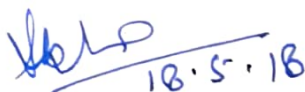


DEDICATED TO
MY BELOVED
PARENTS

**Copyright © Indian Institute of Technology
(Banaras Hindu University), Varanasi-221005,
INDIA. All rights reserved.**

CERTIFICATE

This is to certify that thesis entitled "**Polymeric nanoparticle for the delivery of poorly soluble drugs**" submitted by **Ms. Kushagri Singh** in fulfillment of requirement for the award of the degree of Doctor of Philosophy at the School of Biochemical Engineering, Indian Institute of Technology (Banaras Hindu University), Varanasi, is a record of bonafide work carried out by her under my supervision for the prescribed period in conformity with the rules and regulations of the Ph.D ordinances of the Indian Institute of Technology (Banaras Hindu University). This thesis embodies the results of her investigations conducted during this work as a Ph.D research Scholar.


18.5.18

Supervisor

Dr. Abha Mishra

Associate Professor

School of Biochemical Engineering

Indian Institute of Technology (BHU), Varanasi.

UNDERTAKING BY THE CANDIDATE

I, **Kushagri Singh**, declare that the work embodied in this Ph.D thesis is my own bonafide work carried out under the supervision of **Dr. Abha Mishra** for a period of **5 years and 8 months** from **September 2012 to May 2018** at the School of Biochemical Engineering, Indian Institute of Technology (Banaras Hindu University), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma.

Though care has been taken while writing this thesis, there may still be some errors (typographical or otherwise) which are inadvertent on my part.

Date: 18 05 18

Place: IIT (BHU), Varanasi


(Kushagri Singh)

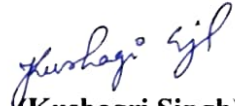
DECLARATION BY THE CANDIDATE

I, **Kushagri Singh**, certify that the work embodied in this Ph.D thesis is my own bonafide work carried out by me under the supervision of **Dr. Abha Mishra** for a period of **5 years and 8 months** from **September 2012 to May 2018** at the School of Biochemical Engineering, Indian Institute of Technology (Banaras Hindu university), 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, given credit to and referred to the research workers wherever their works have been cited in the text and the body of the thesis. I further certify that I have not willfully lifted up some other's work, paragraph, text, data, results, etc. reported in the journals, books, magazines, reports, dissertations, thesis, etc., or available at websites and included in this Ph.D thesis and cited as my own work.


Date: 18.05.18

Place: IIT (BHU), Varanasi


(Kushagri Singh)

CERTIFICATE FROM THE SUPERVISOR


This is to certify that the above statement made by the candidate is correct to the best of our knowledge.

 18/05/2018

Signature of the Coordinator
(Prof. Subir Kundu)

School of Biochemical Engineering
IIT (BHU), Varanasi

संयोजक
Coordinator
जैव रासायनिक अभियांत्रिकी स्कूल
School of Biochemical Engg.
भारतीय प्रौद्योगिकी संस्थान
Indian Institute of Technology
(का०हि०वि०वि०) वाराणसी-221005
(B.H.U.) Varanasi-221005

 18.5.18
Dr. Abha Mishra
Supervisor

**COURSE WORK/COMPREHENSIVE EXAMINATION/CANDIDACY/STATE OF ART
COMPLETION CERTIFICATE**

This is to certify that **Ms. Kushagri Singh**, a bonafide research scholar of School of Biochemical Engineering, has satisfactorily completed the course work/comprehensive examination/candidacy/state of art requirement, which is a part of her Ph.D program.

Date: 18/05/2018
Place: IIT (BHU), Varanasi


Prof. **सुभाष कुन्दु**
Coordinator
जैव रासायनिक अभियांत्रिकी स्कूल
School of Biochemical Engg.
भारतीय प्रौद्योगिकी संस्थान
Indian Institute of Technology
(का०हि०वि०वि०) वाराणसी-221005
(B.H.U.) Varanasi-221005

PRE-SUBMISSION SEMINAR COMPLETION CERTIFICATE

This is to certify that **Ms. Kushagri Singh**, a bonafide research scholar of School of Biochemical Engineering, IIT(BHU) has satisfactorily completed the pre-submission seminar requirement on the topic "**Polymeric nanoparticle for the delivery of poorly soluble drugs**" on 6th January 2018, which is a part of her Ph.D. program.

Date: 18/05/2018
Place: IIT (BHU), Varanasi


Prof. **सुभाष कुन्दु**
Coordinator
जैव रासायनिक अभियांत्रिकी स्कूल
School of Biochemical Engg.
भारतीय प्रौद्योगिकी संस्थान
Indian Institute of Technology
(का०हि०वि०वि०) वाराणसी-221005
(B.H.U.) Varanasi-221005

COPYRIGHT TRANSFER CERTIFICATE

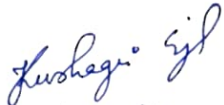
Title of the Thesis: "**Polymeric nanoparticle for the delivery of poorly soluble drugs**".

Candidate's Name: **Ms. Kushagri Singh**

Copyright Transfer

The undersigned hereby assigns to the Indian Institute of Technology (Banaras Hindu University), Varanasi all rights under copyright that may exist in and for the above thesis submitted for the award of the Ph.D. degree.

Date: 18.05.18


(Signature of the candidate)

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 University's copyright notice are indicated.

ACKNOWLEDGEMENT

*I bow my head with great reverence in the pious feet to the founder of this University, Late **Mahamana Pandit Madan Mohan Malviya** a man of great vision for creating such a wonderful and unique University, a temple of learning for thousands of students like me.*

*I bow my head before the feet of the "**Lord Shiva**", the almighty due to whose blessings I could be able to reach at this stage and feel anything in this earth could be possible only with the blessings of this invisible but omnipresent supreme force.*

*I deem it to be a rare opportunity and the proudest privilege of my life to express my profound sense of gratitude, sincere honour and veneration to my supervisor **Dr. Abha Mishra**, Associate Professor, School of Biochemical Engineering, Indian Institute of Technology, Banaras Hindu University, for her sagacious and benevolent guidance, constant encouragement, valuable suggestions, ever ready assistance, constructive criticism, keen interest and ever lasting affection during the entire course of study and in preparation of this manuscript. I am really indebted to her for the affectionate care during entire period.*

My sincere thanks to Prof. Subir Kundu, Coordinator of School of Biochemical Engineering, Indian institute of technology, Banaras Hindu University, for providing all the facilities to carry out my work smoothly.

The help and cooperation received from all the faculty members of the School of Biochemical Engineering especially Prof. S.K. Srivastava, Prof. Mira Debnath(Das), Prof. R.M.Banik, Prof. Pradeep Srivastava, Dr. Ashish Kumar Singh, Dr. Sanjay Kumar & Dr. Vishal Mishra are gratefully acknowledged.

I thank to Dr. Vijay Laxmi Yadav, Department of Chemical Engineering, Indian Institute of Technology, Banaras Hindu University, for her constant encouragement and suggestions. I shall always remember her as a good human and great motivator. My special thanks to Dr. Amit Singh, Pharmacology Department, IMS, BHU, for his valuable suggestions, friendly behaviour and continual help.

I am highly indebted to Prof. A.S.K Sinha, in-charge sophisticated instruments lab, Department of Chemical Engineering, Indian Institute of Technology, Banaras Hindu University, for providing me the FTIR Spectrophotometer & XRD facilities.

I am thankful to Prof. N.K.Mukhopadhyay, Head of Department of Metallurgical Engineering, Indian Institute of Technology, Banaras Hindu University, for giving permission to carry out scanning electron microscopy of nanoparticle samples. I am thankful to Pharmaceutics Department, Indian Institute of Technology, Banaras Hindu University, for providing zetapotential studies.

I am thankful of the non-teaching staff of the School of Biochemical Engineering, especially Dinesh ji, Rama Shankar ji, Arun ji, Usha ji for their help rendered in various ways. I also wish to express my deepest thanks to all my colleagues and many others for supporting me during my Ph.D. to make my task easier.

*This work would have been rather incomplete without the moral support and constant inspiration of my respected and beloved father **Sri. Aditya Narayan Singh**, and mother **Smt. Sunita Singh**.*

*I must appreciate the patience and tremendous love of my son (**Ivaan**). My brothers (**Pragya Nand Singh & Arya Nand Singh**) gave me lot of encouragement, support & shared all the happiness and difficulties with me. My family showed great patience in bearing me during the crunch time of my studies. Great respect to my sister in law (**Meenu Singh**) and my nephew (**Saransh**) for their huge love and prayers.*

I express my indebtedness to the Department of Science and Technology, New Delhi for providing me DST-INSPIRE fellowship in the form of Junior Research Fellowship and Senior Research Fellowship, during the course of this work.

Date: 18.05.18

Place: Varanasi



(Kushagri Singh)

TABLE OF CONTENTS

NO.	CHAPTERS	PAGE NO.
	<i>Acknowledgements</i>	i-ii
	<i>Contents</i>	iii
	<i>List of Abbreviations and Symbols</i>	iv-v
	<i>List of Tables</i>	vi-vii
	<i>List of Figures</i>	viii-x
	<i>Preface</i>	xi-xii
1.	CHAPTER 1: INTRODUCTION	1-5
2.	CHAPTER 2: REVIEW OF LITERATURE	6-40
3.	CHAPTER 3: MATERIAL AND METHODS	41-50
4.	CHAPTER 4: RESULTS AND DISCUSSION	51-100
5.	CHAPTER 5: CONCLUSION	101-104
6.	REFERENCES	105-123
7.	LIST OF PUBLICATIONS	
8.	LIST OF ATTENDED CONFERENCES AND WORKSHOPS	

LIST OF ABBREVIATIONS & SYMBOLS

CS	CHITOSAN
CSNP	CHITOSAN NANOPARTICLES
TCS	THIOLATED CHITOSAN
SEM	SCANNING ELECTRON MICROSCOPY
TEM	TRANSMISSION ELECTRON MICROSCOPY
TPP	TRIPOLYPHOSPHATE
EDTA	ETHYLENEDIAMINETETRAACETICACID
DDA	DEGREE OF DEACETYLATION
DDS	DRUG DELIVERY SYSTEM
FTIR	FOURIER TRANSFORM INFRARED
EE	ENTRAPMENT EFFICIENCY
LC	LOADING CAPACITY
XRD	X-RAY DIFFRACTION
NPS	NANOPARTICLES
PDI	POLYDISPERSITY INDEX
NDDS	NOVEL DRUG DELIVERY SYSTEM
H	HOURS
MINS	MINUTES
MG	MILIGRAMS
ML	MILILETER
ZP	ZETA-POTENTIAL
NM	NANO-METER
API	ACTIVE PHARMACEUTICAL INGREDIENT

M	METER
μM	MICROMETER
Da	DALTON
AmB	AMPHOTERICIN B
VCM	VANCOMYCIN
BCS	BIOPHARMACEUTICAL CLASSIFICATION SYSTEM
AE	ADVERSE EFFECTS
SD	STANDARD DEVIATION
NA	NUTRIENT AGAR
PDA	POTATO DEXTROSE AGAR
R²	CORRELATION COEFFICIENT
TLA	THIOLACTIC ACID
EDC	ETHYL DIMETHYLAMINOPROPYL CARBODIIMIDE
KBr	POTASSIUM BROMIDE
PBS	PHOSPHATE BUFFER SOLUTION

LIST OF TABLES

TABLE NO.	PARTICULARS	PAGE NO.
1	Overview of nanoparticle technology based marketed products.	16
2	Key nanotechnology-based approaches for the enhancement of drug solubility and oral bioavailability.	17
3	Example of Natural and Synthetic Polymer.	20
4	Advantages and Disadvantages of Natural and Synthetic Polymers.	20
5	Concentrations of chitosan, polyanion TPP and Amphotericin B.	43
6	Concentrations of chitosan, polyanion TPP and Ketoconazole.	43
7	Concentrations of chitosan, polyanion TPP and Ciprofloxacin.	43
8	Concentrations of chitosan, polyanion TPP and Vancomycin.	43
9	Concentrations of chitosan, polyanion TPP and Chloramphenicol.	44
10	Concentrations of thiolated chitosan, polyanion TPP and poorly soluble drugs.	48
11	Effect of polymer concentration on particle size and drug entrapment efficiency of nanoparticle.	51
12	Effect of cross linking agent concentration on the particle size and drug entrapment efficiency of nanoparticle.	52
13	Effect of stirring speed on particle size and drug entrapment efficiency of nanoparticle	53
14	Effect of stirring time on particle size and drug entrapment efficiency of nanoparticle.	54
15	External Factors Evaluation (EFE) matrix analysis for Amphotericin B loaded CS1.	85
16	External Factors Evaluation (EFE) matrix analysis for free Amphotericin B.	86
17	External Factors Evaluation (EFE) matrix analysis for Ketoconazole loaded CS1.	88
18	External Factors Evaluation (EFE) matrix analysis for free Ketoconazole.	89
19	External Factors Evaluation (EFE) matrix analysis for Ciprofloxacin loaded CS1.	91
20	External Factors Evaluation (EFE) matrix analysis for free Ciprofloxacin.	92
21	External Factors Evaluation (EFE) matrix analysis for Vancomycin loaded CS1.	94

22	External Factors Evaluation (EFE) matrix analysis for free Vancomycin	95
23	External Factors Evaluation (EFE) matrix analysis for Chloramphenicol loaded CS1.	97
24	External Factors Evaluation (EFE) matrix analysis for free Chloramphenicol.	98

LIST OF FIGURES

Figure No.	PARTICULARS	Page No.
1	The nanometer length scale is a thousand times smaller than bacteria and ten million times smaller than the width of a human hair.	6
2	Synthesis of chitosan from chitin through deacetylation (boiling in presence of 40% NaOH).	23
3	Structure of chitosan along with characteristic properties.	24
4	Pharmaceutical properties of chitosan	25
5	Commercial applications of chitosan	25
6	Advantages of Controlled drug delivery systems	26
7	Coacervation phase separation method	32
8	Spray drying method	32
9	Emulsion-droplet coalescence method	32
10	Iontropic gelation method	33
11	Reverse Micellar method	33
12	Sieving method	34
13	Schematic representation of Ionotropic gelation method for the preparation of chitosan nanoparticles at 37°C	42
14	Graph showing percentage cumulative release of drug with respect to time	49
15	SEM images of chitosan nanoparticles loaded with Amphotericin B, a poorly soluble drug.	55
16	Bar diagram showing effect of chitosan concentration on drug entrapment efficiency of nanoformulations at physiological pH and temperature	56
17	Cumulative drug release percentage profile of free Amphotericin B and Amphotericin B from chitosan nanoparticle and thiolated chitosan formulations up to 10h.	56
18	FTIR analysis shows (a) chitosan and (b) chitosan-TPP nanoparticles and(c) chitosan nanoparticles loaded with Amphotericin B.	57
19	XRD spectra of a) pure chitosan, b) chitosan nanoparticles, c) pure Amphotericin B and d) Amphotericin B loaded chitosan nanoparticles	58
20	Zeta potential distribution plot for Amphotericin B loaded chitosan nanoparticles.	59
21	Zone of Clearance (12mm) in the Lawn of <i>Aspergillus fumigatus</i> by free drug and drug loaded chitosan nanoparticle after 48h of Incubation.	60

Table No.	PARTICULARS	Page No.
22	SEM images of chitosan nanoparticles loaded with Ketoconazole, a poorly soluble drug.	61
23	Bar diagram showing effect of chitosan concentration on drug entrapment efficiency of nano-formulations at physiological pH and temperature	62
24	Cumulative drug release percentage profile of Ketoconazole up to 10h.	62
25	FTIR analysis shows (a) chitosan, (b) chitosan-TPP nanoparticles and(c) chitosan nanoparticles loaded with Ketoconazole.	63
26	XRD spectra of a) pure chitosan, b) chitosan nanoparticles, c) pure Ketoconazole and d) Ketoconazole loaded chitosan nanoparticles.	64
27	Zeta potential distribution plot for the Ketoconazole loaded chitosan nanoparticles.	65
28	Zone of Clearance in the Lawn of fungus <i>Coriolus</i> spp. by free drug and drug loaded chitosan nanoparticle after 48h of incubation.	66
29	SEM images of chitosan nanoparticles loaded with Ciprofloxacin, a poorly soluble drug.	67
30	Bar diagram showing effect of chitosan concentration on drug entrapment efficiency of nanoformulations at physiological pH and temperature.	68
31	Cumulative drug release percentage profile of Ciprofloxacin up to 9h.	68
32	FTIR analysis shows (a) Chitosan, (b) Chitosan-TPP nanoparticles, (c) Chitosan nanoparticles loaded with Ciprofloxacin.	69
33	XRD spectra of a) pure chitosan, b) chitosan nanoparticles, c) pure Ciprofloxacin and d) Ciprofloxacin loaded chitosan nanoparticles.	70
34	Zeta potential distribution plot for the Ciprofloxacin loaded chitosan nanoparticles.	71
35	Zone of clearance in the lawn of <i>Pseudomonas aeruginosa</i> spp. by free drug and drug loaded chitosan nanoparticle after 48h of incubation.	72
36	SEM images of chitosan nanoparticles loaded with Vancomycin, a poorly soluble drug.	73
37	Bar diagram showing effect of chitosan concentration on drug entrapment efficiency of nanoformulations at physiological pH and temperature.	74
38	Cumulative drug release percentage profile of Vancomycin up to 10h.	74
39	FTIR analysis shows (a) chitosan, (b) chitosan-TPP nanoparticles and (c) chitosan nanoparticles loaded with Vancomycin.	75
40	XRD spectra of a) pure chitosan, b) chitosan nanoparticles, c) pure Vancomycin and d) Vancomycin loaded chitosan nanoparticles.	76
41	Zeta potential distribution plot for the Vancomycin loaded chitosan nanoparticles.	77

Table No.	PARTICULARS	Page No.
42	Zone of clearance in the lawn of <i>Bacillus cereus</i> spp. by free drug and drug loaded chitosan nanoparticle after 48h of incubation	78
43	SEM images of chitosan nanoparticles loaded with Chloramphenicol, a poorly soluble drug.	79
44	Bar diagram showing effect of chitosan concentration on drug entrapment efficiency of nanoformulations at physiological pH and temperature.	80
45	Cumulative drug release percentage profile Chloramphenicol up to 10h	80
46	FTIR analysis shows (a) chitosan, (b) chitosan-TPP nanoparticles and (c) chitosan nanoparticles loaded with Chloramphenicol.	81
47	XRD spectra of a) pure chitosan, b) chitosan nanoparticles, c) pure Chloramphenicol and d) Chloramphenicol loaded chitosan nanoparticles.	82
48	Zeta potential distribution plot for the Chloramphenicol loaded chitosan nanoparticles.	83
49	Zone of clearance in the lawn of <i>Streptococcus thermophilus</i> and <i>E.coli</i> by free drug and drug loaded chitosan nanoparticle after 48h of incubation.	84
50	Zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson Crowell Model for Amphotericin B release from Chitosan nanoparticle formulation CS1.	86
51	Zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson Crowell Model for free Amphotericin B.	87
52	Zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson Crowell Model for Ketoconazole release from Chitosan nanoparticle formulation CS1	89
53	Zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson Crowell Model for free Ketoconazole.	90
54	Zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson Crowell Model for Ciprofloxacin release from Chitosan nanoparticle formulation CS1	92
55	Zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson Crowell Model for free Ciprofloxacin.	93
56	Zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson Crowell Model for Vancomycin release from Chitosan nanoparticle formulation CS1.	95
57	Zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson Crowell Model for free Vancomycin.	96
58	Zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson Crowell Model for Chloramphenicol release from Chitosan nanoparticle formulation CS1.	98
59	Zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson Crowell Model for free Chloramphenicol.	99

PREFACE

Effective delivery of active pharmaceutical ingredient or drug has become a major challenge to pharmaceutical industry today. This problem is mainly because of poor solubility of drugs and around 40% of the oral marketed drugs have low bioavailability. Due to rising number of compounds having solubility and permeability issues, finding ways to enhance the solubility of drugs is one of the major concern in the pharmaceutical industry today. Drug absorption, sufficient bioavailability and pharmacokinetic profile of oral drugs are highly dependent on solubility of that compound in aqueous medium. Solubility is one of the important parameters to achieve preferred concentration of drug in systemic circulation for achieving required pharmacological response. Poorly soluble oral drugs having slow drug absorption that leads to gastrointestinal mucosal toxicity, variable bioavailability and undesirable side effects. The solubility behavior of poorly soluble oral drugs remains one of the most challenging aspects of formulation development. Use of nanotechnology in delivery of poorly soluble oral drugs especially antimicrobial agents could improve the bioavailability together with sustained delivery. Numerous advanced functional materials have been investigated.

Among them, nano-carriers strategies have been widely used to enhance the dissolution and oral availability of poorly soluble drugs by encapsulating the drugs into nano-carriers and changing the crystalline form into amorphous together with protection of drugs from enzymatic as well as chemical degradation. Polymeric nanoparticles possess many advantages over conventional and some novel dosage forms such as non-toxicity, biodegradability, stability, sustained delivery of drugs and reproducibility. Polymeric chitosan nanoparticles formulated and loaded with five different antimicrobial agents such as Amphotericin B, Ketoconazole, Ciprfloxacin, Vancomycin and Chloramphenicol. These drug loaded chitosan nanoparticle formulations were then characterized on the basis of different factors and a comparative study was done to check the release status of drug from chitosan nanoparticles, thiolated chitosan nanoparticles and release of free drug itself.

This thesis comprise of mainly five chapters namely Introduction, Review of Literature, Material and Methods, Results and Discussion, and Conclusion. From the discussions on the results that we obtained I believe that it is possible for one to get a better understanding of stable formulation development of chitosan and thiolated chitosan nanoparticles loaded with poorly soluble oral antimicrobial drugs to increase its dissolution as well as absorption through GI tract. This research attempts to develop polymeric chitosan nanoparticles to meet the needs that are required for a successful nano delivery system for poorly soluble oral antimicrobial agents.