DECLARATION BY THE SCHOLAR

I hereby declare that the work reported in the Ph.D. thesis entitled "Development of Metallic Nano-systems (Gold and Silver) for Anti-microbial and Sensing Applications" submitted at Jaypee University of Information Technology, Waknaghat, Solan, Himachal Pradesh, India is an authentic record of my work carried out under the supervision of Dr. Abhishek Chaudhary. I have not submitted this work elsewhere for any other degree or diploma. I am fully responsible for the contents of my Ph.D. thesis.



Mr. Deepak Sharma

Enrollment No. 166555

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology

Waknaghat, Solan, H.P. India-173234

Date: 17/01/23

SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled "Development of Metallic Nano-systems (Gold and Silver) for Anti-microbial and Sensing Applications", submitted by Mr. Deepak Sharma at Jaypee University of Information Technology, Waknaghat, Solan (HP) India, is a bonafide record of his original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.



Supervisor:

Dr. Abhishek Chaudhary

Date: 17/01/23

Assistant Professor

Department of Biotechnology & Bioinformatics Jaypee University of Information Technology Waknaghat, Solan, Himachal Pradesh-173234, India

Acknowledgement

First and the foremost, apotheosis and my reverence to the Lord **Shiva**, the eternal almighty, for his showers of blessings through various accomplishments throughout my life. I am indebted towards him for the courage he bestowed upon me to complete this research work successfully.

No one who achieves success does so without acknowledging the help of others, in same manner, the success and final outcome of this thesis was made possible with the support, guidance and assistance of many people who were always there when needed. I take this opportunity to acknowledge all of them, and extend my heartfelt gratitude for helping me to make this Ph.D. thesis a reality.

I pay my sincere thanks to **Mr. Jai Prakash Gaur** (JUIT Founder), JUIT administration, **Prof. (Dr.) Rajendra Kumar Sharma** (Vice Chancellor), **Prof. (Dr.) Ashok Kumar Gupta** (Dean Academics and research) and **Maj. Gen. Rakesh Bassi** (Registrar) for providing essential amenities, research funding and infrastructure for pursuing the research work.

I feel great serenity to express my highest venerations and heartfelt gratitude to my contemplative, prudent and dignified research supervisor, **Dr. Abhishek Chaudhary**, Assistant Professor, Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, Solan, Himachal Pradesh, India for giving me the opportunity to do research, providing invaluable guidance and freely sharing his knowledge, experience and valuable time for all my endeavors. His dynamism, vision, sincerity and motivation have deeply inspired me. It was a great privilege and honour to work and study under his guidance. His invaluable and benevolent guidance with untiring willingness to help, council, and encouragement at very juncture made me complete my work. I will always remain indebted to him for his selfless persuasion and my thesis could not have been completed without his guidance and support. I also express my deep sense of gratitude towards my DPMC member **Dr. Garlapati Vijay Kumar**, **Dr. Saurabh Bansal** and **Prof. (Dr.) Rajiv Kumar** for their precious suggestion, encouragement and constant support in entire work.

I would also like to extend my gratitude to **Prof. Dr. Sudhir Kumar** Head Department of Biotechnology and Bioinformatics for his continuous support and motivation to accomplish current work. I also would like to thank the faculty members of the department **Dr. Anil Kant Thakur, Dr. Ashok Kumar Nadda, Dr. Gopal Singh Bisht, Dr. Hemant Sood, Dr. Jata Shankar, Dr. Jatindraa Vashistt, Dr. Poonam Sharma, Dr. Rahul Shrivastava, Dr. Raj Kumar, Dr. Teeratha Raj Singh**, and **Dr. Malairaman Udayabanu** for their constant motivation and moral support throughout my doctoral journey. I will always appreciate the help I got from our technical and non-technical staff. I am thankful to **Mr. Ismail Siddiqui, Mr. Baleshwar Shukla, Mrs. Mamta Mishra, Mrs. Sonika Gupta** and **Mrs. Somlata Sharma** for valuable contributions in my research period.

I am extending my heartfelt thanks to **Dr. Gunjan Goel**, Professor, Department of Microbiology Central University of Haryana, Mahendergarh, Haryana and **Dr. Duni Chand**, Professor, Department of Biotechnology, Himachal Pradesh University Shimla for their continuous motivation and support throughout my Ph.D. work. I am also highly thankful to my school teachers **Miss Ritu Gupta** and **Mrs. Deepshikha Gour** for continuous moral support and guidance.

I am thankful to the Himachal Pradesh Council for Science Technology and Environment (HIMCOSTE) for providing me financial assistance in the form of Project Assistance during initial stage of my Ph.D. work.

My acknowledgement would never be complete without the special mention of my seniors Dr. Rishi Mahajan, Dr. Sampan Attri, Dr. Kavita Sharma, Dr. Sanjay Singh, Dr. Deepika Sharma, Dr. Arun Parashar, Dr. Arun Sharma, Dr. Vineet Mehta, Dr. Rohit Sharma, Dr. Prashant Thakur, and Dr. Jonny Dhiman for their encouragement and help during the work. Friends are true gems in life that are always there for you in your best and toughest situations. A special thanks to my crazy friends Nitish Sharma, Lavesh Sharma, Akhil Chauhan, Ranju Rathour, Shivangi Garg, Nikkita Soni, Kanchan Kumari, Neha Luhakhara, Pooja Sharma Neha Kumari, Rajni Chauhan, Priyanka Sharma, Chetna Grover, Swati Sharma, Swati Kanwar, Arvind Yadav, Rohit Shukla, Vikrant Abbot and Kartik Mishra who have refreshed and handled my mood swings during the course of study.

I feel a deep sense of gratitude from the core of my heart towards my parents **Mrs. Kamlesh Sharma** and **Mr. Dayal Dutt**, and my sister **Mrs. Pooja Sharma** for their unconditional love, support and encouragement throughout my academic pursuits. In the end I also thankful and pay regards to those persons whom I am not able to mention here, although they are not forgotten and will always value for their role in this voyage.

Deepak Sharma

LIST OF UNITS, SYMBOLS & ABBREVIATIONS

°C	Degree Celsius
%	Percentage
g	Grams
h	Hours
μg	Microgram
μΜ	Micromolar
mg	Milligrams
ml	Milliliter
mM	Millimolar
min	Minutes
М	Molarity
nm	Nanometer
nM	Nanomolar
Ν	Normality
CuNPs	Copper nanoparticles
DLS	Dynamic light scattering
G-AuNPs	Gentamicin conjugated gold nanoparticles
AuNPs	Gold nanoparticles
FTIR	Fourier-transform infrared spectroscopy
O.D.	Optical density
M-NPs	Metal nanoparticles
MIC	Minimum inhibitory concentration
Pt-NPs	Platinum nanoparticles
PI	Propidium iodide
Q-AgNPs	Quercetin functionalized silver nanoparticles

rpm	Revolutions per minute
SEM	Scanning electron microscopy
SPR	Surface plasmon resonance
TEM	Transmission electron microscopy
UV-vis	Ultraviolet-visible spectroscopy
XRD	X-ray diffraction
ZnO	Zinc oxide nanoparticles

LIST OF FIGURES

Figure No.	Title	Page No.
1.1	Milestone in the development of nanotechnology	3
1.2	Nanomaterial's classification based on dimension	4
1.3	Nanomaterial's classification based on composition	5
1.4	Variations in the colour of gold nanospheres, nanorods, and nanocages with respect to change in size	10
1.5	Different shapes of AuNPs	11
1.6	Approaches of M-NPs synthesis	15
1.7	Various biological sources and biomolecules explored for M-NPs synthesis	21
1.8	Comparison among various synthesis approaches of M-NPs	21
1.9	Mechanisms of metal (silver) nanoparticle synthesis by (a) bacteria, (b) fungi, and (c) algae	23
3.1	UV-vis absorption spectrum of auric chloride solution (1.25 mM), gentamicin sulphate drug (0.05 mM) and G-AuNPs (2.3 nM), inset auric chloride + gentamicin sulphate solution before synthesis and synthesized G-AuNPs	42
3.2	 (a) UV-vis spectrum of G-AuNPs synthesized at various ratios (v/v), (b) UV-vis spectrum of G-AuNPs synthesised at various pH, (c) Variation in G-AuNPs colour synthesized at various ratio, (d) Variation in G-AuNPs colour synthesized at various pH 	43

3.3	(a) UV-vis spectrum of G-AuNPs synthesized at various temperatures, inset variation in G-AuNPs colour synthesized at various temperature, (b) UV-vis spectrum of G-AuNPs at various time intervals	44
3.4	Analysis of G-AuNPs stability at storage conditions (4 °C)	45
3.5	(a) TEM image of G-AuNPs, (b) DLS analysis of G-AuNPs	46
3.6	Evaluation of antibacterial activity by G-AuNPs against (a) <i>E. coli</i> DH5α, (b) <i>E. coli</i> ATCC 25922, (c) <i>E. fergusonii</i> ATCC 35469, and (d) <i>S. aureus</i> MTCC 3160	47
3.7	Effect on ROS levels due to G-AuNPs treatment on microorganisms	49
3.8	Effect on LPO levels due to G-AuNPs treatment on microorganisms	49
3.9	 Measurement of G-AuNPs effect on the permeability of bacterial membrane (1) <i>E. coli</i> DH5α, (2) <i>E. coli</i> ATCC 25922, (3) <i>E. fergusonii</i> ATCC 35469, and (4) <i>S. aureus</i> MTCC 3160 	50
3.10	Fluorescent imaging of microorganisms using propidium iodide dye with and without G-AuNPs treatment.	51
3.11	Analysis of gentamicin drug release from G-AuNPs at cell physiological pH 7.4	52
3.12	Investigation of cytotoxic effects of G-AuNPs, PBS and DMEM on C2C12 cell line	53

4.1	UV-vis spectrum of silver nitrate, quercetin and Q-AgNPs (dilution factor 2.5), inset colour of synthesized Q-AgNPs	61
4.2	Effect of various amounts of quercetin (volume/volume) on Q-AgNPs synthesis	62
4.3	Effect of various pH conditions on the Q-AgNPs synthesis (dilution factor 2.5)	62
4.4	 (a) The UV-vis spectrums of stored (4 °C) Q-AgNPs at various time, (b) Q-AgNPs UV-vis spectrums at various pH conditions, (c) Q-AgNPs UV-vis spectrums after treatment against various solvents, (d) Q-AgNPs UV-vis spectrums after treatment at various temperature 	63
4.5	Q-AgNPs XRD patterns with and without L-Cys	64
4.6	(a) FTIR spectrum of AgNO ₃ , quercetin, and Q-AgNPs, (b) Inset magnified region of Q-AgNPs from 1700-1000 cm ⁻¹	65
4.7	SEM images of Q-AgNPs (a) without L-Cys, (b) with L-Cys, TEM images of Q-AgNPs (c) without L-Cys, (d) with L-Cys, and DLS analysis of Q-AgNPs (e) without (f) with L-Cys	67
4.8	(a) Effects caused in the Q-AgNPs UV-visible spectrum with addition of L-Cys (red line) in comparison to control Q-AgNPs lacking L-Cys (black line), inset Q-AgNPs colour in presence and absence of L-Cys (b) A graphical illustration of the Q-AgNPs biosensing mechanism	69
4.9	(a-f) Effects of varying temperatures on L-Cys sensing (*C corresponds to control (Q-AgNPs without L-Cys), while T corresponds to test (Q-AgNPs with cysteine))	70

4.10	 (a) Variations in the UV-vis spectrum of Q-AgNPs at various time of sensing (b) Normalized UV-vis spectra of Q-AgNPs at various time of sensing (a) Selectivity enclosing of Q. AcNPs towards contains in contract to a spectra of the sensitivity of the sensitity of the sensitivity of the sensi	71
4.11	 (a) Selectivity analysis of Q-AgNPs towards cystelle in contrast to other amino acids through UV-vis spectroscopy (b) Changes in Q-AgNPs colour against different amino acids* * C-control, 1: L-Tyr, 2: L-Lys, 3: L-Asn, 4: L-Ala, 5: L-Gln, 6: L-Asp, 7: L-Phe, 8: L-Val, 9: L-Leu, 10: L-His, 11: L-Met, and 12: L-Cys 	72
4.12	(a) UV-vis spectrum of Q-AgNPs at different aqueous concentration of L-Cys (0.25-15 μ M) (normalized spectra), inset wavelength shifting (b) Regression line displaying a red shift with respective L-Cys concentration, (c) Colour change in Q-AgNPs at different L-Cys concentration in water sample	73
4.13	(a) UV-vis spectrum of Q-AgNPs having different concentration of L-Cys in urine solution (1.25-20 μ M) (normalized spectra), inset wavelength shifting (b) Regression line displaying a red shift with respective L-Cys concentration (c) Colour change in Q-AgNPs at different L-Cys concentration in urine sample	74
4.14	(a) UV-vis spectrum of Q-AgNPs having different concentration of L-Cys in FBS solution (1.25-15 μ M) (normalized spectra), inset wavelength shifting (b) Regression line displaying a red shift with respective L-Cys concentration (c) Colour change in Q-AgNPs at different L-Cys concentration in urine sample	75

LIST OF TABLES

Table No.	Title	Page No.
1.1	Characteristics of different kind of nanoparticles	7
3.1	Measurement of inhibitory zones (diameter in mm) produced by G- AuNPs and gentamicin sulphate drug molecules	48
3.2	Evaluation of MIC against gram-positive and gram-negative bacteria	48
4.1	Description of functional groups as revealed by FTIR analysis of silver nitrate, quercetin and Q-AgNPs	66
4.2	Shifting in the wavelength of Q-AgNPs at varying temperature conditions	71
4.3	Comparison of current research findings with existing research work	76-77