DEVELOPMENT AND EVALUATION OF DASATINIB LOADED POLYMERIC GOLD NANOPARTICLES FOR IMPROVED THERAPY OF CHRONIC MYELOID LEUKAEMIA

Thesis submitted in partial fulfillment
for the Award of Degree
Doctor of Philosophy

By

Sandeep Kumar Reddy A

Department of Pharmaceutical Engineering & Technology
Indian Institute of Technology
(Banaras Hindu University)
Varanasi-221005
India

Roll no. 14161008 **Year: 2019**

Conclusion

Chronic myeloid leukaemia (CML) is a haematopoietic stem cell disorder that affects white blood cells (WBC) and bone marrow with rising morbidity. It is caused mainly 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. The incidence of CML is slightly more in males and there was no evidence of hereditary causes of CML. The exact causes of CML were unknown but exposure to ionising radiation is the only established risk factor associated with CML. The diagnosis of CML is usually detected by elevated WBC count or by splenomegaly. The treatment of CML mainly depends on the genetic profile, disease state, health and age of the patient and the treatment of choice boosted with the development of tyrosine kinase inhibitors. The current treatment of CML carries its own disadvantages like a high rate of morbidity, mortality, multi drug resistance and adverse toxic effects to the surrounding healthy cells, tissues or organs. Thus, it is of great importance to develop alternative treatments, like nanotechnology based drug delivery systems, which may reduce toxicity and side effects of the drugs, and enhance their efficacy.

Present thesis embodies to design, develop, optimize and characterize polymeric GNPs based drug delivery system to target an antineoplastic drug DSB to CML for its improved therapy. This strategy provided not only to enhance chemotherapeutic efficiency but also reduced its associated toxicities observed during conventional treatment. Another important strategy of nanoformulation was the synthesis of GNPs by green reduction method using chitosan and its subsequent stabilization by using biodegradable polymer like PLGA which

exhibited promising properties for sustained release drug delivery. This biodegradable polymer displayed surface erosion mechanism based release of encapsulated drug in a sustained manner. In this research targeted drug delivery of DSB loaded GNPs was attempted to enhance the therapeutic efficiency of the anticancer agent DSB and simultaneously reduce its adverse effects, the prepared nanoformulation exhibited a promising potential in the treatment of CML.

A sensitive, rapid, and precise HPLC analytical method was developed and validated for the quantitative estimation of DSB in the formulation and also in rat plasma with a slight modification of earlier reported method. The chromatogram observed was without any interfering peaks in the rat plasma sample. A good linear relationship was established between peak area ratios of DSB to plasma concentrations. The calibration curve was found to be linear with negligible scatter of experimental points. The developed method was found to be sensitive enough for the quantitative determination of DSB in the biological sample (rat plasma). The simplicity, rapidity, efficacy and short retention time of DSB made the optimized analytical method suitable for the analysis of relatively more number of plasma samples in a very short period of time providing a rapid and economical method for therapeutic monitoring.

The nanoformulation was optimized by adopting quality by design approach. 2-level, 3-factor full factorial design (FFD) was used to assess the effect of independent factors on the responses. It enhances the process capability, formulation design, reduces the variability of the nanoformulation and involves a systematic approach to ensure the quality of the nanoformulation. It develops a thorough understanding of the compatibility of the nanoformulation with all of its critical processes and formulation attributes that are involved in the development

of GNPs. The implementation of this multivariate statistical experimental design in the formulation process provided stable, optimized GNPs after a thorough understanding of the inherent relationships between the factors and the desired responses. The anticipated range of particle size, zeta potential, and entrapment efficiency was found in the desired range. The physicochemical and morphological characterization studies demonstrated smooth and spherical shape of the GNPs without any aggregation. Results of solid state characterization (FTIR, XRD) revealed that all the drug in GNPs nanoformulation was converted into amorphous form due to interaction with the polymer.

The stability study of the optimized nanoformulation of DSB-PLGA-Ch-GNPs was performed as per ICH guidelines to evaluate the effect of stressed storage conditions. The results indicated that no significant change in the physical appearance, PS, % EE and ZP were observed when stored at accelerated (40 ± 20 C / 75 ± 5% RH), room temperature (25 ± 20 C) and refrigerated (4 ±10 C) conditions over a period of 6 months. The *in vitro* drug release profiles of the optimized nanoformulation determined by modified dialysis bag technique using pH 6.8 PBS revealed a sustained drug release pattern because of the strong interaction between the drug and the polymer. The mechanism of drug release from the optimized nanoformulation followed Korsmeyer-Peppas kinetics.

The hemocompatibility study was performed to evaluate the hemolysis of the optimized nanoformulation. The results showed that the optimized nanoformulation was safe, compatible and suitable formulation as there was no sign of hemolysis when it was added to the erythrocyte suspension. *In vitro* cytotoxicity was conducted using K562 human myeloid leukaemia cell lines by SRB assay in order to determine the cell density on the basis of cell protein

content measurement and cell based cytotoxicity. The results showed a confirmed viable cell reduction and evidenced that percentage cell growth and percentage growth inhibition was much higher when treated with GNPs in comparison to pure DSB. This indicates that the optimized DSB-GNPs exhibited a potential cytotoxic effect in the treatment of CML. Internalization of the developed nanoparticles is the most important and crucial feature that plays a key role in the success of targeted drug delivery systems. The success of this feature can demonstrate their therapeutic efficacy through intracellular release. K562 human myeloid leukaemia cell lines treated with the drug and optimized nanoformulation were used for the cellular uptake study by confocal fluorescence microscopy. The results indicated that DSB loaded GNPs resulted in more permeation and accumulation into the leukaemia cancer cells. Hence, it was evident that the conjugated GNPs could remarkably facilitate the drug targeting and cellular uptake of DSB into leukaemia cancer cells and could thus act as an efficient agent to facilitate the DSB drug delivery.

The cell apoptosis assay was determined by flow cytometry to quantify the number of apoptotic cells in the optimized nanoformulation sample by making measurements on each individual cell. The results revealed that with the increase in the GNPs concentration and exposure time, the apoptosis was also increased in the cell population, suggesting that the GNPs could provide effective antileukaemia activity against K562 cells and further induced the K562 cell death with dose and time dependent manner. *In vivo* pharmacokinetic studies was performed following intravenous administration of the pure drug and the optimized nanoformulation in healthy Sprague Dawley rats to measure the bioavailability and the pharmacokinetic paramaters. The non-compartmental

analysis of pure DSB and the optimized nanoformulation was carried out using Kinetica 5.1 software displayed enhanced systemic bioavailability with much higher MRT value. Thus, based on the above observations, it was concluded that the prepared DSB-PLGA-Ch-GNPs offered significant advantages including sustained drug release, making it suitable for DSB delivery with improved biocompatibility, stability, bioavailability, and therapeutic efficiency.