Green synthesis of Iron Oxide nanoparticles using *Datura stramonium* and their interaction with Bovine Serum Albumin

Dissertation submitted in partial fulfillment of the requirement for the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

By

RAHUL CHAUHAN (141805)

SUSHAIN KAUL (141815)

Under the guidance of

Dr. Abhishek Chaudhary

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

WAKNAGHAT

May 2018

TABLE OF CONTENTS

		Page No.
	DECLARATION BY SCHOLAR	**ii**
	SUPERVISOR'S CERTIFICATE	**iii**
	ACKNOWLEDGEMENT	**iv**
	LIST OF ACCRONYMS AND ABBREVIATIONS	**v**
	LIST OF FIGURES	**vi**
	LIST OF TABLES	**vii**
	ABSTRACT	**viii**
1	**CHAPTER 1: INTRODUCTION**	**1**
1.1	**SYNTHESIS OF NANOPARTICLE**	**2**
	1.1.1. Green synthesis	**2**
1.2	**APPLICATION**	**3**
1.3	**CHARACTERIZATION TECHNIQUES**	**3**
	1.3.1 SEM	**3**
	1.3.2 UV-Vis Spectroscopy	**4**
	1.3.3 DLS	**4**
	1.3.4 CD Spectroscopy	**4**
	1.3.5 Fluorescence Spectroscopy	**5**
2	**CHAPTER 2: REVIEW OF LITERATURE**	**6**
3	**CHAPTER 3: MATERIALS AND METHODS**	**11**
3.1	**MATERIAL USED**	**12**
	3.1.1 Chemicals used	**12**
	3.1.2 Other material	**12**
	3.1.3 Apparatus used	**12**
3.2	**METHOD**	**14**
	3.2.1 Green synthesis of iron oxide nanoparticles	**14**
		15

3.2.2 Interaction of nanoparticles with BSA

4 CHAPTER 4: RESULT AND DISCUSSION 16

4.1 OPTIMIZATION 17

4.1.1 Iron oxide synthesis by *Datura stramonium* 17

4.2 CHARACTERIZATION 18

4.2.1 SEM 18

4.2.2 EDS 19

4.2.3 DLS 20

4.3 INTERACTION OF IRON OXIDE WITH BSA 20

4.3.1 UV-Vis spectroscopy 20

4.3.2 Fluorescence spectroscopy 21

4.3.3 CD spectroscopy 23

5 CHAPTER 5: SIGNIFICANCE OF THE PROJECT 24

6 REFERENCES 26

DECLARATION BY THE SCHOLAR

We hereby certify that the work presented in this B-Tech thesis entitled "**Green synthesis of iron oxide nanoparticles using *Datura stramonium* and their interaction with Bovine Serum Albumin**" submitted at **Jaypee University of Information Technology, Waknaghat, 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.

Rahul

Rahul Chauhan

Sushain Kaul

Sushain Kaul

Department of Bioinformatics and Biotechnology

Jaypee University of Information Technology, Waknaghat, India

Dated: 17 May 2018

SUPERVISOR'S CERTIFICATE

This is to certify that work reported in the B-Tech thesis entitled "**Green synthesis of iron oxide nanoparticles and their interaction with Bovine Serum Albumin**" submitted by **Rahul Chauhan and Sushain Kaul** at **Jaypee University of Information Technology, Waknaghat, India,** is a bonafide of their original work carried out under my supervision. This work has not been submitted to elsewhere for any other degree or diploma.

Dr. Abhishek Chaudhary

Assistant Professor

Department of Bioinformatics and Biotechnology

Dated: 17 May 2018

ACKNOWLEDGEMENT

We take this opportunity to express our deepest gratitude and appreciation to all those who have helped us directly or indirectly towards the partial completion of this project.

First and foremost, we would like to express our sincere appreciation and gratitude to our project supervisor Dr. Abhishek Chaudhary, Assistant Professor, Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, for his insightful advice, encouragement, guidance, critics and valuable suggestions throughout the course of our project work. Without his continued support and interest, this project would not have been the same as presented here.

We express our deep gratitude to Dr. Sudhir Syal, HOD, Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, for his constant co-operation, support and for providing necessary facilities throughout this program.

Also, we would like to express our thanks to teaching and non-teaching staff in the Department of Biotechnology and bioinformatics, Jaypee University Of Information Technology, Waknaghat for their invaluable help and support during this period as well as thanks to the IIT Mandi and Punjab University, Chandigarh for providing help with the characterization of our nanoparticles and letting us use their equipment for our project.

Our special thanks to our parents, supporting families and friends who continuously supported and encouraged us in every possible way for the successful completion of this project.

Last but not the least; we thank God Almighty for his blessings without which the completion of this project work would not have been possible.

Rahul Chauhan (141805)

Sushain Kaul (141815)

LIST OF ACRONYMS AND ABBREVIATIONS

CD	Circular Dichroism
cm	Centimeter
DLS	Dynamic Light Scattering
EDS	Energy Dispersive Spectrophotometry
Fe_3O_4	Iron Oxide
FTIR	Fourier Transform Infrared
IONP'S	Iron oxide nanoparticles
JUIT	Jaypee University of Information Technology
mm	Millimeter
µm	Micrometer
MPI	Magnetic particle imaging
MRI	Magnetic resonance imaging
nm	Nanometer
NPs	Nanoparticle
SAIF	Sophisticated Analytical Instrumentation Facility
SEM	Scanning Electron Microscopy
UV-Vis	Ultra Violet Visible
XRD	X-ray diffraction

LIST OF FIGURES

Figure No.	Caption	Page No.
4.1	Color change in reaction solution w.r.t. time	17
4.2	UV-Vis graph representing change in absorbance w.r.t. time	18
4.3	SEM images for iron oxide nanoparticles	18
4.4	EDS graph representing various elements present in solution	19
4.5	DLS graph to depict size of nanoparticles	20
4.6	UV-Vis graph for interaction of nanoparticles with BSA	21
4.7	Fluorescence graph for interaction of nanoparticles with BSA	21
4.8	Graph of I_o/I v/s concentration for quenching constant	22
4.9	Graph representing CD spectra for BSA, leaf extract and nanoparticles	23

LIST OF TABLES

Table No.	Caption	Page No.
3.1	Variable concentration of nanoparticles keeping BSA constant	15

ABSTRACT

Nano biotechnology is the most upcoming and promising field in the area of research in medical sciences .Over the years metal nanoparticles has been of great importance to researchers. Due to this iron oxides have attracted a significant amount of attention among researchers because of their multivalent oxidation states. The iron oxide nanoparticles are being synthesized using various methods. In our work we have synthesized iron oxide nanoparticles by using *Datura stramonium* plant. The synthesized nanoparticles were characterized using ultra violet visible spectrum (UV-Vis), X-Ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM).SEM image tells us about the morphology and size of the nanoparticles. Further UV-Vis, fluorescence and CD spectroscopy and was used to check the interaction of iron oxide nanoparticles with BSA.

Chapter 1:
INTRODUCTION

INTRODUCTION

Nanotechnology, a briskly developing field, is a blessing to the society. It is basically science of miniature components and its further manipulation. Components are smaller than 100 nm in size, and 1 nm equivalent to 10^{-9} meters. It is a proven fact that particles with such miniature size have different physical and chemical properties as contrast to its bulk form that has resulted in improved applications. If we have knowledge about controlling the structural design and formulations of the iron oxide nanoparticles particles, then it has utmost advantage to sectors like electronic industry, food industry, textile industry and medicine etc. [1]. The ever increasing need of these iron oxide nanoparticles in various areas leads to variety of synthesis procedures which are cost cutting and has no toxicity effects towards the environment.

1.1 Synthesis of nanoparticles

The nanoparticle synthesis can depend on two basic parameters i.e. chemical and physical route that is expensive and moreover usage of the nanoparticles gets restricted because of the presence of the compounds that may be toxic to environment. Green synthesis is the method which overcomes the restriction of these physical and chemical methods. Green synthesis, a method for synthesis of nanoparticles with the help of plant metabolites which act as reducers for synthesis which results into a product that are environment friendly and determined by methods these synthesized products cause less energy usage and harmful waste production is also limited[2]. The various synthesis approaches include the bottom up and top down methodology, in bottom up methodology there is formation of nanoparticles from small size particles varying from the scale of angstrom in contrast to this methodology top down methodology leads to the structure formation from the scale of μm. For the synthesis of nanoparticles we have used green synthesis methodology in our project.

1.1.1 Green synthesis

Nanoparticle synthesis can be done with more than one method. One eco-friendly technique that has gained popularity among researchers nowadays is the "**Green synthesis**". This methodology includes usage of extract from plant which act as reducers and stabilize the formed nanoparticles. Green Synthesis from various metal NPs which are gold, silver, iron, copper and platinum have proved their potential in many research areas [3]. In this project, we used leaf extract of *Datura stramonium* for nanoparticle synthesis of iron oxide and its interaction with BSA. This methodology act as a useful alternative to all other physical and chemical methods in terms of usage of

energy, toxicity and eco friendliness. The polyphenolic compounds present in plant reduce the metal ions into nanoparticles and moreover the morphology of nanoparticles i.e. shape can be controlled by changing temperature of medium. [4].

1.2Applications

A Nanoparticle have many important properties like special optical properties, conducting properties, and a large surface area to volume ratio [5]. Due to these properties their usage in various fields is ever increasing and their small size is an added advantage for researchers. It provides the required machinery and technological platform for detailed study and manipulation of various biological entities, where biology provides inspiring models and assembled biology based components to Nano technological grounds. There is wide range nanoparticle applications lying in tissue and medicine engineering, material and manufacturing like for making protective coatings, nanoadjuvents for targeted drug delivery, and on environmental grounds for making biosensors [6].

1.3 Characterization Techniques

Characterization is an important factor after nanoparticle synthesis and it can be done with various analytical methodologies, which includes UV visible spectroscopic technique, X-ray Diffractometry (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) etc. [7]. For characterization of iron oxide nanoparticles, in this project we have done UV-Visible Spectroscopic technique, Transmission Electron Microscopic technique, and Dynamic Light Scattering as given down below:

1.3.1 SEM (Scanning Electron Microscopy)

Scanning Electron Microscopy converge electron beam to produce a series of signals from the surface of dry specimen. These signals cause interaction of sample and beam of electron which gives information about the specimen which includes information about texture (morphological properties), chemical build-up, and material orientation of the sample. In applicative form, collection of data from surface of specimen and a two dimension image is created that will show space arrangements and variation of properties. In scanning mode 1cm to 5 μm areas can generate an image with convention techniques of SEM (20X TO 30,000X of magnification and space arrangement of about 50nm to 100nm) [8].

SEM can select point on sample which can be further analyzed. This methodology can semi quantitatively determine elemental detection using EDS and orientation of crystals.

1.3.2 UV-Vis Spectroscopy

An absorption spectroscopic technique whose spectra lies between ultra-violet(400nm) and visible spectrum (750nm),this technique is applied to molecules present in solution and is best suitable for quantitative analysis. This technique is based on Beer-Lambert law which determines the concentration of analyte by absorbing at a particular wavelength [9]. Deuterium discharge which act as light source for UV and for visible region tungsten halogen lamp is used and during their usage the switch accordingly. From the source light is spread before it gets to the sample. We have done iron oxide NP characterization at JUIT with the help of UV-Visible spectrophotometer and we have obtained spectral curves between absorbance vs concentration which confirms the nanoparticles synthesis.

1.3.3 DLS (Dynamic Light Scattering)

In this methodology size of particles is determined and it goes parallel with the Brownian motion principle. Nanoparticles can be of smaller and bigger size, this size difference will change their trajectory of motion in the medium and the light will be fluctuated accordingly with respect to size [10]. These fluctuations are analyzed which tells us about the hydrodynamic radius of the particle.in our project we used DLS instrument from IIT Mandi for nanoparticle characterization.

1.3.4 CD (Circular Dichroism)

The CD spectra is measured to know about the change in the secondary structure of the BSA in presence of Iron Oxide nanoparticles.CD spectroscopy is a form of light absorption spectroscopy that measures difference in the absorbance of right- and left-circularly polarized light (not the commonly used absorbance of isotropic light) by a substance. For an all α helical serum protein, we get two negative bands at 222 and 208 nm, in its CD spectra. The 222 nm band relates to the strong hydrogen bonding of α helices and is relatively independent of their length. For an all β sheet serum protein, we get, in general, a negative band at around 208 nm and a positive band between 195-200 nm in its CD spectra [11]. The mean Molar Residual Ellipticity (MRE), gives a direct quantitative measurement of loss of α-helical structure of protein. The data

thus obtained suggests that the nanoparticles and their surface fictionalization plays a major role in protein adsorption and its structural change. All these data were interpreted using the newly established theoretical simulation method.

1.3.5 Fluorescence Spectroscopy

Fluorescence and phosphorescence involves photon emission that takes place during molecular relaxation from electronic excited states. These photonic activities involves changing between electronic and vibrational states of polyatomic fluorescent molecules (fluorophores). Fluorescence spectrophotometry is a class of techniques, which assay the state of a biological system by studying its interactions with fluorescent probe molecules. This interaction is observed by measuring the variations in the fluorescent probe optical properties.

Chapter 2:

Review of Literature

REVIEW OF LITRATURE

Nanotechnology, a science that is still in its developing stage is a science which uses all the different fields of science, which are material sciences, chemistry, biology and physics. When even this term was not introduced a long time back Richard Feymann said *"There is plenty of room at the bottom"* at a conference and told that now scientists could work on single atom [12]. After extensive research on the idea, nanotechnology term was given by Norio Taniguchi and from that period there is no looking back, and has made benchmark in the field of research and technology. Within these years there has been a sole research on nanotechnology that has surprised with its vast benefits to the human race. Its majorly affected fields are medicine, food industry, environmental science and IT sector and its influence on these sectors are ever increasing. This has been feasible due to the exploitation of application of nanoparticles which is otherwise not possible on macro scale particles. These nanoparticles have vast usage weather in technology claimed by researches or in daily life products like battery, various types of sensors etc. [13].

Nowadays, these NPs has extensive usage in utmost each sector of economic importance all due to their growing need day by day. With its mounting desire, the threat to environment safety is also increasing and need for sustainable methods for nanoparticle synthesis. The common methodology for making nanoparticles are physical and chemical methodologies. Big concern linked with methodologies like these are the hazardous nature to environment, and their byproduct treatment [14]. Thus we keeping in mind that we need to synthesize nanoparticles with efficient and cleaner method. A substitute to these traditional methodologies for nanoparticles synthesis is "green synthesis", an eco-friendly technique that uses material from plants, their parts, and miniature organisms like bacteria and fungi. [15]

Particles produced by this nature of production are quiet stable and size accurate. These properties are due to its one reaction nature due to which it has superiority over other traditional methods. Moreover, in this method there is low energy consumption making it a very efficient eco-friendly technique for nanoparticle production. It facilitates academic field researches and researches going in other commercially active sectors industrial sector and medicine based sectors etc. [16].

The particles created by these eco-friendly methodologies has their usage in electronics pharmaceutical area, emitters, optics, textile manufacturing units etc. In this project major concentration is on metal nanoparticles synthesis by green synthesis with the help of the presence of polyphenolic compounds in plant extract [17].

This report, demonstrates the nanoparticle synthesis by using plant extract of *Datura stramonium* leaf. This plant has huge applications in medicine field because of its various properties, *Datura stramonium* is known for its high medicinal value.it is commonly found in tropical region of India. Constituents of *Datura stramonium* that are of are of major importance are scopolamine and Datura diol. Mainly parts of *Datura* plant for research purpose is leaf, flower and roots. These parts are highly poisonous and to be used in controlled dosage if taking orally. Its major medicinal properties are anti-bacterial, anti-microbial properties moreover it is also used in treatment of asthma etc. [18].

Extract of plant is composed of phytochemicals basically causes reduces metal ions to nanoparticles.it consist of metabolites such as terpenes, phenolic acids, various alkaloids and different proteins which are causing bio reduction of metal ions into nanoparticles. Moreover these compounds also provide with stability to the formed particles. These metabolites are composed of flavonoids , organic acids and quinones that can cause these particles to quickly get reduced to nanoparticles[19] .The used Metal ion for the nanoparticle synthesis are productive as antimicrobial agents because they are delivered into the cell by the cell membrane where they are assembled and microbial cell are killed. This usually happens due to the oxidation and reduction taking place in wall of cell that causes these metal ion to attach to proteins present in cells which causes damage to cell wall. [20].

Iron oxide NPs have attracted interest of many researchers all over the world due to their property of superparamagnetism. Magnetite and maghemite are two most frequently studied iron oxides. Iron oxide could be derived in four differential phases in natural environment. Which includes $Fe_{1-x}O$ (wustite), $\alpha-Fe_2O_3$ (hematite), $\gamma -Fe_2O_3$ (maghemite) and Fe_3O_4 (magnetite). Conventionally, there has been wide usage as ores of iron and in costly dyes in decorates and coats. In the previous two spans, medical and bio analytics uses of iron-oxide nanoparticles has been instituted mainly by the developing and mounting of Nano-technology and Nano biotechnology[21].

Currently, iron-oxide nanoparticles are one of the utmost universally used medical agents for a large diversity of diagnosis and therapeutical approaches like magnetic resonance imaging (MRI), hypothermia, similar magnetic bioassays , magnetic particle imaging (MPI) , Nano-adjuvants for targeted drug delivery , gene delivery , photo contrasting agents etc[22]. The production of nanoparticles of iron oxide can be done by variety of approaches, some of these are chemical based reduction and precipitation methods, co-precipitation methods(in absence of oxygen), Micro-emulsion method, Chemical Sol Method, Revised co-precipitation approach, Thermal Decomposition, Borohydride Method, Sol-Gel Method, cyclic chemical synthesis process modified solve-thermal method and reduction method [23]. But due to their

expensive substrate consumption, need for sophisticated instruments, controlled temperature requirements and additional cost involving treatment of waste water as a by-product to these methods, these are some of the disadvantages which makes green synthesis which we have applied in our project for nanoparticle synthesis a superior choice over all other method.

Our main aim is to check the iron oxide nanoparticles synthesis with BSA (Bovine Serum Albumin) which has been one of the most widely considered proteins, mainly thanks to its structure homogeneity towards HSA (Human Serum Albumin).It is a mini protein which has single polypeptide chain that is crosslinked by 17 disulfide bonds. BSA has many biochemical properties such as ELISA and immune-histochemistry. Bovine serum albumin is also used in stabilization of some enzymes during DNA digestion [24]. BSA has its wide usage due to structural homology to HSA and its stability which help in increasing signaling assays. Moreover it is biocompatible and does not interfere in nature and it comes with the advantage of low production cost.

Nanoparticles of iron oxide were also produced using an entirely green biosynthesis method by reducing ferric chloride solution by using brown seaweed water extract two seaweeds "*Padinapavonica* (Linnaeus) *Thivyand Sargassum acinarium* (Linnaeus) Setchell 1933 were used in this study"[25]. Extract of this algae was used as a reducing agent of $FeCl_3$ which resulted in photosynthesis of Fe_3O_4-nanoparticles.The Fe3O4-NPs with phytogenic properties were categorized with band observed in surface Plasmon which was close to 402nm and 415 nm; the attained Fe_3O_4-Nanoparticles fall in sizes which ranges from 10nm to 19.5nm and 21.6nm to 27.4nm for *P. pavonica* and *S. acinarium*, respectively. In EDX technique iron signals were reported very strong in spectra. [26].

One recent study showed the interaction between Bovine Serum Albumin and magnetic Nano- particles was determined by FL, UV-vis, Raman scattering, and CD spectroscopic techniques. A model that is completely based on static quenching which is further based on quenching rate constants (Kq) and UV-visible absorption spectra was evidenced practically. The interactions found between magnetic nanoparticles and Bovine Serum Albumin was natural and the electrostatic interactions had a key role in whole process. The study of UV-visible, Raman scattering, and CD spectra strongly pointed that there was a dramatic change in the secondary structure of BSA in existence of magnetic nanoparticles [27]. Future scopes engaging Magnetic nanoparticles will be partial due to the fact that Magnetic nanoparticles has noticeable protein effects. That is why, it is imperious that their biocompatibility and biosafety of Magnetic nanoparticles was given most of concerns and their further studies must be conducted to attain a profounder of their biological effects.

By manipulating these properties of nanoparticles, not simply the problem of drug toxicity has been resolved but to a degree also has managed to the improvement of an effective drug transport system. In an effective drug transport system few things are of utmost importance. Firstly, Target specificity of the drug should be high and drug release should be controlled. [28]. This controlled release limits the side effects caused by these antimicrobials. The nanoscale of these particles minimizes the side effects that can be caused. They also enhance the uptake of the insoluble or less soluble drugs because of their larger surface area and target the drug to the specific site of infection. Another advantage is that they are highly efficient even if taken in small doses. Using these methods have directed to superior results and therefore an opportunity of great progression in this field.

Chapter 3:

MATERIALS AND METHODS

Materials and Methods

3.1Materials Used:

3.1.1 Chemicals

1. $FeCl_3$
2. Ethanol

3.1.2 Other Materials

1. *Datura stramonium* leaves
2. Distilled water
3. Bovine Serum Albumin (BSA)

3.1.3 Apparatus

1. Gloves
2. Cotton
3. Beaker
4. Conical Flask
5. Vials (1.5ml, 2ml, 15ml, 50ml)
6. Magnetic Stirrer
7. Magnetic Bead
8. Glass Rod
9. Hot Plate
10. Micropipette
11. Tips
12. Refrigerator
13. Weighing Balance
14. What man Filter Paper
15. Para film
16. Hot Air Oven
17. Petri plates

18. Measuring Cylinder
19. Quartz Cuvette

3.2Method:

3.2.1 Green synthesis of Iron Oxide nanoparticles

The following procedure was standardized for synthesis of Iron Oxide nanoparticles:

1. Preparation of leaf extract:-
    a. Fresh leaves of *Datura stramonium* were collected from the plants in the university campus.
    b. Leaves were washed using distilled water and kept for drying for some time.
    c. A few leaves were taken and crushed finely using mortar pestle.
    d. 6g of this crushed sample was weighed using weighing balance and put in a conical flask.
    e. 100-150ml of distilled water was added to this conical flask and the mixture was made to boil for 30-40minutes at 100 ^{o}C [29].
    f. The solution was then filtered twice using What man filter paper to obtain pure leaf extract.
    g. The extract was stored at 4 ^{o}C for further use.
2. Preparation of Ferric chloride solution:-
    a. Salt of Ferric Chloride was weighed to prepare 0.5M solution using a weighing balance.
    b. Distilled water was measured using a measuring cylinder according to the concentration and the weighed salt was added to it.
    c. The thus prepared solution was kept aside for further use.
3. Green synthesis of nanoparticles:-
    a. The prepared plant extract and ferric chloride solution were taken in 1:1 ratio (20ml plant extract and 20 ml 0.5 M Ferric Chloride solution)[30].
    b. The extract and the solution were mixed together.
    c. The solution of extract and 0.5M Ferric Chloride salt was put on a hot plate with magnetic stirrer for 35 min at 80-90 ^{o}C.

d. After the reaction was complete, the resulting solution was kept under observation to check any color change for 24 hrs.

e. The synthesized nanoparticle was then confirmed using UV-Vis spectroscopy.

f. The prepared nanoparticles was then kept to use for further characterization and analysis.

3.2.2 Interaction of Iron Oxide nanoparticles with BSA

1. Preparation of BSA stock solution:-

   a. Dry BSA was weighed using weighing balance to prepare 100μM of solution.

   b. Distilled water was measured using measuring cylinder according to concentration and the weighed BSA was added to it and mixed well.

   c. The thus prepared solution was stored at 4 ^{o}C for further use.

2. Checking interaction between nanoparticles and BSA:-

   a. Variable amount of nanoparticles were added to solutions of BSA and water where the amount of BSA was kept constant and water was varied to make a solution of 1.5ml as represented below:

S. No.	Water	BSA	Nanoparticles
1.	1350	150	0
2.	1349	150	1
3.	1348	150	2
4.	1345	150	5
5.	1340	150	10
6.	1330	150	20
7.	1320	150	30
8.	1300	150	50
9.	1280	150	70
10.	1250	150	100

Table 3.1: Variable concentration of nanoparticles keeping concentration of BSA constant

   b. These samples were then stored at 4 ^{o}C and interaction was checked using UV-Vis spectroscopy and Fluorescence spectroscopy.

Chapter 4:

RESULTS AND DISCUSSION

Results and Discussion

4.1 Optimization

4.1.1 Iron Oxide nanoparticles synthesized by *Datura stramonium*:

The most successful and best iron oxide nanoparticles from green synthesis using *Datura stramonium* leaves was found to be in 35 minutes. Color change can be observed in the following pictures. Also we can notice that there is no significant color change between 30 and 35 minutes after which the color was observed to be constant thus indicating the completion of the reaction. The ratio of plant extract to $FeCl_3$ salt solution was optimized to 1:1 and at concentration of 0.5M of $FeCl_3$good results were achieved. Concentration of extract was best found to be 6g of leaf in 150ml of water.

The color change can be observed after every 5 minutes as shown in the picture below (Fig.4.1).

UV-Vis spectrum (Fig 4.2) also shows the increase in the concentration and gives the characteristic peak of Iron oxide nanoparticles i.e. at around 270 nm thus confirming the synthesis of Iron Oxide nanoparticles.

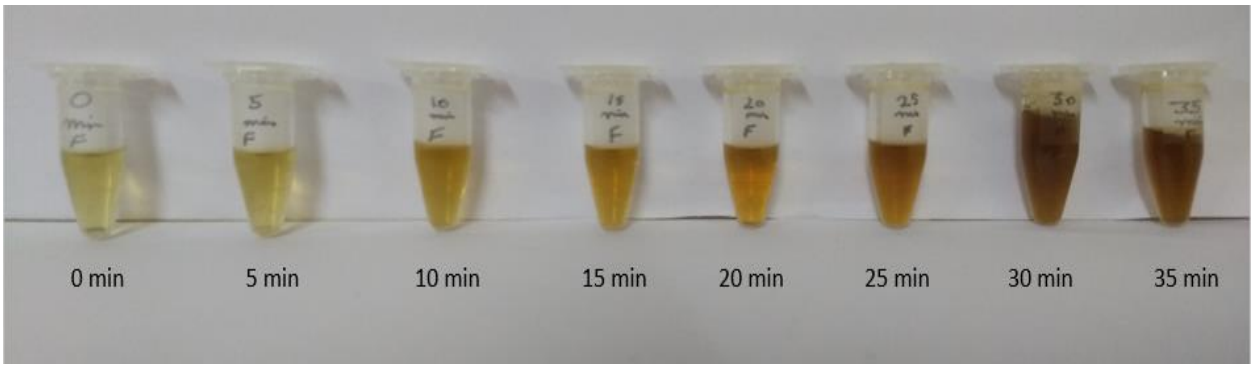

Fig. 4.1: Image representing change in colour of solution as reaction proceeds w.r.t. time

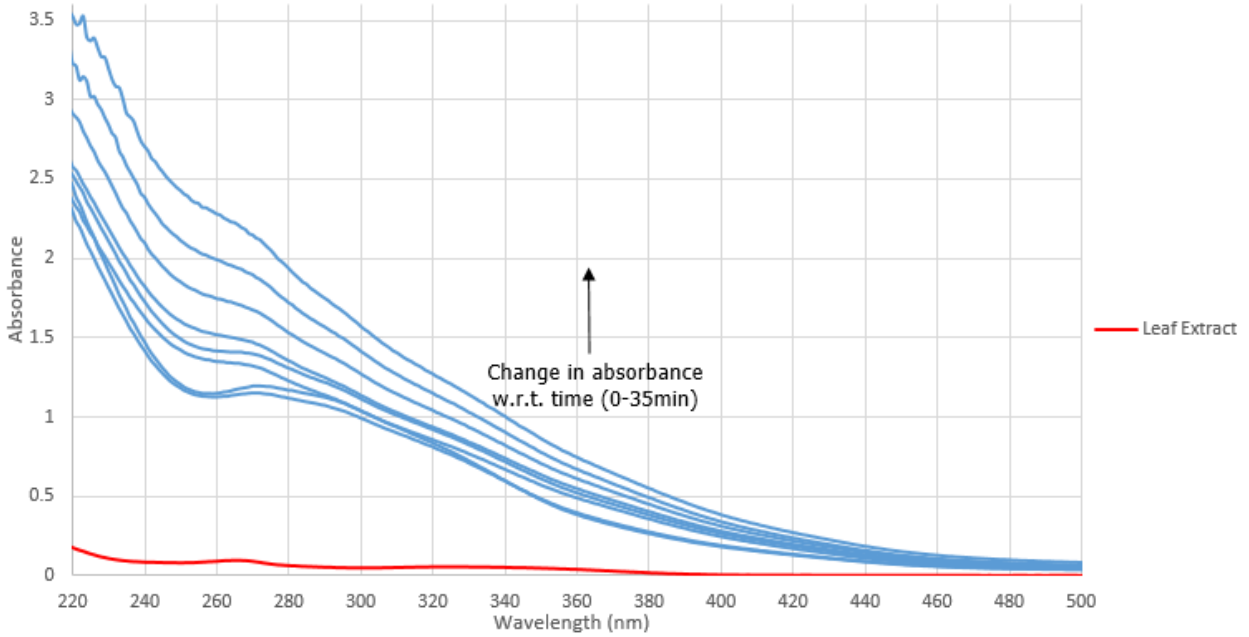

Fig 4.2: UV-Vis graph representing change in absorbance as the reaction proceeds w.r.t. time.

4.2 Characterization

4.2.1 SEM(Scanning Electron Microscopy):

Figure 4.3 shows the SEM micrograph of the iron oxide nanoparticles which was carried out at SAIF, Punjab University Chandigarh. The microscopic analysis showed that the particle size of the Iron Oxide nanoparticles was distributed in a range of 70-80 nm with an average diameter of 75±6 nm.

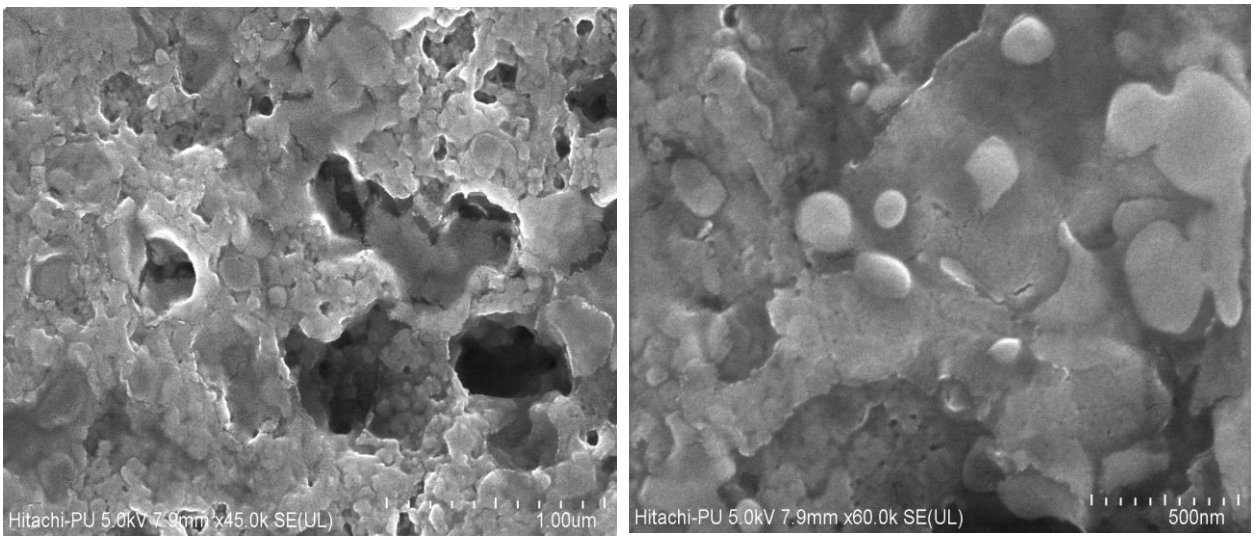

Fig. 4.3: SEM images showing iron oxide nanoparticles at different magnifications

4.2.2 EDS (Energy Dispersive X-Ray Spectroscopy):

Fig. 4.4 presents the results from EDS analysis of the Iron Oxide Nanoparticles. Strong signals at 0.28 keV, 0.58 keV, 0.71 keV and 6.4 keV in the EDS spectrum. The atomic percent values for Fe and O were 1.25 and 22.64, respectively. These results suggested that the as-prepared Iron Oxide nanoparticles were essentially free from impurities. The presence of C (atomic percent 72.05) element at 0.28 keV in the EDS spectrum should be attributed to the plant extract content in the nanocomposite. Therefore, the EDS analyses confirmed the composition and crystallinity of the as-prepared Iron Oxide Nanoparticles.

Spectrum: test 1272

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Carbon	K-series	43.16	59.98	72.05	23.28
Oxygen	K-series	18.07	25.11	22.64	12.28
Iron	K-series	3.47	4.82	1.25	0.88
Chlorine	K-series	6.47	8.99	3.66	0.89
Potassium	K-series	0.79	1.10	0.41	0.27
	Total:	71.96	100.00	100.00	

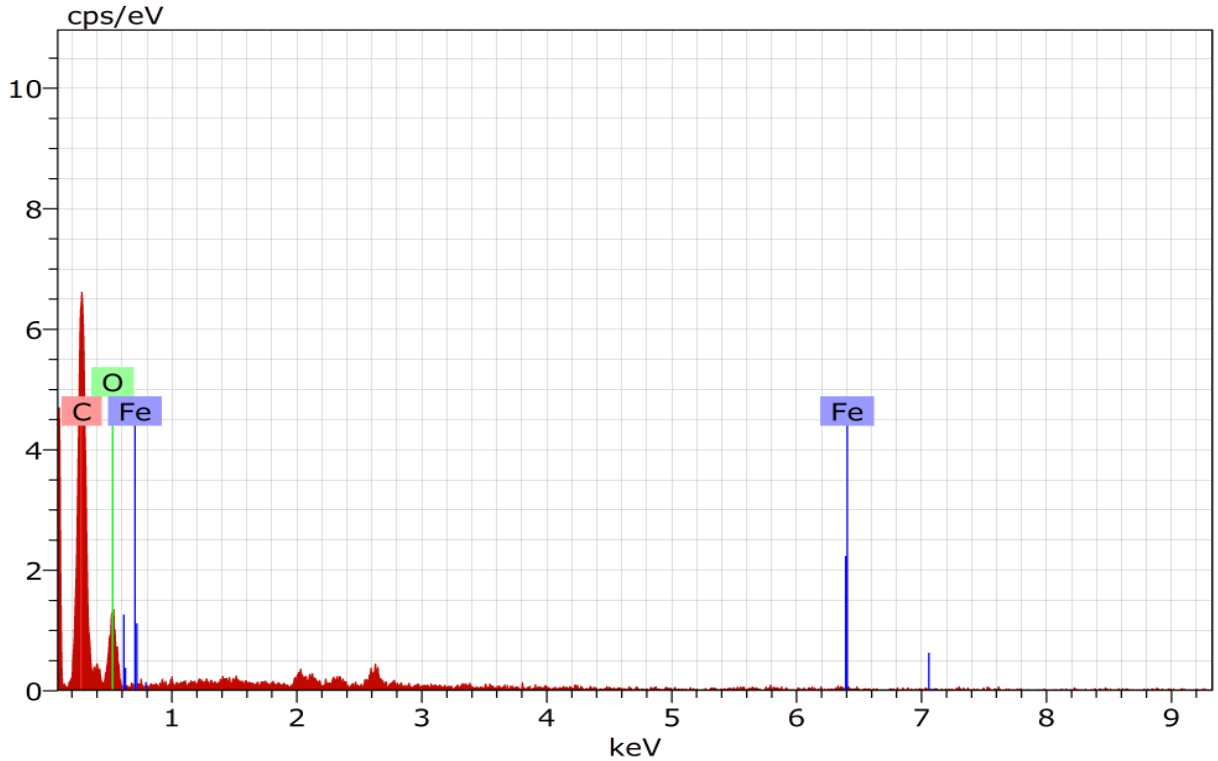

Fig 4.4: EDS graph representing various elements present in the synthesized nanoparticles solution and their relative proportion.

4.2.3 DLS (Dynamic Light Scattering):

The graph shown in figure 4.5 represents the DLS results for the iron oxide nanoparticles. The position of the peak in the graph represents the size of the synthesized nanoparticles. The size depicted here is around 100 nm which is higher than the size depicted by the SEM micrograph. This is because SEM depicts the actual radius of the particles while DLS depicts the hydrodynamic radius of the particles which is higher than the actual radius due to presence of a layer of water around the particles. The small peak which is visible at 10nm can be attributed to the irregularity in size of some nanoparticles which is common in green synthesis.

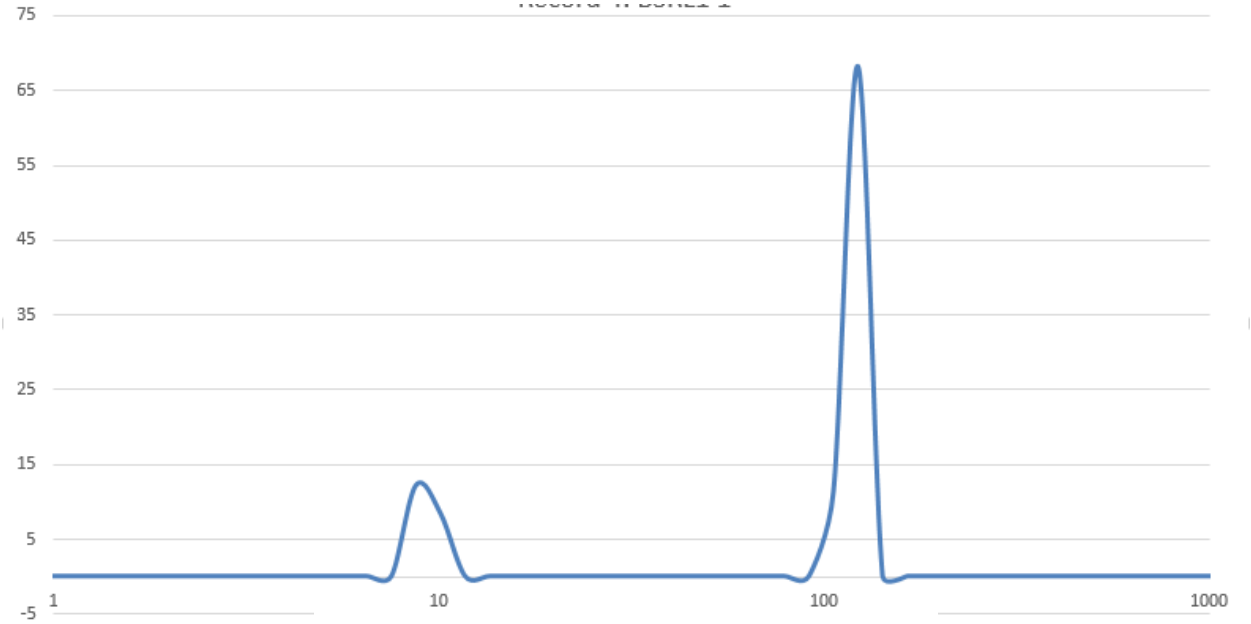

Fig. 4.5: DLS Graph representing the size of the synthesized Iron Oxide nanoparticles

4.3 Interaction of Iron Oxide with BSA

The following analysis were done to understand the interaction between the synthesized iron oxide nanoparticles and BSA:

4.3.1 UV-Vis. Spectroscopy:

The following graph shown in figure 4.6 represents the UV-Vis graph for interaction of iron oxide nanoparticles with BSA at different concentrations as shown in table 1. It can be seen from the graph that there is an upward shift in the spectrum as the concentration

of nanoparticles is increased in the solution. This shows that the interaction between the nanoparticles and BSA is taking place hence causing the upward shift in the peak which is further validated by fluorescence spectroscopy.

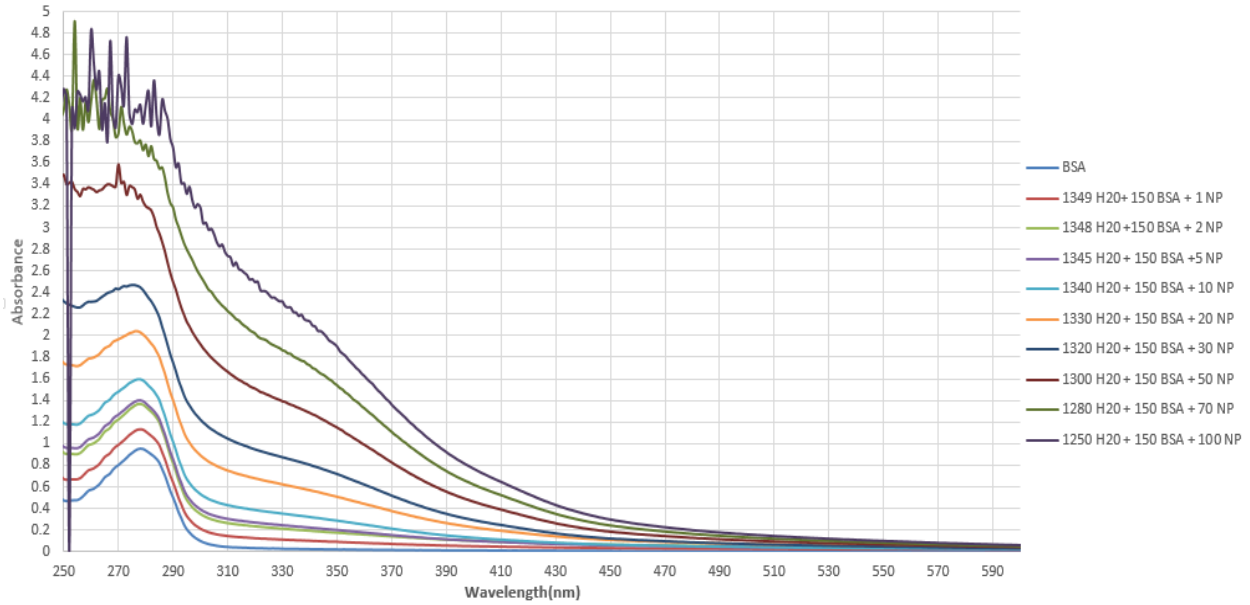

Fig. 4.6: Graph representing UV-Vis spectroscopy results for interaction of nanoparticles with BSA at various concentrations

4.3.2 Fluorescence spectroscopy:

The following graph shown in figure 4.7 represents the fluorescence spectroscopy graph for interaction of iron oxide nanoparticles with BSA at different concentrations as shown in table 1. There can be seen a downward shift in the fluorescence spectra with the increase in concentration of nanoparticles which hence shows the interaction between NP and BSA.

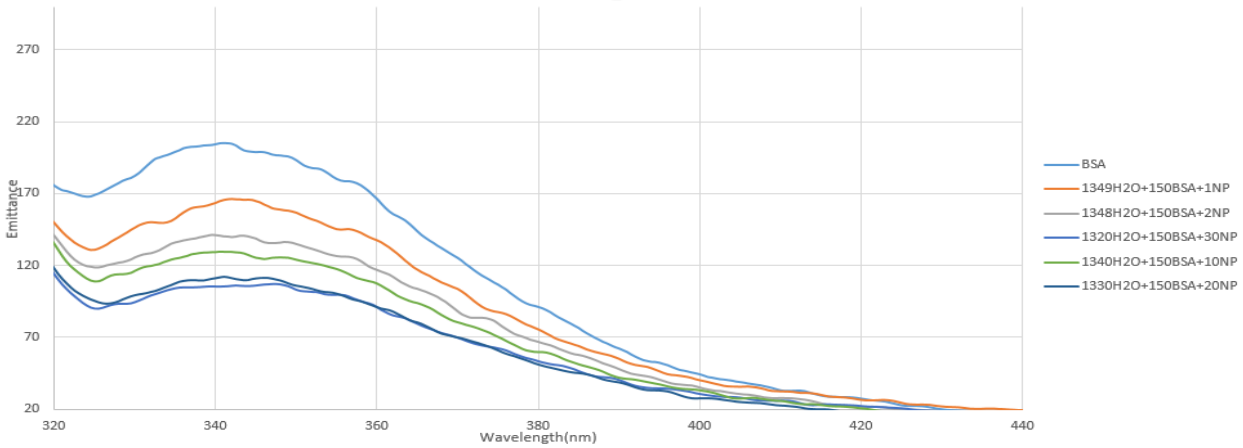

Fig. 4.7: Fluorescence graph showing downward change in the spectra with increase in concentration of nanoparticles

The graph shown in figure 4.8 is plotted for I_o/I against concentration for the samples. In this I_o is the emittance peak of BSA and I is the emittance for each respective sample. The graph was normalized to a straight line and using straight line equation the quenching constant was found out to be $13 lmol^{-1}$.

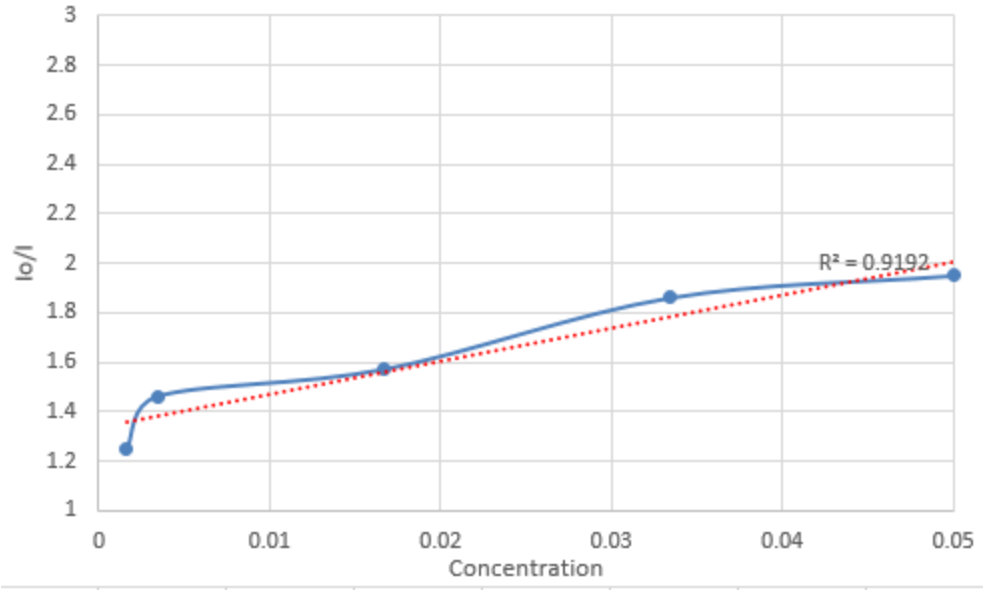

Fig. 4.8: Graph of I_o/I v/s concentration to calculate the quenching constant of the

4.3.3 CD Spectroscopy:

The following graph shown in figure 4.9 represents the CD spectra of the BSA, Extract and synthesized nanoparticles. In our results we notice a drop in the ellipticity as BSA interacts with the nanoparticles as shown in figure 9. The far UV CD spectrum in the image shows two negative minima at 208nm and 222nm. Significant changes in helicity were observed in presence of all the green synthesized iron oxide nanoparticles.

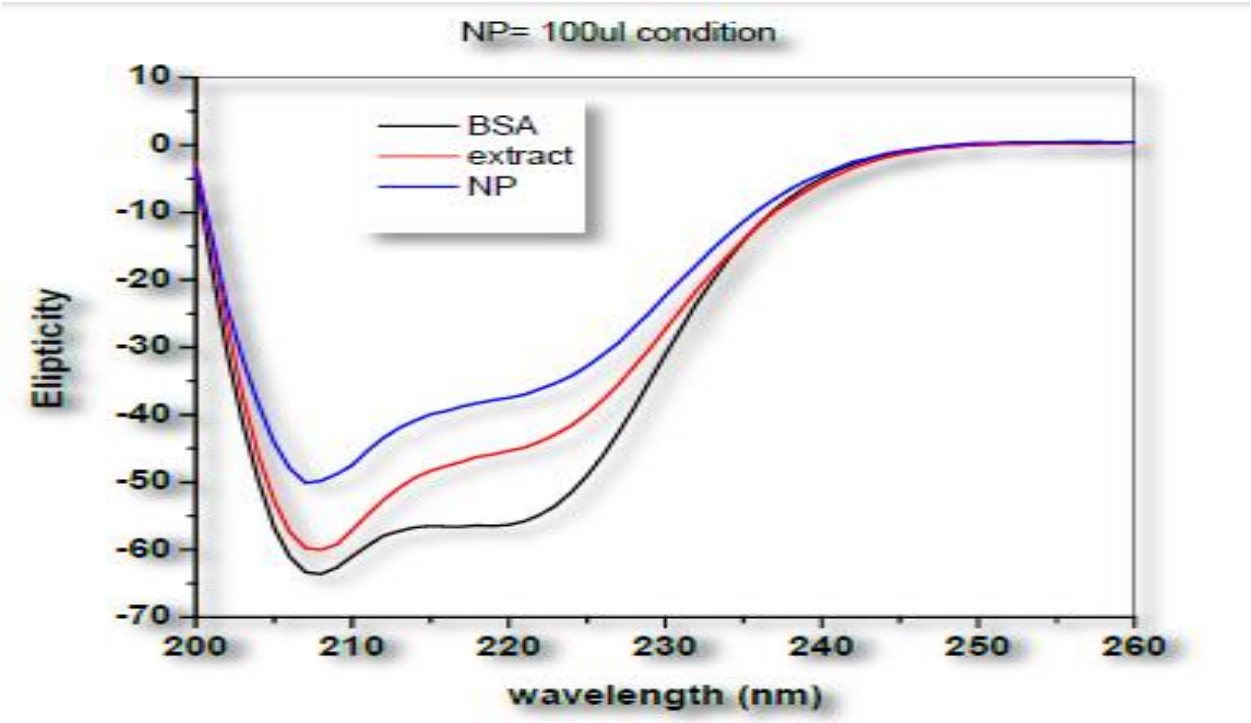

Fig. 4.9: Graph representing CD spectra for BSA, leaf extract and nanoparticles.

CHAPTER 5:
SIGNIFICANCE OF PROJECT

SIGNIFICANCE OF THE PROJECT

In these current years nanoparticles usage have drastically improved in all biological fields which are textile, pharmaceutical, medicine etc. because of its exceptional applications. various methods of its production which include toxic usage of chemicals taking high energy to overshadow this huge drawback, recent biological approaches emerging has been discussed in this project, usage of plant extract for nanoparticle synthesis and their further usage use in various fields such as, targeted drug delivery etc.[31]. One of the main benefit of the plant facilitated nanoparticles synthesis is the existence of all other active components such as antioxidants, flavonoids etc. and in this project we did "Green synthesis of iron oxide nanoparticles using *Datura stramonium* and its interaction with BSA".

With this project, we intent to surge responsiveness for the nanoparticles biosynthesis which is very much cost cutting and eco-friendly approach unlikely to all other processes which make usage of toxic chemicals. These nanoparticles produced could be applied as multimodal agents by utilizing their structural and optical properties in different applications. By studying the interaction of these nanoparticles with BSA there can be large expansion in the field of medicine by making usage commercially into ointments and medicines [32].

REFERENCES

[1] Laurent, S.; Forge, D.; Port, M.; Roch, A.; Robic, C.; Vander Elst, L.; Muller, R. N. Chem. ReV. 2008, 108, 2064

[2] Wang, J.; Wang, Y.; Gao, J.; Hu, P.; Guan, H.; Zhang, L.; Xu, R.; Chen, X.; Zhang, X. Ultrason. Sonochem. 2009, 16, 41

[3] Hyo-Jeoung Lee, Jae Yong Song and Beom Soo Kim, Journal of Chemical Technology and Biotechnology, 2013; 8(11):1971–1977.

[4] Dave P N, Chopda L V. 2014. "Application of iron oxide nanomaterials for the removal of heavy metals". Journal of Nanotechnology, 2014: Article ID 398569

[5] Kulkarni N, Muddapur U. 2014. "Biosynthesis of metal nanoparticles: a review. Journal of Nanotechnology", 2014: Article ID 510246

[6] Qu Shengchun, Yang Haibin, Ren Dawei, et al. 1999. "Magnetite nanoparticles prepared by precipitation from partially reduced ferric chloride aqueous solutions. Journal of Colloid and Interface Science", 215(1): 190–192.

[7] Kandagal, P. B.; Seetharamappa, J.; Ashoka, S.; Shaikh, S. M. T.; Manjunatha, D. H. Int. J. Biol. Macromol. 2006, 39, 234.

[8] G. Xiong, U. Pal, J. G. Serrano, K. B. Ucer, and R. T. Williams, "SEM and DLS study of iron nanoparticles: the impurity and defect perspective," Physica Status Solidi (C), vol. 3, no. 10, pp. 3577–3581, 2006.

[9] Hu, D. H.; Wu, H. M.; Liang, J. G.; Han, H. Y. Spectrochim. ActaA 2008, 69, 830

[10] Maryam Moudi, Rusea Go, Christina Yong SeokYien, and Mohd. NazreInt J PrevMed. 2013; 4(11): 1231–1235

[11]Siddhuraju, P. Becker, K., Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro climatic origins of leaves. J. Agric. Food Chem., 2003, 51 (8): 2144-2155.

[12] Balamurughan, M.G., Mohanraj, S., Kodhaiyolii, S., Pugalenthi, V. Ocimum sanctum leaf extract mediated green synthesis of iron oxide nanoparticles. Spectroscopic and microscopic studies. J. Chem. Pharm. Sci., 2014, 4: 201–204.

[13] Mazur, M., Barras, A., Kuncser, V., Galatanu, A., Zaitzev, V., Turcheniuk, K.V., Woisel, P., Lyskawa, J., Laure, W., Siriwardena, A., Boukherroub, R., Szunerits, S. Iron

oxide magnetic nanoparticles with versatile surface functions based on dopamine anchors. Nanoscale, 2013, 5:2692–2702

[14] Latha, N., Gowri, M. Biosynthesis and characterization of Fe3O4 nanoparticles using Carica papaya leaves extract. Int. J. Sci. Res., 2014, 3(11): 1551-1556

[15] Kim, D., Lee, N., Park, M., Kim, B. H., An K. and Hyeon, T. Synthesis of uniform ferrimagnetic magnetite nanocubes. J Am ChemSoc, 2009, 131: 454-455.

[16] Phumying, S., Labuayai, S., Thomas, C., Amornkitbamrung, V., Swatsitang, E., Maensiri, S. Aloe vera plant-extracted solution hydrothermal synthesis and magnetic properties of magnetite (Fe3O4) nanoparticles. Applied Physics A., 2013, m111 (4):1187– 1193.

[17] Awwad, Akl M., &Nidá M. Salem. A green and facile approach for synthesis of magnetite nanoparticles. Nanoscience Nanotechnology. 2012, 2(6), 208-213.

[18] Li, Shikuo, et al "Green synthesis of silver nanoparticles using Capsicum annuum L. extract". Green Chem., 2007, 9(8), 852-858. doi: 10.1039/b615357g

[19] Pattanayak, Monalisa& P.L. Nayak. Green synthesis and characterization of zero valent iron nanoparticles from the leaf extract of Azadirachtaindica (Neem). World. 2013, 2(1), 06-09.

[20] Lukman, A.I.; Gong, B.; Marjo, C.E.; Roessner, U. & Harris, A.T Facile synthesis, stabilization and anti-bacterial performance of discrete Ag nanoparticles using Medicago sativa seed exudates. J. Colloid Interface Sci., 2011, 353, 433–44.

[21] Lukman, A.I.; Gong, B.; Marjo, C.E.; Roessner, U. & Harris, A.T Facile synthesis, stabilization and anti-bacterial performance of discrete Ag nanoparticles using Medicago sativa seed exudates. J. Colloid Interface Sci., 2011, 353, 433–44.

[22] M. Pattanayak and P. L. Nayak, "Ecofriendly green synthesis of iron nanoparticles from various plants and spices extract," International Journal of Plant, Animal and Environmental Sciences, vol. 3, no. 1, pp. 68–78, 2013

[23] J. Banerjee and R. T. Narendhirakannan, "Biosynthesis of iron oxide nanoparticles from Syzygiumcumini (L.) Seed extract and evaluation of their in vitro antioxidant activities," Digest Journal of Nanomaterials and Biostructures, vol. 6, pp. 961–968, 2011

[24] H. Kumar and R. Rani, "Structural and optical characterization of iron oxide nanoparticles synthesized by micro emulsion route," International Letters of Chemistry, Physics and Astronomy, vol. 19, pp. 26–36, 2013.

[25] M. R. Vaezi and S. K. Sadrnezhaad, "Nanopowder synthesis of iron oxide via solochemical processing," Materials and Design, vol. 28, no. 2, pp. 515–519, 2007.

[26] B. Kumar, K. Smita, L. Cumbal, and A. Debut, "Biogenic synthesis of iron oxide nanoparticles for 2-arylbenzimidazole fabrication," Journal of Saudi Chemical Society, vol. 18, no. 4, pp. 364–369, 2014

[27]Adegoke H I, AmooAdekola F, Fatoki O S, et al. 2014. Adsorption of Cr (VI) on synthetic hematite (a-Fe2O3) nanoparticles of different morphologies. Korean Journal of Chemical Engineering, 31(1): 142–154

[28] Abdul Salam H, Rajiv P, Kamaraj M, et al. 2012. Plants: green route for nanoparticle synthesis. International Research Journal of Biological Science, 1(5): 85–90

[29] Jing-Kun Yan, Yao-Yao, Lin Zhu, Jian-Yong Wu. Green synthesis and characterization of zinc oxide nanoparticles using carboxylic curdlan and their interaction with bovine serum albumin. RSC advances 2016 vol. 17, no.7 .283-300, 2016.

[30] Dhillon GS, Brar SK, Kaur S, Verma M (2012) Green approach for nanoparticle biosynthesis by *datura innoxia* current trends and applications. Crit Rev Biotechnol 32:49–73

[31] Gunalan S, Sivaraj R, Venckatesh R (2011) Green synthesis of iron oxide nanoparticles by *Aloe barbadensis* miller leaf extract: structure and optical properties. Mater Res Bull 46:2560–2566

[32] Feldheim DL, Foss CA (2002) "Metal nanoparticles: synthesis, characterization, and applications". CRC Press, Boca Raton, p 338