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Long-circulating polyhydroxybutyrate-*co*-hydroxyvalerate nanoparticles for tumor targeted docetaxel delivery: Formulation, optimization and *in vitro* characterization

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ABSTRACT

In the present research an attempt was made to develop and optimize docetaxel-loaded polyhydroxybutyrate-*co*-hydroxyvalerate (PHBV) nanoparticles, using modified emulsification solvent evaporation technique and design of experiment (DOE) methodology. Formulation of docetaxel-loaded PHBV nanoparticles was conducted by factor screening studies with Plackett-Burman design (PBD) followed by Box-Behnken experimental design (BBD) to evaluate the effect of independent variables on responses. Five most important independent variables were screened out, which were obtained from failure mode effect analysis (FMEA) and factor screening studies. The effect of formulation parameters on selected responses was depicted by 2-D and 3-D response surface methodology (RSM). The final optimized batch was evaluated by various *in vitro* characterizations. The observed particle size, zeta potential and entrapment efficiency of optimized formulation was found to be 283 ± 2.79 nm, −17 ± 2.64 mV and 44 ± 0.59% respectively. Morphological studies demonstrated the smooth and spherical shape of nanoparticles. *In vitro* drug release follows the Peppas-Korsmeyer model of drug release kinetics. Cytotoxicity study was assessed using MCF-7 for percentage inhibition of human breast cancer cell line. These results indicate that the PHBV Nanoparticles could be a promising drug delivery system for efficient prolong drug release.

1. Introduction

There are several types of nanoparticle systems present today exclusively in cancer treatment strategy (Couvreur, 2013; Couvreur and Vauthier, 2006; Doane and Burda, 2013; Hu and Zhang, 2012). Its advantages include fewer side effects, higher drug efficiency, reduction of dose, desirable distribution and improvement in bioavailability. Polymeric nanoparticles have been extensively studied as particulate drug carriers in the pharmaceutical and medical fields, because of their controlled and sustained release properties, subcellular size and excellent biocompatibility with tissue and cells (Bangham et al., 1965; Tekade et al., 2009; Zhang et al., 2013). Polyhydroxybutyrate-*co*-hydroxyvalerate (PHBV) polymer is biodegradable, biocompatible and non-toxic hydrophobic polyester. PHBV produced with low-cost bacterial fermentation technique (Kuppan et al., 2011; Sendil et al., 1999; Slater et al., 1999; Vilos et al., 2012; Vilos and Velasquez, 2012). This biotechnological method makes it more attractive alternative for large scale pharmaceutical production.

The design of experiments is a test or series of tests in which purposeful changes are made to the input variables of the process so that there will be an improvement in the product and process quality. It includes statistical methodology and interpretation analysis to draw meaningful conclusions from experimental data. It conducts the experiments in a systematic and efficient way, thus reduces design and development time, as well as the cost (Myers et al., 2004; Pyzdek and Keller, 2014). DOE gained wide recognition in the development of drug delivery systems over the traditional approach of optimization. The optimization study begins with several steps includes; (a) defining the objectives of study, (b) identifying the critical quality attributes, (c) screening the factors influencing responses, (d) response surface methodology using experimental design, (e) formulation and evaluation of drug delivery system as per design, (f) statistical analysis and search for an optimum, (g) validation of DOE experimental methodology, (h) scale-up for pharmaceutical production (Gazori et al., 2009; Lawrence, 2008; Wu and Khan, 2010). Among the numerous types of experimental designs, we have chosen Plackett-Burman design (PBD) for factor screening and Box-Behnken design (BBD) for response surface methodology. PBD have the advantages of screening a large number of experimental parameters over fewer runs. BBD is a specially made design, requires only three levels for each factor, *i.e.*, −1, 0 and +1.

Breast cancer is the second leading cause of cancer deaths among women after lung cancer, and overall it holds the fifth position after lung, liver, stomach and colorectal cancer accounting about 508,000 deaths each year worldwide. Chemotherapy is used to treat both early-

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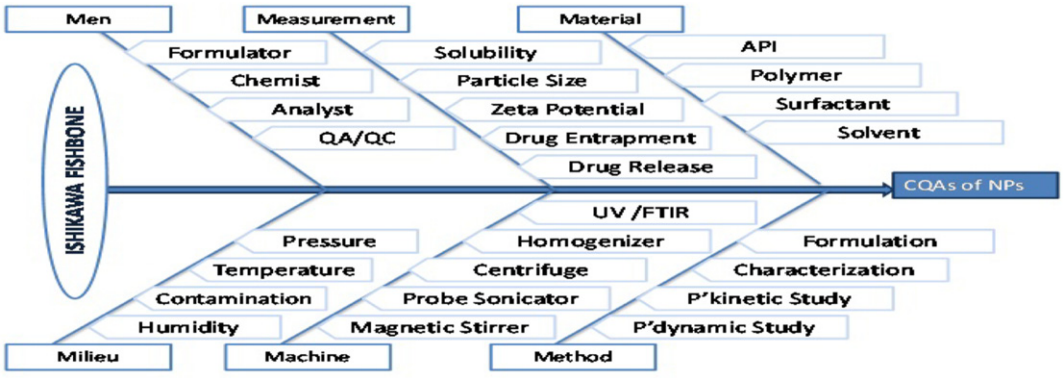

Fig. 1. Ishikawa fishbone diagram is showing risk assessment studies.

stage invasive breast cancer as well as advanced-stage breast cancer to destroy or damage the cancer cells. The scheduling of chemotherapy is based on, the cell type, cell division rate, and the time at which the drug is likely to be effective (Nair and Khawale, 2016; Welch et al., 2016). Antineoplastic drugs have a narrow therapeutic index with greater potential for causing side effects. So, the selection of matrix materials should be made depending on the size of NPs required and drug release profile desired to ensure safety and efficacy of the drug. Docetaxel, an antimitotic agent, belongs to the taxoid baccata family, possess the ability to inhibit microtubular depolarization resulting in blockade of cellular mitotic and interphase functions and, consequently, inhibit the cell division (Liu et al., 2010; Wang et al., 2011). It has a potent anti-tumor activity and generally, a large dose is recommended for treatment. This may induce toxic side effects to normal cells and nontargeted organs as a dose of <10% only reaches the tumor while other being distributed to rest of the body (Acharya and Sahoo, 2011; Wang et al., 2011; Yang et al., 2012). To make the drug more targeted, restrict high dose administration, reduce toxic side effects and improve efficacy by enhancing permeability and retention (EPR) effect is our focus of study.

2. Material and Methods

2.1. Material

Docetaxel was kindly donated as gift sample from Panacea Biotec, Baddi, India. Polyhydroxybutyrate-*co*-hydroxyvalerate (PHBV) and polyvinyl alcohol (PVA) were purchased from Sigma-Aldrich, India. All other chemicals and solvents used throughout the experiments were purchased from Fisher Scientific, Mumbai, India. The solvent used for drug analysis were HPLC grade. Ultrapure Milli-Q water was used throughout the experimentation.

Table 1
Prioritized independent variables based on FMEA study.

CTQ (independent variables)	Responses (dependent variables)			
	Particle size	Zeta potential	Polydispersity index	Entrapment efficiency
Polymer type	Low	Low	Low	Low
Polymer conc.	High	Low	Medium	High
Surfactant type	Low	Low	Low	Low
Surfactant utilizing	High	High	High	Medium
Solvent type	Low	Low	Low	Low
Solvent ratio	Medium	Low	Low	High
Temperature	Low	Low	Low	Low
Homogenizer speed (H. speed)	Medium	Low	Medium	Medium
Homogenizer time (H. time)	High	Low	Low	High
Sonication time (US. time)	High	Low	Medium	Low
Stirring speed (S. speed)	Medium	Medium	Low	Medium
Stirring time (S. time)	Medium	Medium	Low	Low
Centrifugation speed (C. speed)	Low	Low	Low	Medium
Centrifugation time (C. time)	Low	Low	Low	Medium

2.2. Methods

2.2.1. Formulation of Docetaxel-Loaded Nanoparticle

Docetaxel-loaded PHBV polymeric nanoparticles were prepared by modified emulsification solvent evaporation technique (Sahana et al., 2010; Wang et al., 2011). Briefly, 1 mg docetaxel and 15.29 mg PHBV were dissolved in dichloromethane. The first emulsion was prepared by dispersing 3.96 mg/ml PVA aqueous solution in organic phase utilizing IKA high shear homogenizer. Afterward, the formed primary emulsion was mixed with external aqueous phase containing 0.4% w/v PVA under stirring and then the whole mixture was sonicated using ultra probe sonicator (UP50H, Hielscher). Final w/o/w emulsion was stirred overnight on the magnetic stirrer (IKA RH digital) to evaporate organic solvent. The resultant dispersion was centrifuged (RC 4100F, Eltek), washed, lyophilized (Labconco) and stored in a desiccator until further use.

2.2.2. Risk Assessment Studies

The development of dosage form with the concept of QbD requires in-depth knowledge of risk assessment control strategy. These are QTPP (quality target product profile), categorizing CQAs (critical quality attributes), identifying CMA (critical material attributes) and CPP (critical process parameter) (Singare et al., 2010; Singh et al., 2005a; Wang et al., 2014). This information is further used to develop an optimized and robust manufacturing method that can produce a consistent product over time. Risk assessment studies help to identify the material attributes and process parameters which play significant role on the product CQA (Fig. 1). Furthermore, failure mode effect analyses (FMEA) provide an evaluation of rank modes of relative effectiveness to priorities critical to quality (CTQ) variables as high, medium and low. Table 1 shows prioritization levels of the independent variable before drafting DOE to optimize the NPs manufacturing process.

2.2.3. Factor Screening Design Studies

The DOE approach was performed systematically by first employing preliminary screening design and then response surface

Table 2
Independent and dependent variables with their levels in Box-Behnken design.

Levels			
Factors	Low (−1)	Medium (0)	High (+1)
A = Column temperature (°C)	30	40	50
B = Flow rate (ml/min)	0.5	1	1.5
C = Organic phase (%)	20	50	80
Dependent variable (response)			Constraint
Y = Theoretical plate			Maximum