Introduction

Chronic myeloid leukaemia (CML) is a common type of cancer that affects white blood cells (WBC) and bone marrow with rising morbidity [Lee et al., 2011]. It is a haematopoietic stem cell disorder, mainly caused due to translocation of chromosomes and constitutive BCR-ABL oncogene expression that ultimately leads to malignant transformation in the cellular proliferation and control of apoptosis. It is characterized clinically by WBC above the normal range in the blood due to neutrophils in different stages of maturation. Basophilia, eosinophilia, thrombocytosis and a slight increase in the blast cells are common conditions that were found in CML. It represents about 20 % of all leukaemia cases with an incidence of 10 to 20 per million people worldwide of which 17 % of patients were diagnosed with chronic phase CML [Hoffmann et al., 2015]. Male predominance in the incidence is slightly more in CML [Höglund et al., 2013] and there was no evidence of hereditary causes of CML [Björkholm, 2013]. The exact causes of CML were essentially not known but exposure to ionising radiation is the only established risk factor associated with CML [Gluzman et al., 2006].

The diagnosis of CML will usually be suspected from the elevated WBC count or when splenomegaly is detected during the routine blood test or general physical examination [Jabbour and Kantarjian, 2014]. The current state of diagnosis of CML consists of bone marrow biopsy, molecular biology screening, cytogenetic testing like FISH (fluorescence in situ hybridization) or RT-qPCR (reverse transcriptase quantitative polymerase chain reaction) and imaging tests like computed tomography or ultrasound examination of the spleen [Baccarani et al., 2015]. The treatment of CML mainly depends on the genetic profile, disease state, health and age of the patient. The current research for the treatment of CML is being done on stem cell transplantation,

but it carries its own disadvantages like a high rate of morbidity, mortality and also requires appropriate young stem cell donor. Alternatively, the treatment of choice boosted with the development of tyrosine kinase inhibitors (TKI) [Mathisen et al., 2014].

Over the past half century, chemotherapy has extensively enhanced the cancer treatment. But sadly, lack of selectivity for the conventional chemotherapeutic agents, less than one percent of the chemotherapeutic drugs were taken up by the tumorous cells and its microenvironment and the remaining drug reaches to the surrounding healthy tissues [Van et al., 2010]. Since chemotherapeutic agents are usually intended for a specific site in the body, conventional method of delivering the drug is devoid of efficiency and need a significant amount of drug for administration thereby leading to adverse toxic effects to the surrounding healthy cells, tissues or organs. Another problem associated with the failure of cancer chemotherapy is MDR (multi drug resistance) which is the most important problem that may restrain the efficient drug accumulation within the cancerous cells and cause a low intracellular drug concentration. As cancer cells are diverse in their chemosensitivity, a number of sensitive cells show a comparatively high percentage of response with chemotherapy. In case of drug resistant cells, the chemotherapeutic agents have modest activity and result in a very low percentage of response. Hence developing drug delivery systems that are efficacious selectively is one of the ultimate challenges confronting chemotherapy at present.

For the newly diagnosed patients, TKI therapy by Dasatinib (DSB) is the first line treatment. United States Food and Drug Administration (US FDA) approved DSB (second generation TKI) which is a novel, potent and multi-targeted inhibitor of tyrosine kinases (Src and BCR-ABL) activity in the leukaemia cells [Mian et al., 2015

and Obr et al., 2014]. But the main problem associated with DSB treatment is hematologic and non-hematologic adverse effects due to endothelial hyperpermeability which ultimately leads to peripheral edema and pleural effusion [Steinberg et al., 2007]. These adverse effects are likely because of its interaction with non-tumor related processes and cells, leading to dose reduction or treatment discontinuation [Latagliata et al., 2013]. DSB is a BCS - II drug, having low solubility and high permeability. It is having low bioavailability, shorter plasma half life, lack of specificity, early degradation, rapid elimination, and is recommended in high dose. All these characteristics associated with DSB may be effectively eliminated by employing gold nanoparticles (GNPs) as a drug carrier [Bertrand et al., 2014, Jain, 2010].

Gold nanoparticles (GNPs) have turned out to be an excellent drug nanocarrier for anti-cancer drugs as they can effectively carry and protect a high therapeutic payload and selectively delivers the drug at the tumor site by active/ passive targeting methods thereby enhancing the therapeutic index [Muddineti et al., 2015]. They have become a potential carrier for targeted drug delivery because of its bioavailability, biocompatibility, and biodegradability [Cobley et al., 2011]. They can be engineered in different ways to detect a stimulus and act in response instantly by releasing the therapeutic payload into the cells or tissue [Dreaden et al., 2011]. They have been extensively utilized in the biomedical field for various applications like drug carriers [Sharma et al., 2015], imaging probes [Chattoraj et al., 2016], biosensing [Anker et al., 2008], molecular imaging [Nguyen et al., 2013], and as photothermal therapy radiosensitizers [Heidari et al., 2015]. The other significant advantages of the GNPs include reduced dosage, modulated pharmacokinetics, reduced toxicity, controlled biodistribution, and most importantly, improved patient compliance [Kumar et al., 2013, Vigderman and Zubarev, 2013].

In recent times, the synthesis and use of GNPs have fascinated the scientific community. Various physicochemical methods have been developed for the synthesis of GNPs. The significant step involved in the synthesis of GNPs is the reduction of the Au (III) ions to their metallic form which is usually carried out with the help of chemical reducing agents by chemical reduction method. The incorporation of these chemical reducing agents not only decreases the purity of the obtained GNPs but also are harmful to human beings. They are not suitable for long-term environmental sustainability [Birla et al., 2009] and biocompatibility is required for their biomedical applications [Rai et al., 2008]. In order to overcome these limitations, alternatively, green reduction methods have been developed for the synthesis of GNPs. These methods are getting preference due to its various advantages like non-toxic, simple process, easy reduction of their salts at room temperature, eco-friendly, zero energy based, cost-effective and suitable for large scale production [Singh et al., 2016].

In comparison to chemical synthesis of GNPs, the green synthesis of GNPs requires a shorter time [Manivasagan et al., 2015] due to reduction in the number of steps involved in the surface modification of GNPs in order to make them biologically active with the attachment of some functional groups [Singh et al., 2016]. A natural polymer chitosan (Ch), is an attractive biopolymer widely used in the drug delivery systems because of its non-toxic, biocompatible, biodegradable nature, cost-effective, eco-friendly, suitable for large scale production, possess sustained release and tumor inhibiting properties [Adena et al., 2018, Singh et al., 2016, Xue et al., 2015]. Synthesized Ch-GNPs possesses antimicrobial, antitumor, antiviral and other unique bioactivities and are highly biocompatible with biomedical and pharmaceutical applications [Agnihotri et al., 2004].

The stabilization of colloidal GNPs by preventing flocculation is one of the most significant and essential features and enables extensive physical and biological applications [Boisselier et al., 2009]. In order to improve the stabilization of colloidal GNPs, the dispersion status of gold colloid is preserved through steric stabilization by coating GNPs with thiol-terminated polymers and biodegradable polymers [Ofir et al., 2008]. Among thiol-terminated polymers, thiolated polyethylene glycol (PEG) is most widely used for the reason that it has good solubility, biocompatibility and antifouling property [Dreaden et al., 2009, Liu et al., 2007, Simpson et al., 2011]. Functional PEGylated GNPs is widely used in targeted drug delivery. PEG acts as a steric stabilizer in the GNP system under complex conditions. It avoids unexpected effects between the reducing agent and other electrolytes by screening the charge on GNPs. It also provide an antifouling spacer arm on the gold surface [Wang et al., 2013].

Biodegradable polymers are approved by the US FDA in the development of nanoparticles and are most commonly used for the stabilization of GNPs and improving its dispersity [Ehlerding et al., 2016]. Among them, poly lactic-co-glycolic acid (PLGA) is widely used mainly because it is biocompatible and its degradation rates can be tailored to release the encapsulated payload for a prolonged period of time. It is a safely administrable polymer and best candidate for sustained release drug delivery system [Sharma et al., 2016].

In the development of DSB-GNPs, QbD (Quality by Design) is employed which is a proactive, modern and scientific approach to product design and development. It enhances the process capability, formulation design, reduces the product variability and involves a systematic approach to ensure the quality of the final product. It is a very efficient away to enhance value of research and minimize the development time and cost. QbD develops a thorough understanding of the compatibility of the final product

to all of its critical process and formulation attributes that are involved in product development. It helps to identify the root cause of a quality issue by efficient analysis and provides insights throughout the process of product development and aims at achieving desired quality product with anticipated and predetermined specifications [Dhat et al., 2017, Garg et al., 2017, Lawrence et al., 2014].

The aim of the present research work was therefore, to synthesize and stabilize potent, non-toxic, cost-effective, eco-friendly, selectively targeted, sustained release drug loaded gold nanocarriers by green reduction method using minimum raw materials and time, and preserving its stability and bioactivity during fabrication and release. DSB-GNPs were optimized by QbD approach and the optimized DSB-GNPs were characterized by Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), high resolution scanning electron microscopy **(**HR-SEM), energy dispersive x-ray spectroscopy (EDXS), transmission electron microscopy (TEM), selected area electron diffraction (SAED), atomic force microscopy (AFM), dynamic light scattering (DLS) and zeta potential (ZP). Further, they were evaluated for stability at different storage conditions, *in vitro* drug release and release kinetics, *in vitro* hemocompatibility, cellular uptake by confocal fluorescence microscopy, *in vitro* cytotoxicity in K562 cell lines, cell apoptosis and *in vivo* pharmacokinetics in Sprague Dawley rats in order to test and provide evidence for the developed nanoformulation to be used as potential delivery system of DSB for more effective treatment of CML.