (Dabur Pharma, Ghaziabad, India), and Nanotax® (CritiTech, Lawrence, KS, USA).

In the present case study, we aimed to develop paclitaxel nanoparticle formulations using a QbD approach to understand the influences of formulation and process parameters on the critical quality attributes (CQAs) of paclitaxel nanoparticles and to establish a design space.

EXPERIMENTAL

Materials

Paclitaxel, PLGA (lactide:glycolide ratio of 50:50, acid and ester terminal groups, 7–17 kDa and 24–38 kDa of molecular weights), dichloromethane, polyvinyl alcohol (PVA), sodium dodecyl sulfate (SDS), polysorbate 80, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), methanol, formic acid, and phosphate-buffered saline (PBS) tablets were purchased from Sigma–Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS), penicillin–streptomycin, L-glutamine, and Dulbecco's modified Eagle's medium (DMEM) were purchased from BioChrom AG (Berlin, Germany). All chemicals were either extra pure or chromatography grade.

Preparation of Paclitaxel Nanoparticles

The preparation of nanoparticle formulations was based on simple oil-in-water (o/w) emulsification-solvent evaporation method.[26] Briefly, paclitaxel and PLGA were dissolved in 5 mL of dichloromethane. The obtained oil phase was poured into 40 mL of aqueous phase containing a surfactant and then immediately homogenized. After homogenization, dichloromethane was evaporated under vacuum. The resulting suspension containing nanoparticles was centrifuged at 24,000*g* for 30 min (Z 383 K, Hermle; Wehingen, Germany) at ambient conditions, the supernatant was discarded and the pellet was washed twice to eliminate unloaded drug and residual surfactant. Nanoparticles were dispersed in 1 mL of deionized water and finally lyophilized for 24 h at −60°C with a vacuum pressure of 0.04 hPa (Heto PowerDry PL3000, Jouan; Allerød, Denmark). The obtained nanoparticles were kept in a dessicator at 4°C for further tests.

Risk Identification: Ishikawa Diagram

For the identification of the formulation and the process parameters regarding the given manufacturing method, and for the evaluation of their potentials to influence the CQAs of paclitaxel nanoparticles an Ishikawa diagram was constructed.[27] On the basis of prior scientific knowledge, average particle size, zeta potential, and encapsulation efficiency were considered as CQAs of paclitaxel nanoparticles as these parameters are likely to affect the therapeutic efficacy of nanoparticulate drug delivery systems. Ishikawa diagram showed eight formulation and process variables, which may affect nanoparticle properties and these variables were included in subsequent studies.

Risk Analysis: Plackett–Burman Design

A Plackett–Burman statistical experimental design was performed for screening of formulation and process parameters, which may influence the CQAs of paclitaxel nanoparticles.[19] Eight factors were tested at 12 runs. The parameter level selection was based on preliminary experiments and literature knowledge. Some potential risk factors such as organic solvent type and organic-to-aqueous phase ratio were not included in experimental design as variations in these factors caused problems during manufacturing process. The selected factors and their levels are given in Table 1.

Minitab® 16 (Minitab Inc.; State College, PA, USA) software was used to generate and randomize design matrix and for statistical analyses (Table 2). Multilinear regression analysis and ANOVA were performed to test the significance of the model and the factor coefficients.

The experimental runs (formulations) were prepared in triplicate. The dependent variables (CQAs) were average particle size (Y_1), zeta potential (Y_2), and encapsulation efficiency (Y_3).

Optimization of Paclitaxel Nanoparticles: Box–Behnken Design

Following Plackett–Burman screening design and identifying critical formulation and process variables, a response surface method, three-factor, three-level Box–Behnken design was applied for the optimization of paclitaxel nanoparticle formulation. The low and high levels of factors were directly adopted from the previous Plackett–Burman design and the medium levels were set as the midpoint of low and high levels (Table 3).

In addition, five factors, which were evaluated in Plackett–Burman design, were set at a fixed level in Box–Behnken design as their effects on the response variables seemed statistically insignificant or indispensable according to the results of Plackett–Burman design (Table 4).

Table 1. The Factors and Their Levels Used in Plackett–Burman Design

	Levels	
Factors	Low	High
X_1: Paclitaxel amount in organic phase (mg)	1.0	2.0
X_2: PLGA amount in organic phase (mg)	20	40
X_3: PLGA molecular weight (kDa)	7–17	24–38
X_4: PLGA terminal group type	Acid	Ester
X_5: Surfactant type	SDS	PVA
X_6: Surfactant concentration in aqueous phase (%)	1	3
X_7: Homogenization rate (rpm)	11,000	16,000
X_8: Homogenization duration (min)	1	3