Introduction

Breast cancer is the second leading cause of cancer deaths among women after lung cancer. Incident of carcinoma of the breast is higher in premenopausal women group of developed countries because of increase in urbanization and life expectancy. Overall breast cancer holds fifth position after lung, liver, stomach and colorectal cancer accounting about 508000 deaths each year worldwide [Jemal et al., 2008]. Breast cancer is difficult to diagnose and treat as it grows from the heart of the organs and is not identified as a foreign body by our immune system. Over time, if untreated it can grow and invade nearby healthy tissue and then underarm lymph nodes by entering through lymphatic vessels and further gotten into the blood stream from where it spread to different parts of the body. Its chance of being cured increases with early detection and treatment. Chemotherapy is widely used to treat both early-stage invasive breast cancer as well as advanced-stage breast cancer. In chemotherapy we use cytotoxic anti-cancer medicines to weaken and kill cancer cells by halting or stopping the process of cell division. The scheduling of chemotherapy is set 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]. Today various antineoplastic drugs with narrow therapeutic index are available in the market. They not only cause severe side effect but also exhibits limitations of solubility, macrophage uptake and multidrug resistance. To enhance the activity of chemotherapeutic agents, better targeting drug delivery system is required. Targeting drug delivery improves therapeutic index and minimizes the side effects of drugs. Targeting drug delivery carriers enhances the accumulation of drug at tumor site.

The ideal drug delivery system possesses broad range of properties such as biocompatibility, ease of fabrication, high drug loading capacity and low cost production.

Drug delivery using nanotechnology is relatively a new idea in medicine that incorporates existing knowledge and modern equipment. [Elzoghby et al., 2016, Lim et al., 2016, Lu et al., 2016]. Nanoparticles (NPs) play a significant role in improving targeted drug delivery, they not only influence pharmacokinetics of drug but also minimize the related adverse effects [Couvreur, 2013, Couvreur and Vauthier, 2006, Doane and Burda, 2013, Hu and Zhang, 2012]. Polymeric nanoparticles have been extensively studied as particulate drug carriers in the pharmaceutical fields, due to their controlled and sustained release properties, subcellular size and excellent biocompatibility [Bangham et al., 1965, Tekade et al., 2009, Zhang et al., 2013]. Antineoplastic drugs have a narrow therapeutic index with greater potential for causing side effects. So, the selection of polymeric materials should be made depending on the size of nanoparticles required and drug release profile desired to ensure safety and efficacy of the drug. Polyhydroxybutyrate-co-hydroxyvalerate (PHBV) is a hydrophobic, biodegradable polymer obtained by microorganisms (bacterial fermentation) from polyhydroxyalkanoates of polyhydroxybutyrate and hybroxyvalerate [Chen et al., 2016, Moorkoth and Nampoothiri, 2016]. Recently, it has been intensively used as a biomaterial for biodegradable tissue engineering and controlled release drug delivery systems. PHBV is rigid and crystalline polymer having lower glass transition temperature. The advantages of PHBV over attractive polymers like PLGA and PLA are that it is economical with a low production cost. It provides longer encapsulation time for

anticancer agents and only releases drug once it enters into the tumor. PHBV has shown promising usefulness as a sustained release polymer and also in protecting the drug from premature degradation.

The selected model drug docetaxel is a semi-synthetic drug of taxoid family isolated from the needles biomass of yew tree *Taxus baccata* [Smith et al., 2016]. It is approved as a first-line drug for the treatment of locally or advanced metastatic breast, gastric, ovarian and lung cancer. It is an antimitotic agent that acts by disrupting the microtubular network [Piccart, 2003, Simon and Bunn Jr, 2003]. The drug has a potent anti-tumor activity, and generally, a large dose is recommended for the management of cancer [Liu et al., 2010, Wang et al., 2011]. This may induce toxic effects to normal cells and nontargeted tissues as the dose of less than 10% only reach the tumor while other being distributed to rest of the body. To make the drug more targeted, enhanced permeability and retention (EPR) effect helps in restricting high dose administration and improving anticancer efficacy. EPR effect has become the gold standard in cancer targeting, and it is applicable for all rapidly growing solid tumors. The prepared nanoparticles passed through tumor's large vascularized leaky blood vessels and accumulated in the tumor micro environment. Once it reaches to tumor environment, they sustain longer due to impaired lymphatic drainage system and extravasation. However, docetaxel belongs to class IV drug with poor solubility and poor permeability as per biopharmaceutical classification system (BCS) and is considered a big challenge for formulation development [Gao et al., 2008, Kuppens et al., 2005].

Traditionally, the methodology towards designing of a dosage form involved achieving multiple objectives under the given set of limitations carried out through various trials which involve a good deal of effort and time. A very efficient way to enhance value of research and minimize the development time and cost is through design of experiments (DOE) approach. A DOE based quality by design (QbD) aims at achieving desired quality product with anticipated and predetermined specifications. International conference on harmonization (ICH) guideline Q8 (R2) also recommends QbD method for the pharmaceutical process and development. To find the optimum size and properties, different parameters (such as polymer, surfactant, solvent, homogenization, sonication, stirring and centrifugation) were applied during the preparation process with the implementation of statistical experimental design techniques [Gidwani and Vyas, 2016, Kaur et al., 2016]. An experimental design is a set of experimentation to simultaneously evaluate different factors at given number of levels in predefined numbers of experimental runs. This technique helps in planning, conducting, analyzing and interpreting data from the experiments. The factors are independent variables of formulation process that are directly under the control of formulator. On the other hand, responses are the dependent variables which are the direct function of factors. The important critical factors used for screening are either found from historical data or from experiments. These factors are further subjected to response surface methodology (RSM) to examine their behavior in more detail. RSM is used to identify possible inter-relationship between independent and dependent variables. It utilizes polynomial equations and multidimensional plots to elucidate response optimizer for the identification of optimal factor settings, to hit specific targets [Vardhan et al., 2017].

The main goal of this study is to prepare novel pegylated PHBV based nanoparticles containing docetaxel by utilizing DOE optimization technique to increase the chemotherapeutic concentration in diseased tissue. The drug carrying vehicle would be targeted to tumor sites as well as cancer cells employing passive targeting (EPR effect). This strategy causes to enhance the chemotherapeutic efficiency of drug and reduces its serious responding adverse effects. Chemical structure and properties of the prepared polymeric nanoparticulate system were examined with ^{1}H-NMR, fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), and differential scanning calorimetry (DSC). NPs size, its distribution, surface charge and shape were characterized by dynamic light scattering (DLS), zeta analyzer, scanning electron microscope (SEM), transmission electron microscope (TEM), and atomic force microscope (AFM). Further experimentations for *in vitro* drug release, cytotoxicity, cellular uptake, hemolysis, platelets aggregation, pharmacokinetics, *in vivo* anticancer activity were carefully studied with prepared nanoformulations. The most prominent key in this research is pH responsive drug delivery of the prepared formulation leading to release of drug at the tumor site. Poly (L-histidine) coat was used as an outer shell of nanoparticles. In addition, due to high protonation capacity of PLH in acidic medium, it exhibited relatively high internalization into cancer cells. To have an ideal nanoparticulate delivery system, the prepared pH-responsive formulations were further coated with polyethylene block polyethylene glycol (PE-PEG) for surface modification. Thus, it is expected that most of the applied drug would release inside the cell. The influence of pegylation was observed through *in vivo* evaluation in C3H/Jax tumor model bearing the mammary strain of female mice.