Contents

			Page No
	List	of Figures	i-iii
	List	of Tables	iv-v
	List	of Abbreviations and Symbols	vi-vii
	Prefa	ace	viii-ix
1.	Introduction		01-06
2.	Literature review		07-41
	2.1	Chronic myeloid leukaemia (CML)	07
		2.1.1 Diagnostics and therapeutics	08
		2.1.2 Current therapeutics	11
	2.2	Nanomedicine	12
	2.3	Focus on GNPs	13
	2.4	Synthetic strategies of preparing GNPs	15
		2.4.1 Chemical methods	16
		2.4.2 Physical methods	19
		2.4.3 Green methods	20
	2.5	Controlling the size of the GNPs	22
	2.6	Characterization of GNPs	23
	2.7	GNPs based theranostics	24
		2.7.1 Labelling and visualizing	24
		2.7.2 Targeting and delivery	27
	2.8	Nanotechnology commercial impact	32
	2.9	Drug and excipients	34
		2.9.1 Dasatinib	34
		2.9.2 Chitosan	37
		2.9.3 Poly vinyl pyrrolidone (PVP)	39
		2.9.4 Poly ethylene glycol (PEG) thiol	40
		2.9.5 Poly(lactic-co-glycolic acid) (PLGA)	41
3.	Plan	of study	42-44
4.	Mat	erials and methods	45-71
	4.1	Materials	45
		4.1.1 Chemicals	45
		4.1.2 Equipments	46
		4.1.3 Animals and cell lines	47
		4.1.4 Software	47
	4.2	Preformulation studies	47

	4.2.1 Physical observation	47
	4.2.2 FTIR interpretation	48
	4.2.3 Analytical method development for DSB by UV-Visible spectroscopy	48
	4.2.4 Analytical method development for biological samples (rat plasma) using HPLC and its validation	49
4.3	Formulation development	51
	4.3.1 Preparation of DSB loaded PVP stabilized chitosan capped GNPs (DSB-PVP-Ch-GNPs)	52
	4.3.1.1 Factorial design for formulation development	53
	4.3.1.2 Graphical drawing, optimization and statistical analysis	53
	4.3.1.3 UV-Vis absorption spectrophotometry	54
	4.3.1.4 Determination of PS and ZP	54
	4.3.1.5 Determination of entrapment efficiency	55
	4.3.1.6 FTIR and XRD studies	56
	4.3.1.7 TEM	56
	4.3.1.8 <i>In vitro</i> drug release study	56
	4.3.2 Preparation of DSB loaded PEG stabilized chitosan capped GNPs (DSB-PEG-Ch-GNPs)	57
	4.3.2.1 Risk assessment studies	57
	4.3.2.2 Factorial design	58
	4.3.2.3 Graphical drawing, optimization and statistical analysis	59
	4.3.2.4 Physical characterization of DSB-PEG-Ch-GNPs	59
	4.3.2.5 Solid state characterization	60
	4.3.2.6 Physicochemical and morphological characterization	61
	4.3.2.7 Stability study	62
	4.3.2.8 <i>In vitro</i> drug release studies	62
	4.3.3 Preparation of DSB loaded PLGA stabilized chitosan capped GNPs (DSB-PLGA-Ch-GNPs)	63
	4.3.3.1 Cause-effect relationship: Ishikawa fish bone	65
	4.3.3.2 Risk assessment screening: PBD	66
	4.3.3.3 Optimization of nanoformulation: 2 ³ Full factorial design	66
	4.3.3.4 Physical characterization of DSB-PLGA-Ch-GNPs	67
	4.3.3.5 Solid state characterization	67
	4.3.3.6 Physicochemical and morphological characterization	68
	4.3.3.7 Stability study	68
	4.3.3.8 <i>In vitro</i> drug release study	68
	4.3.3.9 <i>In vitro</i> hemocompatibility study	68

		4.3.3.10 Cellular uptake study by confocal fluorescence microscopy	69
		4.3.3.11 <i>In vitro</i> cytotoxicity assay	69
		4.3.3.12 Cell apoptosis assay	70
		4.3.3.13 <i>In vivo</i> pharmacokinetic study	70
		4.3.3.14 Statistical analysis	71
5.	Resul	Its and discussion	72-133
	5.1	Preformulation studies	72
		5.1.1 Physical observation	72
		5.1.2 FTIR interpretation	72
		5.1.3 Analytical method development for DSB by UV-Visible spectroscopy	73
		5.1.4 Analytical method development for biological samples (rat plasma) using HPLC and its validation	74
	5.2	Preparation of DSB loaded PVP stabilized chitosan capped GNPs (DSB-PVP-Ch-GNPs)	78
		5.2.1 Optimization process variables by PBD	79
		5.2.2 Box-Behnken experimental design	81
		5.2.3 FTIR and XRD studies	86
		5.2.4 TEM	88
		5.2.5 <i>In vitro</i> drug release study	89
	5.3	Preparation of DSB loaded PEG stabilized chitosan capped GNPs (DSB-PEG-Ch-GNPs)	90
		5.3.1 Risk assessment studies	90
		5.3.2 Optimization process variables by PBD and BBD	91
		5.3.3 Solid state characterization of DSB-PEG-Ch-GNPs	99
		5.3.4 Physicochemical and morphological characterization	100
		5.3.5 Stability study	105
		5.3.6 <i>In vitro</i> drug release studies	107
	5.4	Preparation of DSB loaded PLGA stabilized chitosan capped GNPs (DSB-PLGA-Ch-GNPs)	108
		5.4.1 Risk identification and risk assessment screening	108
		5.4.2 2 ³ full factorial design	112
		5.4.3 PS, % EE and ZP	116
		5.4.4 Solid state characterization of optimized nanoformulation	118
		5.4.5 Physicochemical and morphological characterization	121
		5.4.6 Stability study	124
		5.4.7 <i>In vitro</i> drug release study	126
		5.4.8 <i>In vitro</i> hemocompatibility study	127
		5.4.9 Cellular uptake study by confocal fluorescence microscopy	128

	5.4.10 <i>In vitro</i> cytotoxicity assay	130
	5.4.11 Cell apoptosis assay	130
	5.4.12 In vivo pharmacokinetic study	132
6.	Conclusion	134-136
7.	References	139-158
8.	Publications	159



THIS WORK IS DEDICATED TO MY FAMILY



Copyright © Indian Institute of Technology (B.H.U.), Varanasi, India, 2019 All rights reserved



Department of Pharmaceutical Engineering & Technology Indian Institute of Technology (Banaras Hindu University) Varanasi - 221 005

CERTIFICATE

योगः कर्मस कौशलम

It is certified that the work contained in the thesis titled "DEVELOPMENT AND EVALUATION OF DASATINIB LOADED GOLD NANOPARTICLES FOR IMPROVED THERAPY OF CHRONIC MYELOID LEUKAEMIA" by SANDEEP KUMAR REDDY A has been carried out under my supervision and that this work has been not submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Course work, Comprehensive, Candidacy, SOTA and Pre-submission seminar.

(Prof. B. Mishra)

Department of Pharmaceutical Engineering & Technology Indian Institute of Technology (BHU), Varanasi



Department of Pharmaceutical Engineering & Technology Indian Institute of Technology (Banaras Hindu University) Varanasi – 221 005

DECLARATION BY THE CANDIDATE

I, Sandeep Kumar Reddy A, certify that the work embodied in this Ph.D. thesis is my own bonafide work and carried out by me under the supervision of Prof. B. Mishra from July, 2014 to June, 2019 at the Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (B.H.U.), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma.

I declare that I have faithfully acknowledged and given credits to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not willfully copied any other's work, paragraphs, text, data, results, etc., reported in journals, books, magazines, reports, dissertations, theses, etc., or available at websites and have not included them in this Ph.D. thesis and have not cited as my own work.

Date: Place:

(Sandeep Kumar Reddy A)

CERTIFICATE FROM THE SUPERVISOR

It is certified that the above statement made by the student is correct to the best of my/our knowledge.

(Prof. B. Mishra) Supervisor (Prof. S. K. Shrivastava) Head of the Department



Place:

Department of Pharmaceutical Engineering & Technology Indian Institute of Technology (Banaras Hindu University) Varanasi - 221 005

COPYRIGHT TRANSFER CERTIFICATE

Title of the Thesis: "DEVELOPMENT AND EVALUATION OF DASATINIB LOADED GOLD NANOPARTICLES FOR IMPROVED THERAPY OF CHRONIC MYELOID LEUKAEMIA"

Name of the Student: Mr. SANDEEP KUMAR REDDY A

Copyright Transfer

The undersigned hereby assigns to the Indian Institute of Technology (B.H.U.), Varanasi all rights under copyright that may exist in and for the above thesis submitted for the award of the "Doctor of Philosophy".

(Sandeep Kumar Reddy A) Date: EDUCATION

Note: However, the author may reproduce or authorize others to reproduce material extracted verbatim from the thesis or derivative of the thesis for author's personal use provided that the source and the Institute's copyright notice are indicated.

Acknowledgements

First of all, I bow with reverence to thank the ALMIGHTY who has enriched me with such a golden opportunity and infused the power in my mind to fulfill the task assigned to me. Words are less to express my sincere heartfelt to Bharat Ratna Mahamana Pandit Madan Mohan Malaviya Ji, founder of the prestigious temple of knowledge.

It is definitely a pleasing privilege for me to express my insightful gratefulness and indebtedness to my revered supervisor Prof. Brahmeshwar Mishra. His conscientious suggestions, creative ideas, scientific acumen, critical evaluation, boundless enthusiasm, perspicacious remarks and homely atmosphere in which he makes one work, are some of the supreme qualities that will be cherished as a quintessential example of the brilliant guidance. I shall remain indebted to him for his diligent efforts and professional competencies, which contributed towards the completion of the research work. The overwhelming enthusiasm that he has inoculated in me is not just limited to this work but will take me a long way in my life.

I am indeed obliged and sincerely thankful to Prof. S. K. Shrivastava, Head, Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (BHU) for kindly allowing me to use the facilities required to complete my research work.

I owe my gratitude to my RPEC members Mr. A. K. Srivastava, Dr. Abha Mishra for their encouragement and insightful comments leading me to the completion of the research work. I wish to express deep regards to all the faculty members of the department Prof. S. K. Singh, Prof. Sanjay Singh, Dr. S. Hemalatha, Dr. K. Sairam, Dr. Senthil Raja A., Dr. A. N. Sahu, Dr. S. K. Mishra, Dr. Ruchi Chawla, Dr. Ashok Maurya, Dr. M. S. Muthu, Dr. P. K. Nayak, Dr. G. P. Modi, Dr. S. K. Jain, Dr. V. Tiwari and Dr. A. K. Agrawal for their kind cooperation at all moments during the progress of my research. I shall fail in my duty if I do not convey my regards to all the non-teaching staff members of the department for their support and help during the research work.

I take this opportunity to sincerely acknowledge MHRD, New Delhi for providing financial support in the form of Research Fellowship that immensely helped me to achieve my goals. I also thank Department of Science & Technology, New Delhi, Department of Biotechnology, New Delhi and Indian Council of Medical Research, New Delhi for awarding me travel grant to attend international conference.

I am indeed thankful to have got welcoming, and friendly fellow mates Mr. Ramoji Kosuru, Mr. Harsh, Mrs. Mansi, Mrs. Pramila, Mrs. Sarita, Mr. Satheesh Kumar and Mr. Kasi Viswanadh who were ever ready to provide me with all possible help and they created a happy working environment and foster camaraderie within the laboratory. I also extend my gratitude to all those, who have been directly or indirectly related to my project work.

I owe my deepest gratitude to my Parents Mr. A. Subba Reddy and Mrs. A. Sumalatha for being a constant source of encouragement and support throughout my life. It is their love and trust on my abilities which has made me a person I am today. My heartfelt thanks to my sister Dr. A. Chaitanya Reddy for her constant support, encouragement, and wishes for my career.

I also acknowledge the warm support extended to me by Mrs. S. Sunanda Manohar Reddy, Mr. K. Rama Subba Reddy, Mr. K. Sudhakar Reddy, Mr. P. V. Bhaskar Reddy, Mr. S. V. Sekhar Reddy, Dr. S. Haritha, Mrs. G. Himaja, Mr. S. Harsha, Mr. K. Sravan, Mrs. P. Ramya, Ms. K. Anusha and Ms. T. Anuhya.

My profound thank to MSN Laboratories Pvt. Ltd., Hyderabad for providing gift sample of drug during the research work. Finally, I would like to thank everybody who was important to the successful realization of the thesis, as well as expressing my apology that I could not mention personally one by one.

Date: (Sandeep Kumar Reddy A)