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ESTADO DE PUEBLA**

DEPARMENT OF BIOLOGICAL SCIENCES

*“BIOSYNTHESIS OF IRON OXIDE NANOPARTICLES
BY EICHHORNIA CRASSIPES (WATER HYACINTH)
BIOMASS AS REDUCTANT AGENT”*

Thesis to obtain the degree of:

Biotechnology Engineer

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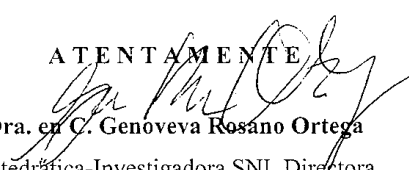
H. Puebla de Zaragoza a 28 de marzo de 2017

A QUIEN CORRESPONDA:

Por este conducto me permito informar a ustedes que la **tesis de Licenciatura en Ingeniería en Biotecnología** de la **C. Dahlia del Castillo Trujillo**, con **ID 96298** y **matrícula no. 74600109**, respectivamente, titulada *"Biosynthesis of iron oxide nanoparticles by Eichhornia crassipes (water hyacinth) biomass as reductant agent"*, bajo mi dirección **está terminada y cumple con los requisitos necesarios para obtener el grado de Ing. en Biotecnología**. Por lo anterior, espero que puedan presentar su examen profesional de licenciatura a la brevedad posible.

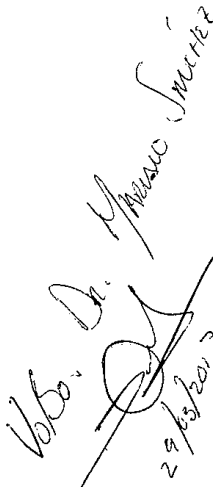
En espera de su respuesta, reciba mis más altas y distinguidas consideraciones.

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Dedicatory

To my parents, Sonia Trujillo and Rafael Del Castillo because of their great example and for being a strong inspiration. I am so fortunate to have you and feel forever thankful to you.

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Abstract

Iron oxides nanoparticles have been strongly investigated due to their diverse applications from environmental as pollution absorbents to medical such as hyperthermia and drug delivery vehicles. Current methods for synthesis are mainly chemical, leading to toxic byproducts and reactants. This work attempts to optimize an extracellular synthesis method using water hyacinth biomass, one of the most invasive aquatic plants in the world, collected from the dam Manuel Avila Camacho, Puebla. The part of the plant used was the leaf due to the high tannin concentration which works as a reductant agent and the salt precursor was iron chloride (FeCl_3).

Uv-Visible spectroscopy was used for identify the iron oxide surface plasmon band. High resolution transmission electron microcopy (HRTEM) was used to determine lattice parameters and identify present species, together with X-ray Diffractometer (XRD).

Three species of iron oxide were obtained by this method; Fe_{1-x}O (Wustite), Fe_2O_3 (Hematite) and $\gamma\text{-Fe}_2\text{O}_3$ (Maghemite), the structures found were face-centered cubic, rhombohedral rhomb-centered and primitive cubic structure respectively. Three solution were evaluated, 5, 7 and 10 pH. Hematite was the most frequent specie. A better stability, high production rate and a more homogeneous size (less than 5 nm) of particles were observed at pH10. Obtained nanoparticles could be used for removal of certain pollutants such as Arsenic from water stream, however further research will be required.

Keywords: Iron oxide nanoparticles, biosynthesis, *E. crassipes*, Nanoremediation.

Resumen

Las nanopartículas de óxido de hierro han sido fuertemente investigadas debido a sus múltiples aplicaciones desde ambientales tales como absorbentes de contaminantes hasta médicas como transporte para antibióticos o tratamientos como hipertermia para tumores cancerígenos. Los actuales métodos para síntesis son mayoritariamente químicos, estos procesos derivan sustancias tóxicas como subproductos y reactivos. Este trabajo se enfatiza en una síntesis extracelular, usando biomasa de lirio acuático (*E. crassipes*), una de las plagas más invasivas a nivel mundial, colectada en la presa de Manuel Ávila Camacho, Puebla. La parte de la planta utilizada fue la hoja debido a la concentración alta que tiene de taninos, los cuales actúan como agente reductor, mientras que la sal precursora fue cloruro férrico (FeCl_3).

Se utilizó espectroscopía ultravioleta con el objetivo de identificar el óxido de hierro, microscopía de transmisión electrónica de alta resolución para determinar los parámetros de celda e identificación de especies de hierro presentes que fueron confirmados posteriormente con un difractómetro de rayos-X.

Tres especies fueron identificadas: Fe_{1-x}O (Wustita), Fe_2O_3 (Hematita) y $\gamma\text{-Fe}_2\text{O}_3$ (Maghemita), con estructuras cúbica centrada en las caras, romboédrica y estructura cúbica primitiva respectivamente. Tres diferentes soluciones fueron evaluadas: pH 5, 7 y 10. En todas las soluciones, hematita fue la especie más frecuente. A pH 10, se observó una mejor estabilidad, mayor producción y un tamaño de partícula más homogéneo ($< 5 \text{ nm}$). Las nanopartículas obtenidas podrían ser utilizadas para remoción de ciertos contaminantes del agua, tales como arsénico. Sin embargo, se requiere una investigación más profunda para dichas aplicaciones.

Palabras claves: Nanopartículas de óxido de hierro, biosíntesis, *E. crassipes*. Nanoremediación.

General index

CHAPTER ONE: BACKGROUND.....	20
1.1. NANOTECHNOLOGY	20
1.1.1. NANO AND BIOTECHNOLOGY.....	21
1.1.2. NANOTECHNOLOGY CURRENTLY IN THE MARKET.....	23
1.1.2. 1 Nanotechnology in Mexico	
1.1.3. NANOREMEDIATION.....	26
1.2. SYNTHESIS METHODS.....	27
1.2.1 BOTTOM UP METHODS OF SYNTHESIS IRON NANOPARTICLES.....	29
1.2.1.1 BIOSYNTHESIS.....	29
1.2.1.1.1 Plant Metabolites	
1.2.1.1.2 Plant extracts	
1.2.1.1.3 Plant biomass	
a) Why the use of <i>Eichhornia crassipes</i> is so interesting for a green-synthesis ?	
1.2.1.1.4 Bacteria for Iron Nanoparticle Synthesis	
1.2.1.1.5 Peptides for Nanoparticle Synthesis	
1.2.1.2 CO-PRECIPITATION.....	48
1.2.1.3 HYDROTHERMAL / HIGH TEMPERATURE REACTIONS	49
1.2.1.4 SOL-GEL REACTIONS AND POLYOL METHOD.....	50
1.2.1.5 MICROWAVE-ASSISTED SYTHESIS.....	51

1.2.1.6	CONSTRAINED ENVIRONMENT REACTIONS.....	51
	I) Reverse Micelle Or Emulsion Technology	
	II) Magnetoliposomes	
	III) Sonochemical or sonolysis method	
1.2.1.7	AEROSOL / VAPOR TECHNIQUES.....	55
	I) Spray Pyrolysis	
	II) Laser Pyrolysis	
1.2.2	TOP DOWN METHODS FOR SYNTHESIS OF IONPS.....	58
1.2.2.1	LASER ABLATION.....	59
1.2.2.2	ELECTRO EXPLOSION	60
1.2.2.3	ATTRITION MILLING	61
1.3	IRON OXIDE NANOPARTICLES	62
1.3.1	MAGNETISM IN IRON OXIDES NANOPARTICLES.....	65
1.3.2	MODIFICATION OF IRON OXIDES NANOPARTICLES.....	68
1.3.3	APPLICATIONS OF IRON OXIDES	70
1.3.3.1	PROTEIN IMMOBILIZATION.....	70
1.3.3.2	MEDICAL SCIENCE.....	72
	1.3.3.2.1 Targeted Drug Delivery	
	1.3.3.2.2 Gene Delivery	
	1.3.3.2.3 Hyperthermia	
	1.3.3.2.4 Magnetic Resonance Imaging	
1.3.3.3	DATA STORAGE APPLICATIONS	78
1.3.3.4	PIGMENTS.....	79
1.3.3.5	CATALYST	79
1.3.3.6	IRON NANOPARTICLES AND ITS ROLE FOR ENVIRONMENTAL REMEDIATION	80
	1.3.3.6.1 Mechanisms Of Adsorption Of Ions	
	1.3.3.6.2 Mechanisms Of Desorption Of Ions	

	1.3.3.6.3	Effect Of pH In Adsorption	
1.3.4		TOXICITY OF IRON OXIDES NANOPARTICLES	85
1.4		MANUEL ÁVILA CAMACHO DAM_.....	86
1.5		CHARACTERIZATION OF NANOMATERIALS.....	87
	1.5.1	ULTRAVIOLET-VISIBLE LIGHT.....	89
	1.5.2	TRANSMISSION ELECTRON MICROSCOPY SPECTROSCOPY	89
	1.5.3	X-RAY DIFFRACTION	92

CHAPTER TWO: METHOLOGY.....95

2.1	MATERIALS, REAGENTS AND EQUIPMENT USED	95
2.2	PROCESSING OF BIOMASS	96
2.3	BIOSYNTHESIS OF IRON OXIDE NANOPARTICLES	96
2.4	CHARACTERIZATION OF NANOPARTICLES	97
2.4.1	HRTEM.....	97
2.4.2	X-RAY DIFFRACTION (XRD).....	98
2.4.3	ULTRAVIOLET VISIBLE SPECTROSCOPY (UV-VIS).....	98
2.4.4	ENERGY-DISPERSIVE X-RAY SPECTROSCOPY (EDS).....	98
2.4.5	RECOVERY OF POWDER FROM FEO BIOSYNTHESIS SUSPENSION	
2.4.6	SIZE DISTRIBUTION	98
2.4.7	STRUCTURE AND PHASE	99

CHAPTER THREE: RESULTS AND DISCUSSIONS

3.1	BIOSYNTHESIS	100
	-CHARACTERIZATION:	
3.2	UV – VIS SPECTROSCOPY	101
3.3	SIZE OF NANOPARTICLES	103
3.4	PRODUCTION RATE	107
3.5	PLASMA CLEANING	107
3.6	TEM AND HRTEM	108
3.7	<i>X-RAY DIFFRACTION</i>	123
3.8	ENERGY- DISPERSIVE X-RAY SPECTROSCOPY (EDS).....	125
3.9	MAGNETIC PROPERTIES AND POSSIBLE APPLICATIONS OF THE OBTAINED IRON OXIDES SPECIES.....	126

CHAPTER FOUR: CONCLUSIONS AND FUTURE PERSPECTIVES OF THE PROJECT.....

APENDIX

CHAPTER FIVE: BIBLIOGRAPHY

LIST OF TABLES

Table 1: Nanotechnology products by sector in Mexico (Zayago <i>et al.</i> ,2012).....	26
Table 2: Published works of biosynthesis of nanoparticles by using <i>E. crassipes</i> as a reducing agent. Adapted from: (Peña, 2016).....	44
Table 3 : Analytical techniques for assessment of physicochemical properties of nanomaterials. Adapted from: Ali <i>et al.</i> ,2016.....	88
Table 4: Iron oxide phases found by percentage	121
Table 5: Main elements found in sample from a SEM image	125

LIST OF FIGURES

Figure 1: Systematic study the developments of biotechnology up to its current stage. Taken from: Verma <i>et al.</i> , (2011)	22
Figure 2: Available products since 2007 in each major category and in the Health and Fitness subcategories. Taken from Vance (2015)	24
Figure 3: Nanomaterial additives into consumer products by categories . Taken from: Vance <i>et al.</i> , 2015	25
Figure 4: Three different methods for (superparamagnetic) iron oxide synthesis (Ali <i>et al.</i> ,2016)	28
Figure 5: Main types of metabolites found in plants, involved in the synthesis of metal nanoparticles. A- terpenoids (eugenol); B,C-flavonoids ; D-a reducing hexose with open chain form; E-F- Tryptophan and tyrosine respectively. Taken from: Makarov <i>et al.</i> , (2008)	31
Figure 6: Some of the possible size and shapes of nanoparticles synthesis from plant extracts : Taken from: Kuppusamy <i>et al.</i> ,2016; Chandrant <i>et al.</i> ,2006; Dubey <i>et al.</i> , 2010).	32
Figure 4: Most common condensed tannins. Taken from: Hagerman (2002).	34
Figure 8: Products of hydrolysable tannins.	35
Figure 9: Biosynthesis of metallic nanoparticles by tannin radicals as a reducing agent. Adapted from: Rosano (2009)	36
Figure 10: Representation of metal nanoparticle synthesis in a plant extract, where the metal ion bind to the reducing metabolites and stabilizing agents so the metal atoms can be reduced. Taken from: Makarov <i>et al.</i> , 2014.....	38
Figure 11: Size distribution plot of Iron Oxides nanoparticles with different pH. Taken from: Herrera-Becerra (2007).....	41
Figure 12: Water hyacinth single (right) and as a mat (left). Taken from: Aboul-Enein <i>et al.</i> ,2011	42
Figure 13: Clinically isolated leukemia cells (A), and treatment with crude extract for 24 h (B). Taken from: Aboul-Enein <i>et al.</i> ,2011.....	43
Figure 14: Phytochemical analysis of major secondary metabolites present in in metabolic extract of <i>Eichhornia crassipes</i> , taken from : Shanab <i>et al.</i> , 2010.	44

Figure 15: LaMer diagram formation of mono- dispersed nano- and microparticles with the nucleation and crystal growth mechanisms. Taken from: Chang & Waclawik (2014).	49
Figure 16: Graphic representation of nanoparticle synthesis in microemulsions a) by mixing microemulsions , b) by adding a reducing agent, and c) by bubbling gas trough the microemulsion. Image taken from: (Teja & Koh, 2009).	53
Figure 17: Schematic representation of the encapsulation of magnetite and monomer droplet to latex particle by sonochemical method dived miniemulsion polymerization pathway. Sodium dodecyl sulfate used as a stabilizer. Taken from Teo et al., (2009).	55
Figure 18: Aerosol process routes.	56
Figure 20: Morphology of particle preparing by spray method. Taken from: Okuyama and Lenggoro (2003).	57
Figure 21: Representation of laser pyrolysis device (Dumitrache et al., 2005; Teja & Koh, 2009)	58
Figure 22: Illustration of formation of FeO nanoparticles by laser ablation. Taken from: Zeng et al., 2005.	60
Figure 23: Formation of nanodroplets. Taken from: Niu et al., 2010.	60
Figure 24: Iron oxides in the global system. Taken from: Cornell & Schwertmann, 2003.	62
Figure 25: Crystallographic data of hematite, magnetite and maghemite the black ball is Fe ²⁺ , the green ball is Fe ³⁺ and the red ball is O ²⁻ . Taken from: Wu et al., 2015.	63
Figure 26: Different types of iron oxides and oxyhydroxides known so far. (Cornell and Schwertmann, 2003).	64
Figure 27: Physical and magnetic properties of Iron oxides. Taken from: Teja and Pei-Yoong (2009).	65
Figure 28: Magnetic domains in bulk material. Taken from: Estelrich (2015)	66
Figure 29: Alignment of individual atomic magnetic moments in different types of materials . Tc meaning Curie temperature and T _n meaning Neel temperature. Taken from Teja and Kho.,2009.	67
Figure 30: Silane for surface functionalization on magnetic nanoparticles. Taken from: Xu et al (2014).	69
Figure 31: (Strept) avidin-biotin technology for protein immobilization onto iron magnetic nanoparticles. Taken from: Xu et al (2014).	72
Figure 32: Required phases of biocompatibility for Fe ₃ O ₄ magnetic nanoparticles for medical applications. Taken from Popescu et al., 2014	73
Figure 33: Kinetics of nanoparticle accumulation monitored by MRI in targeted groups of animals. Taken from : Chertok et al.,(2008). (A) targeted and (C) non targeted	77
Figure 34: Kinetic series of Magnetic resonance imaging scans of nanoparticle accumulation in 9L gliosacome. Taken from: Chertok et al.,(2008).	78
Figure 35: Recovery of Hg(II) using thiol-functionalized mesoporous silica-coated magnetite nanoparticles taken from: Hakami et al.,2012.	80
Figure 36: Environmental contaminants that can be transformed by iron nanoparticles. Taken from: Zhang (2003).	81
Figure 37: Core-shell model of zero-valent iron nanoparticles. Taken from: Theron et al., 2008	82
Figure 38: Potential remediation of groundwater, using nZVI . Source: Zhang (2003).	82
Figure 39: Types of nanoparticles used for environmental remediaton and type of media treated. Taken from Khan et al., 2009.	83

Figure 40: Current metals in Valsequillo Dam, Puebla. Taken from: Celdeño-Valdéz et al., (2008).....	87
Figure 41: Comparison of bright field TEM, dark field TEM and HAADF-STEM. Taken from: Klein et al (2015).	91
Figure 42: Size obtained by TEM and x-ray diffraction peak broadening analysis. Taken from: Akbari et al.,2010.....	93
Figure 43: Bragg's law reflection. Taken from: (Dutrow & Clark, 2016).....	94
Figure 44: Transmission electron microscope (TEM) JEOL JEM-2010F , physics institute, UNAM.	97
Figure 45: Example of a crystallographic table for rhombohedral hematite	99
Figure 46: UV-Vis absorption graphic of pH 5 of iron oxides nanoparticles.....	101
Figure 47: UV-Vis absorption graphic of pH 7 of iron oxides nanoparticles.....	102
Figure 48: UV-Vis absorption graphic of pH 10 of iron oxides nanoparticles.....	102
- Figure 49: Dark field image for measuring size of particles for pH10..	104
Figure 50: pH 7 sample	104
Figure 51: Image from sample pH5.....	105
Figure 52: Histogram of iron oxide nanoparticles distribution size of pH 10.	106
Figure 53: image one from sample Ph5_0042	109
Figure 54 : Image two from sample ph5_0042.....	110
Figure 55: Image two from sample pH5_009.....	111
Figure 56: Image one from pH7_002 in HRTEM and FFT together with interplanar distances (right).	112
Figure 57: Image two from pH7_002	113
Figure 58: Image from sample pH7_004.....	114
Figure 59: Image from sample pH7_e.....	114
Figure 60: Image from sample pH7_f HRTEM	115
Figure 61: Image from sample ph10_0025 in HRTEM (left)and FFT together with the interplanar distances (right).....	116
Figure 62: Image from sample ph10_0023 in HRTEM (left)and FFT together with the interplanar distances (right).....	117
Figure 63: Image from sample ph10_0028 in HRTEM (left) and FFT together with the interplanar distances (right).....	118
Figure 64: Image from sample ph10_0022 in HRTEM (left) and FFT together with the interplanar distances (right).....	119
Figure 65: Image 1 from sample ph10_0019 in HRTEM (left) and FFT together with the interplanar distances (right).....	120
Figure 66: Image 2 from sample ph10_0019 in HRTEM (left) and FFT together with the interplanar distances (right).....	121
Figure 67: Crystal structures of (A) Wusite, (B) Hematite and (C) Maghemite	122
Figure 68: DRX analysis with various components identified.	123
Figure 69: DRX analysis representing four different iron oxides species.....	124
Figure 67: SEM image.....	125
Figure 68: EDS graphic	125

HYPOTHESIS

Iron oxide nanoparticles are biosynthesized using water hyacinth (*Eichhornia crassipes*) biomass as a reductant agent

GENERAL OBJECTIVE

Standardization of the bioreduction method for FeO nanoparticles synthesis by water hyacinth (*E. crassipes*) biomass.

PARTICULAR OBJECTIVE 1

Biosynthesis of iron oxide (FeO) nanoparticles, evaluating pH in solution.

PARTICULAR OBJECTIVE 2

Characterization of nanoparticles by:

- Uv-vis spectroscopy
- Transmission electron microscopy
- High resolution transmission electron microscopy
- Electron dispersion spectroscopy

In order to obtain shape, dispersion, and phase of biosynthesized iron oxide nanoparticles

Problem justification

Conventional nanoparticle synthesis such as pyrolysis and attrition are known to have drawbacks such as high cost of manufacturing and large energy requirement (Thakkar et al.,2010) whereas chemical methods as sol-gel and chemical reduction has important disadvantages such as the use of toxic/hazardous reactants, precursors and byproducts (Thakkar et al.,2010; Herlekar et al., 2014).

Hence, it is desirable to optimize a synthesis method for the production of iron oxide nanoparticles, which is not only green but simple to develop as it does not require expensive equipment or reactants. An important advantage of the synthesis method is that the active biological component acts itself as a reductant and a capping agent, thereby reducing the overall cost of the process (Senapati et al.,2005).

This technique do not use toxic solvents; it use water, ferrous salt precursor and a buffer. Generally, green synthesis methods focused on the use of plant extracts or biomass reduce metal ions in a shorter time than microbes, depending on the concentration of phytochemicals (Rai et al.,2008) and aseptic conditions are not needed which elevates cost (Sathishkumar et al.,2008).

Furthermore, some of the plants used before for synthesis of iron oxides, such as alfalfa, green tea (Kuang et al.,2013), sorghum bran (Njagi et al.,2011), eucalyptus (Wang et al.,2014) pomegranate (Rao et al.2013) and mulberry (Machado et al., 2013) are part of the human or animal diet/consumption of certain regions, making them not ideal for this purpose.

However, when using an invasive, undesirable and highly reproductive plant such as Eichhornia crassipes as a reductant agent, benefits such as the availability of the resource need to be taking into account, specifically when one of the main future goals is industrialized the obtained nanoparticles. Water hyacinth bioreduction method used in this thesis was developed by Rosano-Ortega (2006).

Here, three different pH are evaluated to seek the optimal to produce stable and homogeneous particles and diverse techniques are used to characterize the products such as DRX, UV-vis and TEM. In order to achieve this objective, a collaboration was held by different institutes. Institute

of physics, at UNAM, academic center of entailment and technology transfer in BUAP, and department of biological sciences and research department at UPAEP.

Research limitations

This project had slight limitations concerning to:

- Physical-geographic space: As some of the instruments required for characterization of obtained nanoparticles are not available in the current university, high demand, location and waiting list of other research facilities which collaborate in this project could delay the process of obtaining results and further interpretations of this data.

Declaration of Authorship

I, Dahlia Del Castillo Trujillo, declare that this thesis titled "Biosynthesis of iron oxide nanoparticles by water hyacinth (*Eichnornia crassipes*) biomass as reductant agent" is my own.

I confirm that:

1. ⌘ This work was done wholly or mainly while in candidature for a research degree at this University.
2. ⌘ Where I have consulted the published work of others, this is always clearly attributed.
3. ⌘ Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
4. ⌘ I have acknowledged all main sources of help.
5. ⌘ Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Signed:  Dahlia Del Castillo Trujillo

Date: May 2017

CHAPTER 1: BACKGROUND

1.1 NANOTECHNOLOGY

Nanotechnology, according to the National Nanotechnology initiative (NNI), refers to the “...understanding and control of matter at dimensions between 1 and 100 nanometers, where unique phenomena enable novel applications”. It is worth adding that this *nanoworld*, is a multidisciplinary field currently encompassing engineering, physics, chemistry and biology sciences (Fakruddin et al., 2012) in the research and development of technology at atomic, molecular or macromolecular level (Abu-Salah et al ,2010).

Early discoveries of nanostructures began after the term “nanotechnology” gained serious attention around 1990’s after the theoretical physicist Richard Feynman proposed it for the first time, in his famous speech on top-down nanotechnology titled “There’s plenty of room at the Bottom” in 1959. Following the concept and advances, Harry Kroto, Richard Smalley and Richard Curl won the Nobel Prize in chemistry for their discovery of fullerenes in 1985, while carbon nanotubes discovery was attributed to Sumio Iijima in 1991. This was just the beginning for important and progressive developments and discoveries of the nanoscale world.

Nanomaterials can present new and unexpected properties when compared with bulk. For example, opaque substances become transparent, stable material becomes combustible, some insoluble materials become soluble or even more surprising is the fact that some elements which express no magnetism in volumetric samples, such as Pd and Rh, present magnetic moments in a nanometric scales (Issa et al.,2013; Rao, 2014). These unique physicochemical properties presented in nanostructures, can be explained by the number of atoms in the surface of the material and the total number of atoms which conforms the whole system. Superficial atoms are more reactive because they are not saturated (Moran & Rodriguez, 2012).

Nanoparticles properties are shape and size dependent (Wahajuddin and Arora, 2012; Iravani et al., 2011; Corain, 2011). Some of the most common developments in applications of nanoparticles englobe bio and chemical sensors (Holzinger et al.,2014; Reimhult et al.,2015; Sriarm et al.,2015; Bülbül et al.,2015), catalysts (Chatuvedi et al., 2012), photonics and imaging (West & Halas, 2003), generation of broad spectrum of antimicrobial agents (Yah et al., 2015) innovative and more efficient data storage technologies (Tartaj et al.,2003; Reiss & Hütten, 2005), new agrochemical agents for improving crop productivity as well as promises for reducing pesticides (Sekhon et al., 2014 ; Parisi et al., 2015). In medicine nanotechnology can be implemented for diagnosis, prevention and treatment with drug delivery (Park ,2007; Gao et al.,2016). And in surfaces such as water repellent, wrinkle resistance, UV-bloquing textiles (Qu et al.,2016), biodegradable plastics and glass with self-cleaning ability (Yunus et al., 2012) have been deeply investigated. Nevertheless, there is an increasing effort and research to use nanotechnology for environmental technology for environmental protection through pollution prevention, treatment, and cleanup of long-term problems such as toxic anthropogenic waste (Karn et al., 2009).

Nanomaterials can be classified in several groups according with Pakowski (2006): Nanoparticles, nanoporous materials, nanotubes and nanowires, nanostructured surface and films.

Interestingly, current trends in the area (Rao, 2014) indicate that atomic-scale might not be a limiting factor for development. However, it may take time to achieve it because inevitable, as systems decrease in size, quantum effects start to dominate.

1.1.1 NANO AND BIOTECHNOLOGY

The term biotechnology was used for the first time by a Hungarian Engineer, Karl Ekey in 1919. However, biotechnology has been implemented since the beginning of human life, starting with developments/discoveries in the ancient period i.e., before the year 1800 (Verma *et al.*, 2011). Figure 1 representing biotechnology history with the most important advances of this science.

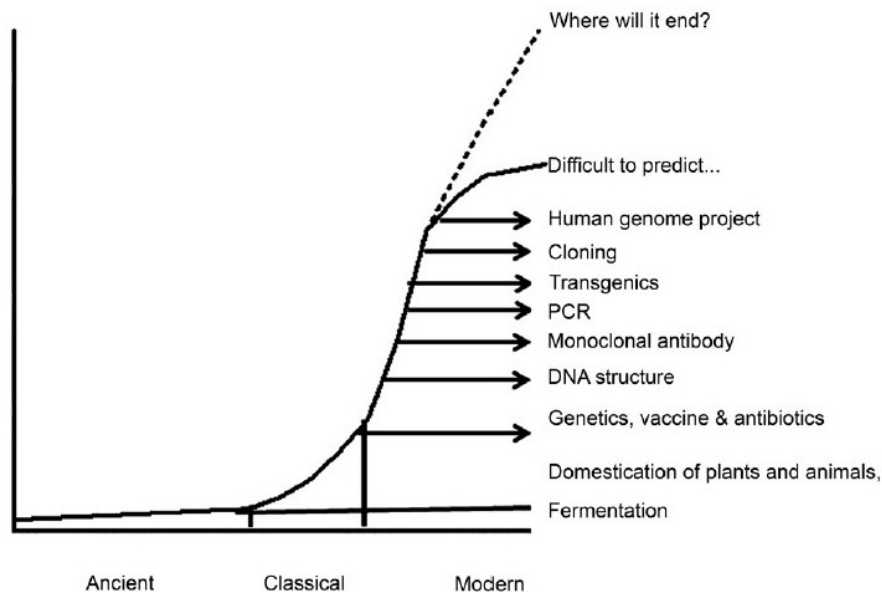


Figure 1: Systematic study the developments of biotechnology up to its current stage. Taken from: Verma *et al.*, (2011)

Biotechnology then, refers to “the use of living organism/s or their product/s to modify or improve human health and human environment” (Verna *et al.*, 2011). In concrete, application of biological material. It is evident that microbiology and biochemistry have contribute to biotechnology development. Furthermore, as is evident in *figure 1*, biotechnology is also moving toward the small scales.

Nanobiotechnolgy or bionanotechnology is the application of nanotechnology in biological fields (Fakruddin *et al.*, 2012; Thangavelu *et al.*, 2016) by using principles and techniques for understanding and transforming biological systems in order to create novel devices and systems at the nano-scale (Abu-Salah *et al.*, 2010). Applications of nanobiotechnology include synthesis of nanoparticles by using living organisms which had been of great interest and had opened new opportunities for novel applications (Makarov *et al.*, 2014). Additionally, another important and progressively area of biotechnology has been proposed for the improvement of biosynthesis of nanomaterials: Genetics (Kang *et al.*, 2008). By altering an organism genome for obtaining desirable characteristics of nanoparticles.

The majority of applications of nanobiotechnology tend to incline to medicine. For example, fluorescent polymers have been coated with nanosphers which emits a signal when encounter specific molecules or tracks down metabolites which could be associated with certain health

problems such as tumors (Doria et al., 2012). Similarly, proteins that self-assemble to create new functional and programmable nanomaterials are another developing branch in nanobiotechnology (Fei & Sarah, 2009).

Agriculture, on the other hand, is a field that has been improved over time in terms of productivity thus, the implementation of nanotechnology in this area is not excepted. Engineered nanoparticles have been served as nanocarriers containing genes, herbicides, chemicals, or even targeting a specific plant location for releasing its content (Sekhon, 2014; Thangavelu *et al.*, 2016). Nanoencapsulated fertilizers may save fertilizer consumption and fight environmental pollution (Jones, 2006).

Toxicology of nanoparticles needs to be carefully explored due to their scale; they can produce potential damage in species at cellular level. There are still unanswered questions about its fate on ecosystems and human health. Moreover, effects can vary in a considerable way due to the shape of nanoparticles (Petersen et al., 2016). Nanoecotoxicology is a new research field involving analysis for predicting toxicology and should be performed before industrialize any product containing nanoparticles. Now a day it is estimated to be more than 800 of products based on nanotechnologies and with potential to increase in the years to come (Maynard et al. 2006).

1.1.2. NANOTECHNOLOGY CURRENTLY IN THE MARKET

The nanotechnology Consumer Products Inventory (CPI) is the largest online database of nanotechnology consumer products currently in the market. Actually there are 1814 consumer products from 622 companies in 32 countries which can be readily purchased by consumers and claimed to contain nanomaterials. As this database began in 2005, the market of products containing some sort of nanomaterials has increased 30 times by 2015 (Vance *et al.*, 2015). Health and fitness products represent the 60% by 2009, and by 2015 this same category is 42% of the total product including personal care products such as toothbrushes, lotions and hairstyling tools as presented below in *Figure 2*.

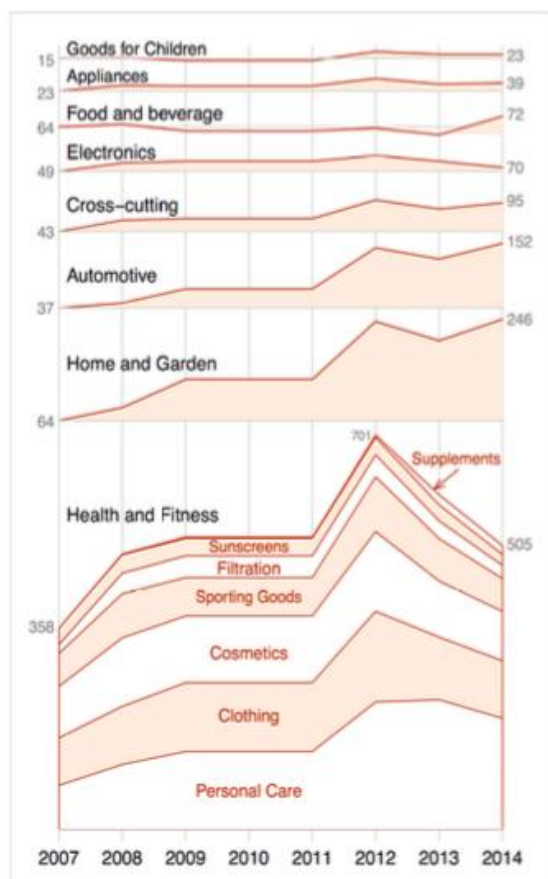


Figure 2: Available products since 2007 in each major category and in the Health and Fitness subcategories. Taken from Vance (2015)

Silver is the most popular consumer product with 435 products due to their known antibacterial behavior (Tsuzuki, 2009). Silver nanoparticles, titanium dioxide, nano-organics and gold nanoparticles are some of the most commonly used nanoparticles in cosmetics products. Zinc oxide nanoparticles have the ability to reflect and adsorb UV radiation (Iravani, 2014) so they are currently used for sunscreens. In dietary supplements, a variety of nanomaterial compositions for example silver, nano-organics, calcium, gold, silicon dioxide, magnesium and ceramics have been advertised (Vance *et al.*, 2015). Figure 3 representing the majority of the number of products per category according with consumption, with antimicrobial protection being the more common product.

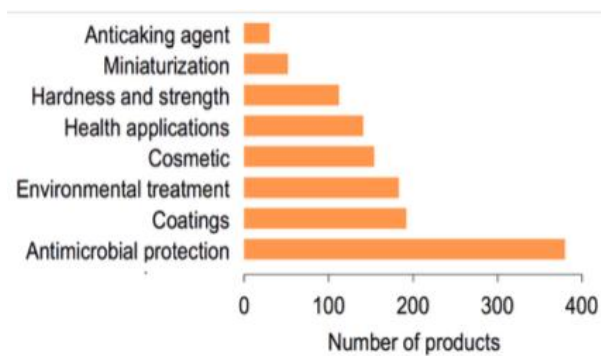


Figure 3: Nanomaterial additives into consumer products by categories . Taken from: Vance *et al.*, 2015

Nanoparticles need to be produced in a large-scale in order to get to products in the market and thus need to be economically viable. It is important to stabilize nanoparticles because their properties are shape and size dependent. Also, synthesis methods need to be carefully chosen in order to develop heterogeneous or homogeneous particles, depending on their use. There are no standardized methods for assessing consumer risk from using products containing nanomaterial nor a characterizing for determining the environmental relevant concentrations (Holden *et al.*, 2014) together with the possible exposure scenarios from the expected use of the product.

1.1.2. 1 Nanotechnology in Mexico

Mexico is currently occupying the second place in nanotechnology development in Latin America, after Brazil and before Argentina (Robes-Belmont, 2012; Zagayo *et al.*, 2013).

In 2012, Mexican Secretary of Economy released several guidelines for the regulation of nanotechnology and nanomaterials which form a bilateral agreement with the U.S. regulations which is a relevant platform for restriction regulation and a pro-trade stance (Foladori & Zayaco, 2014). In *table 1* different nanotechnology products by sector are listed (Záyago *et al.*, 2012).

Table 1: Nanotechnology products by sector in Mexico (Zayago *et al.*, 2012)

Chemical	17	Cosmetic	3
Water	8	Pharmaceutics	2
Electrical components	8	Biomedical	2
Plastic and cave	8	White goods	2
Construction	7	Equipment and machinery	2
Commerce	6	Odontology equipment	2
Food	5	Mining	2
Nanomaterials	5	Paper	1
Industrial equipment	4	Petrochemical	1
Electronics	4	Cleaning service	1
Iron and steel	4	Textile	1
Automotive	3		
Biotechnology	3	total	101

Mexico is currently supporting nanotechnology and since 2001, Mexican government recognize it as a strategic area in policies of science and technology. This approach clusters three important sectors: Academy, government and companies (bussines).

1.1.3 NANOREMEDIATION

The application of reactive nanomaterials containing chemical properties and catalysis, is referred as “Nanoremediation” due to the capacity for transforming and detoxifying pollutants of concern (Karn *et al.*, 2009).

Recently, nanoparticles have been used as adsorbent media due to their high efficiency in comparison to bulk materials. A nanoadsorbent should have several characteristics when aims to remove a pollutant, such as high surface area, a simple synthesis and a high pollutant removal capacity (Das *et al.*, 2014). Adsorptive media filtration is one of the most extant technologies due to its high efficiency, low cost, ease of process and its versatility for various water streams (Mohan *and* Pittman, 2007). Nanoadsorbents may create highly active adsorption sites, producing a major adsorption capacity per unit surface area.

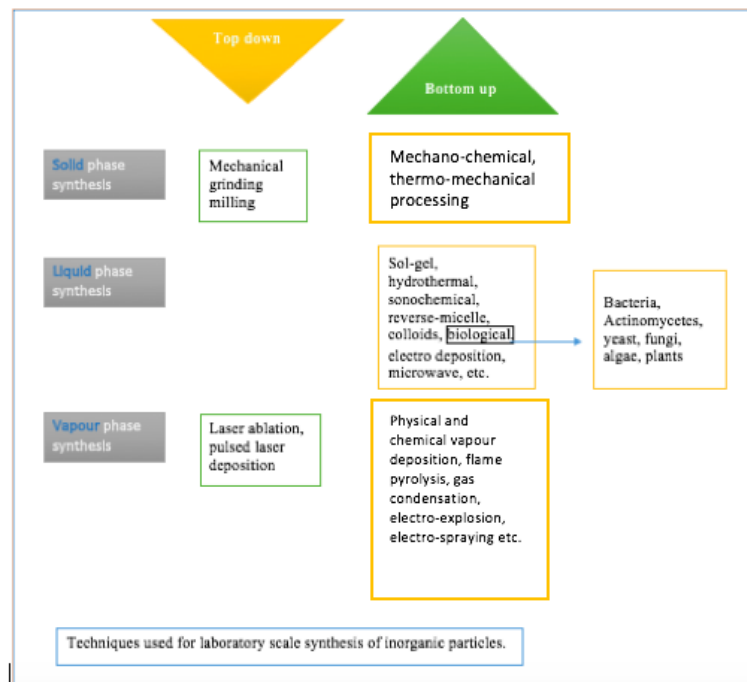
An important issue to consider when utilizing a nanoadsorbent due to their small size, is the difficulty to attach them into filters or/and the separation of nanomaterials, hence causing

secondary pollution. In order to address this inconvenient, research has been focused to develop support materials such as carbon nanotubes, graphene, and porous beads that are able to retain the nano-adsorbents more efficiently (Chandra *et al.*, 2010). Several studies have implemented the use of certain materials as bases for nanoparticles such as mesoporous silica beads, carbon and titanium dioxide, chitosan beads, ceramic membranes, kaolin and bentonite, or even oyster shells as a basis for nano-adsorbents (Alipour *et al.*, 2014).

1.2 SYNTHESIS METHODS

The majority of nanoparticles are synthesized by two approaches: the top down and the bottom up methods (Kim, 2008) which include the chemical, physical and biological pathways (Zhao *et al.*, 2007). Top down method, generates nanoparticles from breaking down bulk materials, whereas bottom-up method nanostructures are formed by assembling of atoms and molecules.

Nevertheless, there is a different classification of synthesis by phase (Tsuzuki, 2009); vapor phase synthesis may include physical and chemical vapour deposition, flame and spray pyrolysis, laser ablation, electro-explosion, electro-splashing, cluster-beam just to name a few. Liquid phase synthesis includes sol-gel, hydrothermal, solvothermal, sonochemical, reverse micelle, colloids, biological, electro deposition, microwave etc. Solid phase synthesis refers to mechanochemical processing and thermo-mechanical processing.



Two challenges can be faced when synthesizing nanoparticles. First, chemical challenge lies on a homogenous composition and narrow size distribution. Second, the selection of a process which can be reproducible in industry without time consuming or costly purification procedures.

A variety of factors may control shape and size, i.e. adjusting pH, temperature, ionic strength, nature of the salts (perchlorates, chlorides, sulfates, and nitrates), injection flux and the addition of chelating organic anions in the case of metal nanoparticles (Laurent, 2008).

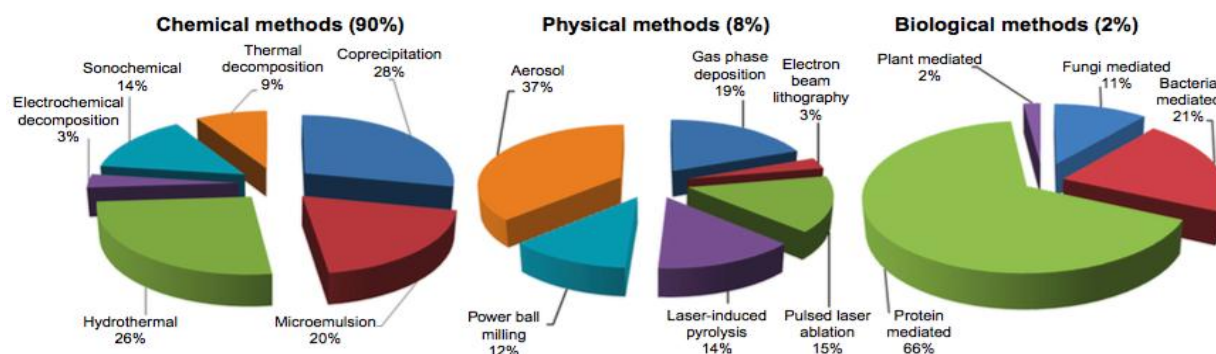


Figure 4: Three different methods for (superparamagnetic) iron oxide synthesis (Ali et al.,2016)

Aggregation occur during separation from a bulk material to nanoparticle solution due to Van der Waals forces, altering size and diameter into greater dimensions (Ehrampoush *et al.*, 2015). Agglomeration can be prevented by using several organic or inorganic materials which modify particle surface (Zhao, 2005).

Nanoparticle growth depend of two mechanisms; surface reaction and monomers diffusion to the surface (Sugimoto, 2001). Nucleation is the initial process occurring during crystal formation. During nucleation, ions, atoms or molecules are arranged in a pattern which allows additional particles to be deposit and thus leads to a crystal growth. Shape variation depends of variation in electrostatic surface density of the nanoparticles.

1.2.1 BOTTOM UP METHODS OF SYNTHESIS IRON NANOPARTICLES

1.2.1.1 BIOSYNTHESIS

There is no doubt that nanomaterials will play an important role in future technologies. However, because of the negative environmental impact almost every new technology generates, it is vital to develop clean, nontoxic, environmental safe with increase energy efficiency methods. A tentative possibility is to use microorganisms which are known to be able to synthesize inorganic molecules (Plaza *et al.*, 2014). Biosynthetic methods can be classified according to the place where nanoparticles are formed: intracellular and extracellular synthesis (Velusamy *et al.*, 2016).

Microorganisms are able to perform certain detoxification methods when exposed to abnormally high amounts of a compound. In metal exposure, some microorganisms react by reducing ions and therefore can be inherently utilized as possible eco-friendly nanofactories (Sastry *et al.* 2003).

Nanoparticles have been synthesized from bacteria (Kahani *and* Yagini, 2014; Scarano *et al.*, 2003), photosynthetic algae (Chad *et al* 2016), diatoms (Schröfel *et al* ., 2011), yeast (Kowshik *et al.*, 2002), fungi (Kitching *et al.*, 2015; Rautaray *et al.*, 2003) and human cells (Anshup *et al.*, 2005) just to name a few.

Phytosynthesis (using plants for synthesis) method produce not only non- toxic compounds but a well-defined size and shape. Plant metabolites such as terpenoids, sugars, polyphenols, phenolic acids, proteins, alkaloids, alcohols, carboxyls, ketones, hydroxyls and sulfhydryls are able to stabilize and reduce metal ions into nanoparticles (Kumar *et al.*,2012; Nisha *et al.*, 2014, Makarov *et al.*,2014; Shankar *et al.*, 2004; Shankar *et al.*, 2003; Morales-Diaz *et al.*, 2016). It can be assumed that virtually any biological compound that contains on its structure this groups are suitable to convert metal ions in NPs. Biological agents develop a double function in biosynthesis: as reductant agents and as a stabilizing agents (Morales-Diaz *et al.*, 2016).

Plant metabolites will have an effect on the morphological diversity of nanoparticles such as triangles, hexagons, pentagons, cubes, spheres, ellipsoids, nanowires, nanorods. Plants are able to translocate ions in different parts of their structure, in various tissues and organs (Maracov *et al.*,2014).

1.2.1.1.1 Plant Metabolites

Bioreduction of metal ions, yielding nanoparticles can be accomplish by metabolites. Plant metabolites can include, terpenoids, polyphenols, sugars, alkaloids, phenolic acids, and proteins (Morales-Diaz *et al.*, 2016) . Metabolites are byproducts of metabolism. Their formation depends on enzymes that act on a parent structure. However, as metabolites are intermediates, they tend to be present in small amounts only and usually conduct a rapid chemical change. Metabolites have a finite existence, thus they can not accumulate in cells (Harris, n.d.).

The study of metal bioaccumulation in plants has revealed metals are deposited in form of nanoparticles (Makarov *et al.*,2014). When heavy metals are absorbed by roots, they tend to reduce by plants defense system in order to avoid toxicity, the phytochelatinases and other enzymes such as tannins, are located on the vacuoles, and are able to storage this metals in form of nanoparticles of just few atoms (Gardea *et al.*, 2002).

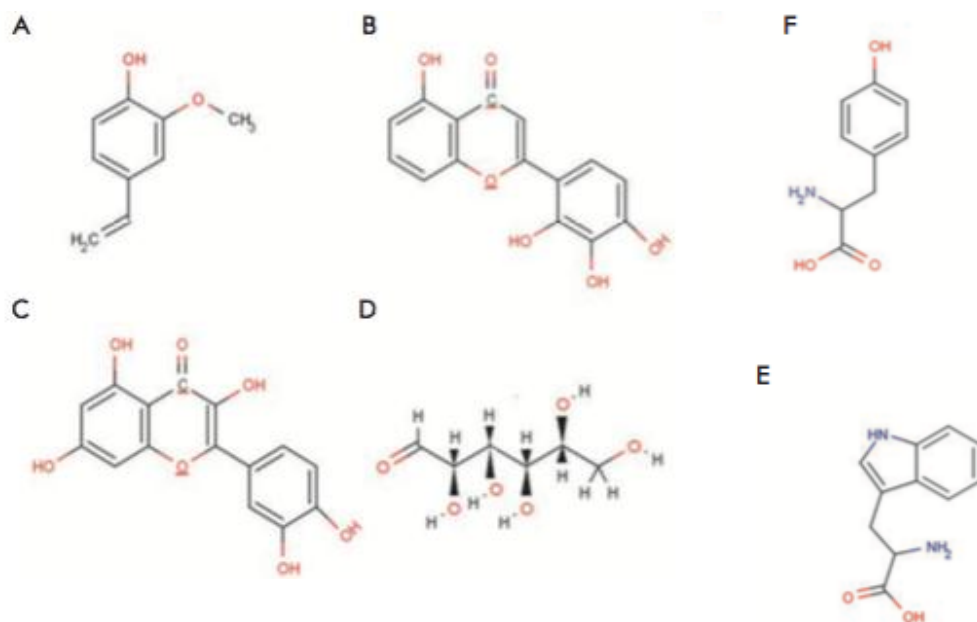


Figure 5: Main types of metabolites found in plants, involved in the synthesis of metal nanoparticles. A-terpenoids (eugenol); B,C-flavonoids ; D-a reducing hexose with open chain form; E-F- Tryptophan and tyrosine respectively. Taken from: Makarov *et al.*, (2008)

Metallic nanoparticles have been synthesized from natural resources due to the physic and chemical properties of plants. Gold, silver, platinum, zinc, copper, titanium oxide, magnetite and nickel are some of the metals which have been successfully synthesized with plants metabolites (Kuppusamy *et al.*, 2016). Different parts of plant for example stem, root, fruit, seed, callus, leave, peel and flowers are able to synthesize various shapes and sizes of metallic nanoparticles as presented in Figure 6.

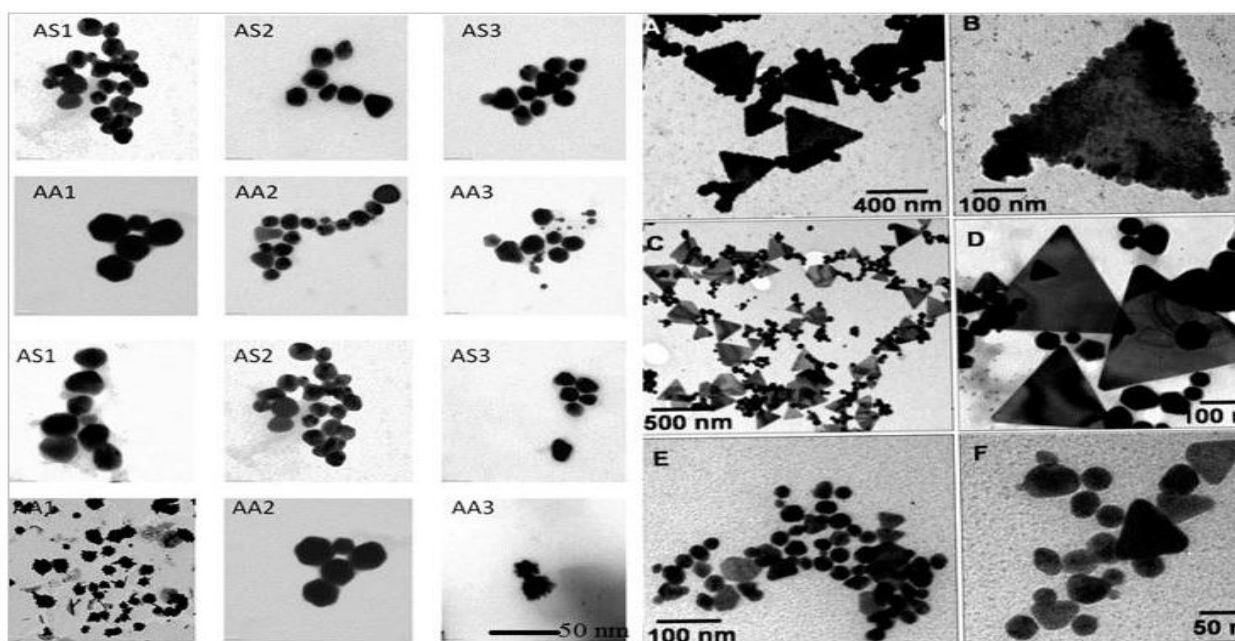


Figure 6: Some of the possible size and shapes of nanoparticles synthesis from plant extracts : Taken from: Kuppusamy *et al.*,2016; Chandrant *et al.*,2006; Dubey *et al.*, 2010).

a) Terpenoids

Terpenoids are polymers derived from five-carbon isoprene units which have been associated with nanoparticle formation because of their strong antioxidant activity. Silver nanoparticles have been produced by using geranium leaves extracts (Shankar *et al.*,2003) . Similarly, Shingh and colleagues (2010) achieved the bioreduction of HAuCl_4 and AgNO_3 nanoparticles from eugenol, the main terpenoid of *Cinnamomum zeylanisum*.

b) Flavonoids

Flavonoids are a polyphenolic compounds including six different classes: anthocyanins, isoflavonoids, flavonols, chalcones, flavones and flavonones, which act as reductants able to actively chelate and form nanoparticles (Makarov *et al.*,2014). Considering the various functional groups flavonoids have, tautomeric transformations may occur from the enol-form of the flavonoids, to the keto-form, releasing a reactive hydrogen atom during this process, that could reduce metal ions to form nanoparticles (Makarov *et al.*,2014).

Kiruba and collaborators (2013) obtained iron nanoparticles by using leaf extract of *Dodonaea viscosa*. By a FT-IR study, they determined that flavonoids were present in leaf, and attribute to their reductive properties the formation of the nanoscale particles. On the other hand, a different

research (Venkateswarlu *et al.*, 2014) reported a biosynthesis of ferromagnetic Fe₃O₄ by *Syzygium cumini* seed extract (a byproduct of agriculture). *S. cumini* is known to be constituted by polyphenols including flavanoides acid derivatives (Hossain *et al.*, 2012). FT-IR analysis demonstrate presence of polyphenols, flavonoids and other biomolecules of the seed extract which may participate on the formation of Fe₃O₄.

c) Mono and polysaccharids

The ability to form nano structures by monosaccharids and polysaccharids depends of the capacity of the individual molecule to adopt an open-chain form to provide access of a metal ion to an aldehyde group (Makarov *et al.*, 2014). For example, maltose and lactose have reducing potential because of their open chain conformation in contrast with sucrose. In addition, Mahdavi and co-authors (2013) successfully biosynthesized Fe₃O₄ nanoparticles using brown seaweed *Sargassum muticum* extract containing sulphated polysaccharides. Polysaccharides induced the reduction of Fe³⁺ and also served as a stabilizer to prevent agglomeration. Hydroxyl, sulphate and aldehyde groups behaved as the reducing agents, present in seaweed extract composition.

d) Tannins

Tannins are water soluble polyphenols present in many plants. Their complex structure is conformed with several neighboring hydroxyl groups achieving a molecular weight from 500 (gallic acid esters or hydrolysable tannins) to 2,000 (condensed tannins and proanthocyanidins) (Ramashayam *et al.*, 2012). Hydroxyl groups can form complexes with iron and other metallic cations (Andjelkovic *et al.*, 2006). Tannins can be found in large quantities in fruits, wood, leaves, seed, wood, bark and stem tissues (McDonald *et al.*, 1996). This polyphenolic biomolecule prevents attacks of pathogens, herbivores and rot (Herrera-Becerra *et al.*, 2010) operating as a defense mechanism, among others diverse effects on biological systems, such as metal ion chelators, protein precipitating agents and biological antioxidants (Hagerman, 2002).

Tannins are divided in two large groups: condensed and hydrolysable.

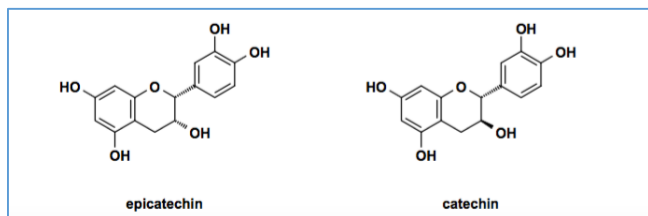


Figure 7: Most common condensed tannins. Taken from: Hagerman (2002).

Condensed tannins or proanthocyanidins are polymeric flavonoids, metabolites known to have a heterocyclic ring. Flavan-3-ols(-)-epicatechin and (+) catechin are the most widely studied condensed tannins as presented in figure 4 (Hagerman, 2002).

Hydrolysable tannins, on the other hand, are gallic acid derivatives (Figure 8)

- a) Gallotannins are the simplest hydrolysable tannins, their structure is a simple polygalloyl ester of glucose or a pentagalloyl glucose, which has five identical ester linkages involving aliphatic hydroxyl groups of the core sugar (Hagerman, 2002). Their chemical properties (which are structure-dependent) are susceptible to hydrolysis and biochemically, they tend to precipitate proteins.

b) Ellagitannins

The simple tannins are esters of hexahydroxydiphenic acid (HHDP) which spontaneously lactonizes to ellagic acid in aqueous solution. Gallotannins can form ellagitannins by oxidative coupling of galloyl groups (Hagerman, 2002). There are more than 500 hydrolysable tannins characterized until now, ellagitannins being the largest group, subdivided at the same time, in simple ellagitannins, C-glycosidic ellagitannins, complex tannins (condensates of C-glycosidic tannins with flavan-3-ol), and oligomers up to pentamers (Okuda et al.,1995). Ellagitannins produce ellagic acid upon hydrolysis (Yoshida et al.,2010).

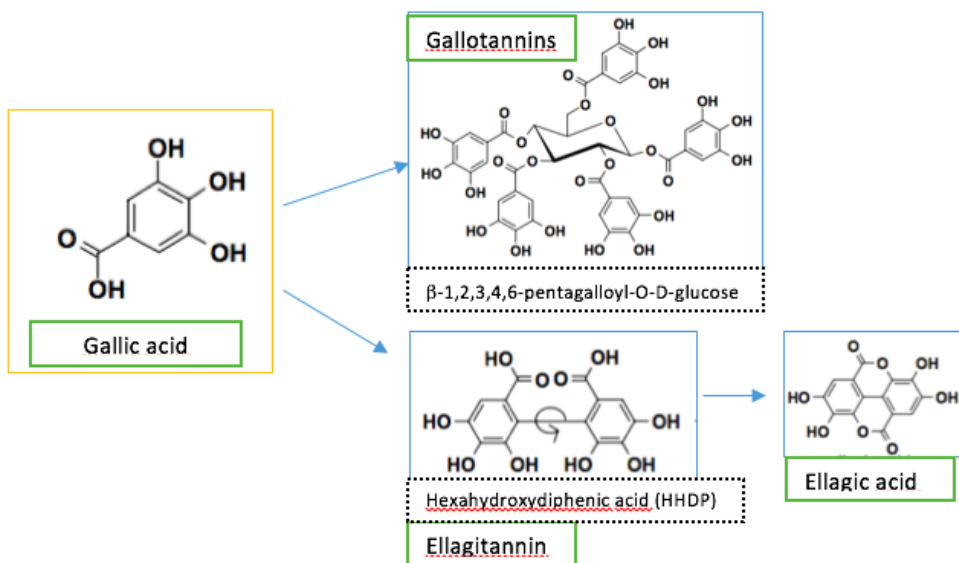
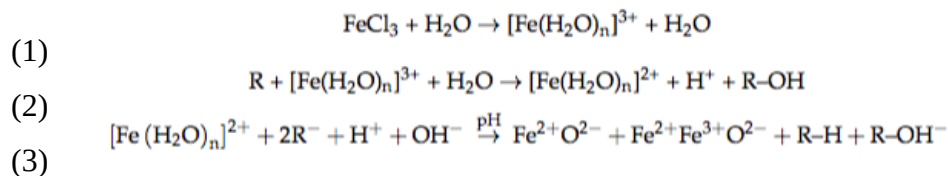


Figure 8: Products of hydrolysable tannins.

Iron is able to bind polyphenols through ester bounds with their functional groups, orto-dihydroxy or thrihydrocy benzene. Because of this complexes, trough an oxidation–reduction process, it is possible to reduce an iron salt, forming iron oxides nanoparticles. The adjacent phenolic groups are found all along the backbone of tannins, therefore, reaction can occur in all dimensions (Ramashayam *et al.*, 2012).

Herrera-Becerra (2010) proved the formation of $\text{Fe}_{0.902}\text{O}$ and Fe_2O_3 nanoparticles smaller than 10 nm at an optimal 10 pH. Authors attributed the biosynthesis to tannic radicals, formed by the HCl-lysis of hydrolysable tannins found in alfalfa. The biosynthesis process which induce metal ions (in this particulate case, iron ions) to form nanoparticles is presented in the following equations (R represent radical tannins) (Herrera-Becerra *et al.*, 2010).



Similarly, Rosano (2009) reported the synthesis of Mn, FeO, Co and Cr nanoparticles presenting different morphologies by a bio-reduction method, using *Eichhornia crassipes* (water hyacinth). The reduction was attributed to the tannin radical (Figure 9). Gallic acid (a type of phenolic acid, found in hydrolysable tannins) is able to decompose into ellagic acid and glucose by HCl or H_2SO_4 .

Biomass of water hyacinth was pretreated before exposed to metal salt, by .01 M HCl in order to form radicals of organic compounds which interacts with metallic ions and reduce them into an elemental state. Stabilization of nanoparticles is determined by Van der Waals forces which acts in the adsorption of organic molecules with nanoparticles surface, producing a steric barrier. This steric barrier avoids contact between metallic centers of nanoparticles hence, prevents agglomeration (Trindade *et al.*, 2001).

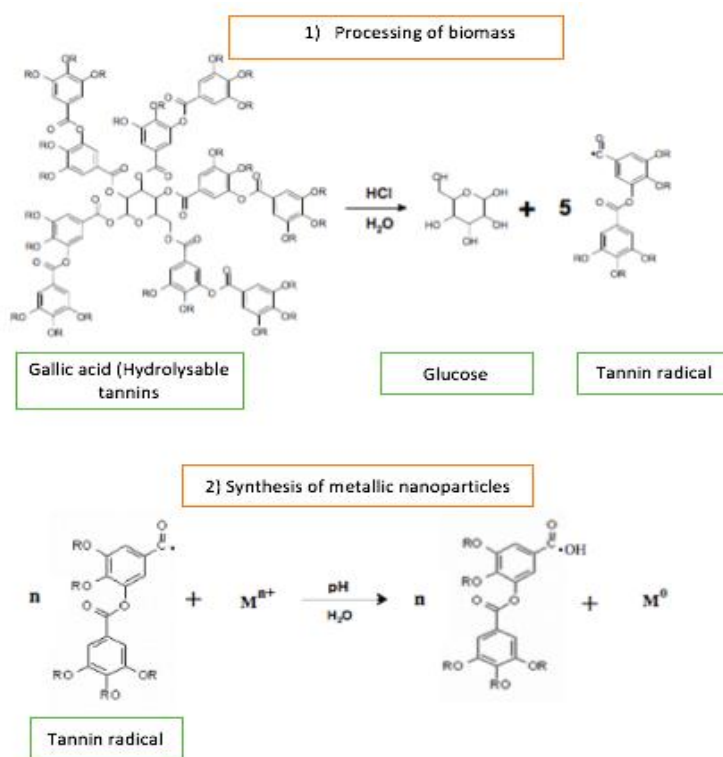


Figure 9: Biosynthesis of metallic nanoparticles by tannin radicals as a reducing agent. Adapted from: Rosano (2009)

1.2.1.1.2 Plant extracts

Market or industrial requirement of heterogeneous size and shape of nanoparticles will limit the biosynthesis in whole plant (Makarov *et al.*, 2014). However, when using plant extracts which has been a common biosynthesis technique, a more controlled size and shape can be achieved. By

changing pH, concentration of plant active substance and/or temperature, different sizes and shapes can be obtained (Rosano-Ortega, 2009; Marakov *et al.*, 2014).

Metal ions can be reduced in a shorter time depending on plant specie or phytochemical concentration (minutes or hours) comparted with microbes which need obligatory aseptic conditions increasing costs (Rai *et al.*, 2008; Sathishkumar *et al.*, 2010).

Iron nanoparticles have been successfully biosynthesized with tea extract (Hoag *et al.*, 2009; Nadagouda *et al.*, 2010; Shahwan *et al.*, 2011; Kuang *et al.*, 2013; Huang *et al.*, 2013), eucalyptus leaf extract (Wang, 2013; Wang *et al.*, 2014; Madhavi *et al.*, 2013), pomegranate leaf extract (Rao *et al.*, 2013), plantain peel extract (Venkateswarlu *et al.*, 2013) and banana peel ash extract (Thakur and Karak., 2014) just to name a few. Polyphenols play a vital role as bioreductants, conferring antioxidant properties thus determining their content in several plant parts is crucial.

In *Figure 10*, a representation of a metal nanoparticle biosynthesis using plant extract is presented. After reduced to metal atoms, the resulting metal ion-plant metabolite complex forms a small metal nanoparticle. Then, the nanoparticle grow and coalescence from small into larger particles until they form a stable shape and size.

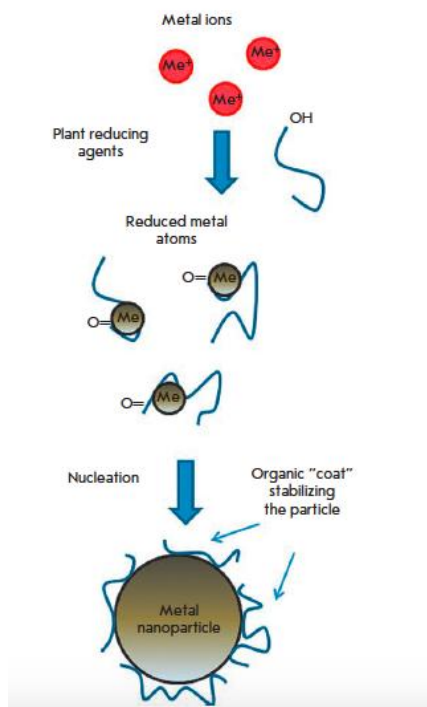


Figure 10: Representation of metal nanoparticle synthesis in a plant extract, where the metal ion bind to the reducing metabolites and stabilizing agents so the metal atoms can be reduced. Taken from: Makarov *et al.*, 2014.

For nanocrystals there are still some gaps whether they are formed during plant synthesis, if inside the plant by reduction of a metal salt or outside and then transported into other plant compartments. It is believed that nanoparticles are formed in root tips when absorbing minerals dissolved in water. Metal salts ascend as ions and then are reduced to elemental form as nanoparticles (Gardea-Torresdey *et al.*, 2005).

For both plants and microorganisms, the production of nanoparticles is due to defense mechanism against toxicity. An advantage of using plants instead of microorganism is they don't need so much time and effort for preventing pollution as growing medium requires, select of the best organism (bacteria, actinomycetes, yeast, fungi), found optimal conditions for cell growth and enzymatic activity etc. (Iravani, 2011). Using Biomass due to its potential for reducing metal ions is well documented as well as the chemical structures for metal binding. Plant molecules such as tannins can be promising as an alternative for nanoparticle production (Herrera *et al.*, 2007; Rosano-Ortega *et al.*, 2006).

1.2.1.1.3 Plant biomass

IONPs tend to form aggregates in aqueous solutions (Song and Carraway., 2005). This can be prevented when mixed with supporting materials such as carbon (Lui et al., 2008). A rich-highly economical and renewable source of carbon is indeed, plant biomass. Plant biomass synthesis can be even easier than utilizing plant extract due to the lack or little pretreatment.

Biomass has been used as a biotemplate allowing nanoparticle formation acting as a structural support in synthesis for superparamagnetic iron oxide nanoparticles (Cai *et al.*, 2010). Herrera-Becerra and Zorilla (2007), synthesized 10-nm IONPs with alfalfa (*Medicago sativa*) in 48 hours, while López –Tellez (2013) used orange peel to reduce Fe (II) metal ions which were formed on the peel surface after 6-hour contact. Ehrampoush and colleagues (2015) used IONPs synthesized with tangerine peel extract for removing cadmium. Tangerine extract help preventing agglomeration and also as an adsorbent for cadmium ions present on a contaminated solution. Rosano-Ortega and colleagues (2006) synthesized stable Mn quantum dots by bio-reduction using water hyacinth. Nanoparticles in a size range 1-2 nm at pH 5 were obtained with a easy procedure and little pretreatment of biomass.

When utilizing the whole plant for synthesis, several limitations need to be considered. In a review made by Makarov *et al* (2013), authors claimed that size and shape of the nanoparticle strongly depend on their localization of the plant. Different tissues may move nanoparticles to other regions, and the recovery is known to be pretty low and difficult. However, in vitro synthesis using the plant biomass, provides more flexible control over shape, size and recovery of the particles. Factors such as pH, reaction temperature, concentration of metal salt etc. can be crucial and had been studied and correlated with nanoparticle structure. Another advantage is that reactions take place almost instantaneously, in comparison with the whole plant use which implicates a delay for the uptake and diffusion of metal ion throughout the plant.

Elemental iron (Fe(0)) nanoparticles present an excellent reactivity due to its electron donating ability, specially toward hazardous materials including heavy metal ions, inorganic anions, and

halogenated organic compounds (Pondert *et al.*, 2000; Kanel *et al.*, 2005; Barnes *et al.*, 2010). However, is difficult to stabilize them in air due to their susceptible behavior to oxidize and their tendency to aggregate in aqueous systems (Liu *et al.*, 2008). Therefore, previous research has focus on finding stability of Fe (0) NP in air by coating or mixing with polymeric materials (Phenrat *et al.*, 2006 ; Liu *et al.*, 2008) which also prevents agglomeration. Zhang and collaborators (2012) used Fe₃O₄ nanoparticles as an iron source in order to fabricate Fe(0) NP in one-step pyrolysis supported on a natural cellulose fiber. Carbon source proposed was wood fiber which serves as a supported matrix, reductant and stabilizer. This elemental-iron nanoparticles, were prepared at 800 °C, and remained stable for at least 3 months. Most of the particles presented a diameter around 50 nm. Interestingly, when pyrolysis was applied for 500 °C, reacted violently to air, producing α -Fe₂O₃.

- pH in biosynthesis

A crucial variable in plant biosynthesis is pH. A charge change in solution affect ability of phytochemicals of binding and reducing metal cations and anions during the nanoparticle synthesis, which will finally affect their properties (Makarov *et al.*, 2014). When altering pH, the chemical composition of the crystal surface change and thus size and shape will be altered too (Laurent *et al.*, 2007). Size of nanoparticles can be controled by controlling pH in solution and the ionic strenght as shown in *figure 11* (Wang *et al.*, 2007).

Optimal pH to produce magnetite nanoparticles is in the range of 9.7 to 10.6, while for side-products such as goethite and maghemite, pH values must lie below 8.5 (Andrade *et al.*, 2010). However, another research recalls the optimal pH for hematite and wustite was pH 10, obtaining particles from 1.4 to 4.9 nm.

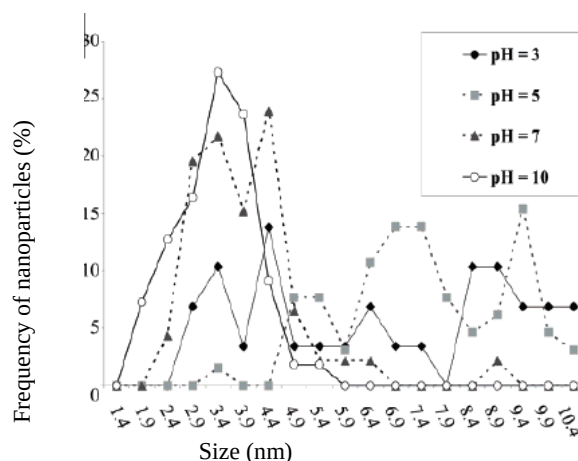


Figure 11: Size distribution plot of Iron Oxides nanoparticles with different pH. Taken from: Herrera-Becerra (2007).

a) Why the use of *Eichhornia crassipes* is so interesting for a green-synthesis?

Eichhornia crassipes , commonly known as water hyacinth (Liliales: pontederiaceae) is a worldwide invasive plant, native of the Amazon basin (Barret and Forno ,1982) known for its high rate growth even in severe pollute water (So *et al.*, 2003). It has spreaded to tropical and subtropical regions by humans. It invaded about 62 countries in Africa, Asia, and North America.

The growth and propagation of this plant has cause major conservation problems with considerable socioeconomic repercussions due to competing with other aquatic plants, interference with navigation, water flow and eutrophication (Téllez *et al.*, 2008). According with the International Union for Conservation of Nature (IUCN), is situated of the 100 most dangerous invasive species.

The impact of *E. Crassipes* has impacted also in health, it has act as an agent for spreading serious diseases in tropical countries, while in the physic-chemical impact relies on a decline in pH and temperature, nutrient levels, biological oxygen demand, treat of local native species diversity,

disrupting food chains and nutrient cycling by altering ecosystem structure (Rai and Mushi 1979; Shanab *et al.*, 2010).

Mechanical removal has the inconvenient of the dead plant accumulation at the bottom of the water body of which contains allelochemicals which can be carried away on canal banks and its dissolving in irrigation waters may led to inhibition of seed germination and growth (Shanab *et al.*, 2010). Several biological control agents have been successfully used in 33 countries (Julien and Griffiths, 1998). The best agents have been *Neochetina bruchi* Hustache , *N. eichhorniae* Warner, and *Niphograpta albiguttalis* (Julien *et al.*, 2001).



Figure 12: Water hyacinth single (right) and as a mat (left). Taken from: Aboul-Enein *et al.*, 2011

Some current uses of Water Hyacinth are listed below:

- Antibacterial behavior

Boral and collaborators (2012) found that extracts from water hyacinth may be useful for developing alternative compounds for antibiotic-resistant pathogens which cause several infections. Water hyacinth inhibit successfully, *salmonella typhi*, *acinetobacter sp.*, *schigella sp.*, *Escherichia coli*, *sclerotium rolfsii* and *fusarium proliferatum* which cause several problems worldwide.

- Bioremediation opportunity

Water hyacinth is able to adsorb calcium, magnesium, sulfur, ferric, manganese, aluminum, boron, copper, molybdenum, zinc, nitrogen, phosphorus and potassium (Dandelot *et al.*, 2008),

thus being effective for bioremediation processes, with the drawback of the need to control that plant because, as saying before, has a fast growth rate.

- Herbicidal activity

Macrophytes such as *E. crassipes*, both release and accumulate bioactive phenolic compounds secondary metabolites, which can due to the large quantities released, inhibit algal, fungal and bacterial growth (Mogestu *et al.*, 1960).

- Therapeutic activities

Crude extract of water hyacinth has shown effects against several tumor types such as liver cancer cell lines, and hormone-dependent tumor types as cervix and breast cancers (Aboul-Enein *et al.*, 2011).

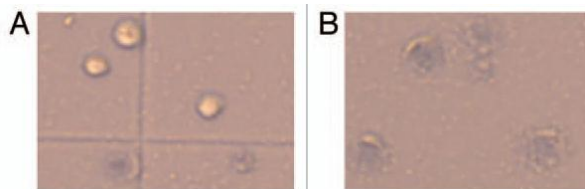


Figure 13: Clinically isolated leukemia cells (A), and treatment with crude extract for 24 h (B). Taken from: Aboul-Enein *et al.*, 2011

Several compounds such as alkaloid, phthalate derivatives, propanoid and phenyl derivatives have been identified in extract of water hyacinth (Abul-Enein *et al.*, 2011). Hydroxyl groups and unsaturated bonds in chemical structure of *E. crassipes* are responsible for its strong antioxidant activity (Shanab *et al.*, 2010).

Water hyacinth has the capacity of bio accumulate heavy metals. Rosano (2009) report 10,089 mg/Kg of toxic elements by dry weight which indicates that can be used for phytoextraction purposes. However, this may present several problems when the plant degrades and reincorporate metals and other compounds into the ecosystem, increasing concentrations in the bottom of the water stream (Gao-Lei and Bo L., 2004) which may incorporate and persist on the food chain, causing toxicity to several organisms (Zaranyka and Ndapwadza., 1995; Shanab *et al.*, 2010).

The root act as the very first barrier for heavy metal capitation and translocation. Furthermore, metal concentrations decrease in the following order: Roth> stems> leaves > fruits> seeds (Rosano *et al.*, 2007). It is believed that the organic molecules responsible for the biosynthesis of metallic nanoparticles are tannins (Gardea-Torresday *et al.*, 2005).

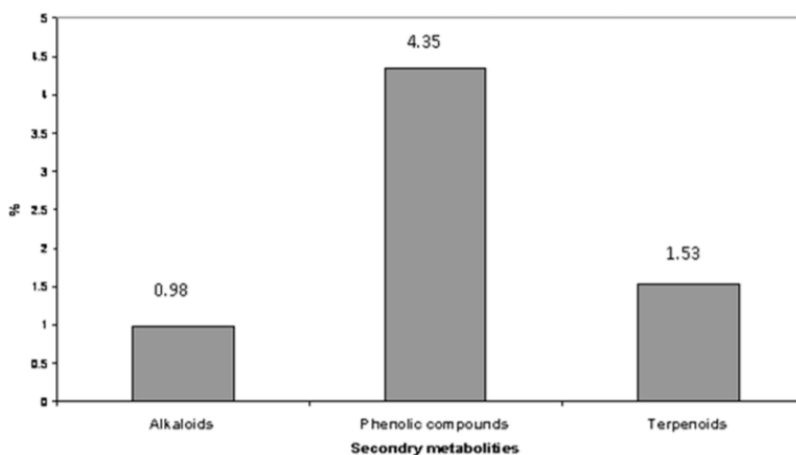


Figure 14: Phytochemical analysis of major secondary metabolites present in in metabolic extract of *Eichhornia crassipes*, taken from : Shanab *et al.*, 2010.

Due to its secondary metabolites, *E. crassipes* has been successfully implemented as a reductor agent in nanoparticle biosynthesis. Several works had been published which use water hyacinth in the biosynthesis of nanoparticles (Table 2):

Table 2: Published works of biosynthesis of nanoparticles by using *E. crassipes* as a reducing agent. Adapted from: (Peña, 2016)

Metal	Metalic salt	Size	Shape	Reference
Mn, Cr, Fe, Co	MnSO ₄ , K ₂ Cr ₂ O ₇ , FeCl ₃ ,CoCl ₂	2 nm, 3-10 nm, 3-10 nm	Cubic and spherical	Rosano-Ortega (2009)
Ag	AgNO ₃	10-70 nm	Spherical	Kiruba et al.,(2012)

Au	AuCl ₃	27.45-74.35 nm	Spherical	Jannathul and Lalitha (2013)
Ag	AgNO ₃	120-240 nm	Cubic and spherical	Thombre et al.,(2014)
Ag	AgNO ₃	18-30 nm	Spherical	Prusty and Parida (2014)
Ag	AgNO ₃	2-9 nm	Cuboctaedron	Munive-Olarte (2014)

1.2.1.1.4. Bacteria for Iron Nanoparticle Synthesis

In an attempt to develop reliable, nontoxic, and ecofriendly synthesis of nanoparticles, one option has been explored for using microorganisms such as bacteria. Researchers have begun to use biomass or cell extracts of bacteria for synthesizing nanoparticles. The reason bacteria and other organisms form nanoparticles, is merely for overcoming toxicity, for example most metal ions are toxic for bacteria, the reduction of this ions is a defense mechanism (Iravani, 2014).

Temperature, pH, light, ion concentration and shape of the formed crystal are some of the factors that varies from bacterial species in synthesis. Some will synthesize extracellular like *Bacillus subtilis* (Saifuddin et al 2009) or intracellular such as *Pseudomonas stutzeri* showing a detoxification in the periplasmic space (Slawson et al, 1992). The stabilization of the nanoparticles by this living biofactories, showed to be good, without forming aggregated even for many weeks at room temperature (Wen *et al.*,2009), a proposed reason of this stabilization is the due to proteins and enzymes secreted.

A future approach for bacterial synthesis of nanoparticles are the genetic modified bacteria. GMO bacteria may overexpress a particular gene for a particular protein or enzyme which could produce more reductive agents and might potentially control size, shape, stability and yield of nanoparticles. Kang and colleagues (2008), genetically modified *E. Coli* in order to controlled synthesis of Cadmium sulfate CdS nanocrystals. They increased the production of gamma-glutamylcysteine synthase that catalyzes the precursor of phytochelatins (PCs) which serve as

binding template and nucleation site for metal ions and prevents aggregation. They found that the overexpression of glutathione synthetase in *E. Coli* with metal stress, results in an improve of biosynthesis of cadmium nanoparticles.

A notorious disadvantage of using bacteria for synthesis is the recovery of the nanoparticles, which is not well documented. However, there are some processes such as ultrasound treatment or reactions with suitable detergents may be required for recovery. Further, the recovery of precious metals from wastes in mines could be considered as well (Iravani, 2014). Enzymatic lysis could be utilized as well but is considered to be expensive and not efficient when planning a recovery for industrial production of nanoparticles. Surfactants and some organic solvents could also be used for extraction and stabilization, nevertheless they are not particularly recommended because of their toxicity (Iravani, 2014).

An example of an iron-reduced bacteria is the sulfate-reducing bacteria, capable of producing magnetic iron sulfide (FeS) nanoparticles, which could adsorb radioactive metals providing a suitable safe matrix for the long-term hazardous radioactive ion storage (Watson *et al.*,1999). GS-15 or *Geobacter metallireducens* oxidized simple organic compounds and is known to be an iron-reducing microorganism, which produced copious quantities of magnetite, in a size range of 10 to 50 nm, coupling the organic matter to the reduction of ferric iron. Lovley and coworkers (1987) investigate the ability of *Pelobacter acetylenicus* and *P. venetianus* to reduce Fe(III) and *Alteromonas putrefaciens* ability to reduce Fe(II) and Mn(IV). *P. carbinolicus* growing in an ethanol medium, reduce Fe(III), as it was the sole electron acceptor. Thermus species can reduce amorphous Fe(III)–oxyhydroxide to magnetic iron oxide (Lui *et al.*,1997). A variety of mesophilic microorganism use Fe(III) as a terminal electron acceptor, thus, can reduce some metalloids and Fe(III) (Ahmad *et al.*,2003). Atul and coworkers (2008) found that *Actinobacter sp.* extracellular synthesized maghemite (γ -Fe₂O₃) and greigite (Fe₃S₄) under ambient conditions. Interestingly they found that specific biochemical transformations happened when challenging the bacteria with different metal ions, which resulted in induction of different proteins.

An application of this Fe(III)-reducing microorganisms could be used for removal of several metals from waters and waste streams and immobilization of metals in subsurface environments.

1.2.1.1.5. Peptides for Nanoparticle Synthesis

According with Makarov and coworkers (2013) some peptides and proteins can be used for the “green” synthesis of metal nanoparticles in combination with plant extracts. Some amino acids such as tryptophan, tyrosine, arginine and lysine are able to reduce metal ions. Virus and virus-like particles can be utilized also for synthesis, considered as effective biomatrices due to its mutual spatial orientation of active peptide groups and general structural regularity. A viral particles surface consists of dense arrays of capsid proteins, carefully packed. This surface is reactive and thus capable of form a metal ion interaction.

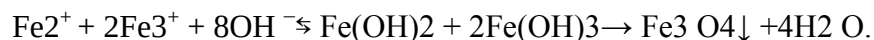
Moreover, Glusker and colleagues (1999), demonstrated that size, morphology and amount of nascent nanoparticles can be greatly affected by the amino acid sequence of a protein. For example, a synthetic amino acid GASLWWSEKL reduces metal ions into less than 10 nm particles, furthermore when N-and C-terminal amino acids were replaced in the peptide SEKLWWGASL, nanospheres and nanotriangles ~ 40 nm in size were generated.

Recently, researches in Beijing (Zhang *et al.*, 2015) developed a novel engineered *E. Coli* filamentous virus named M13, containing a specific peptide at its end, able to synthesize zero valent (ZV) iron nanoparticles. The engineered peptide carried one more negatively charged residue than the wild one, favouring nanoparticle synthesis. Nanoparticles were uniformly dispersed with a diameter of 5-10 nm.

Protein ferritin, is known to synthesize iron oxide nanoparticles in a size range of 8 nm diameter within a hollow polypeptide cage (Harrison *et al.*, 1989). On the other hand, nobel type of bionanoreactor utilized for biomimetic iron oxide nanoparticle synthesis was developed by Shenton and coworkers (2001) based on enzyme found in bacterial and fungi, *lumazine synthase*. This enzyme served as a mineralization template in the fabrication of iron oxide nanocrystals. The capsid shell of the *lumazine synthase* is charged positively and negatively so the authors suggest the simultaneously sequester of both cationic and anionic reactants for nanoparticle synthesis.

1.2.1.2 CO-PRECIPITATION

Co-precipitation is one of the most common methods which consist of mixing ferric and ferrous ions in a 1:2 molar ratios in a non-oxidizing oxygen environment. Reaction mechanism showed below (Wu *et al.*, 2015).



The optimal pH for this reaction should be between 8 and 14, due to the thermodynamics of the reaction (Jolivet *et al.*, 2004). In presence of oxygen, magnetite is transformed into maghemite. Two stages are involved during co-precipitation synthesis (only kinetics factors control the growth of the crystal).

a) When concentration of the species reaches critical saturation, which leads to a short burst of nucleation.

b) Diffusion of the solutes to the crystal surface which leads to the slow growth of nuclei.

Size and shape of nanoparticles can be modified and controlled by adjusting pH, ionic strength, temperature, nature of salts (perchlorates, nitrates, chlorides and sulfates) or ferrous and ferric ions ratio (Laurent *et al.*, 2008).

Nanoparticles prepared in liquid phase, are most of the time formed via coprecipitation, together with other solvents. Nanoparticles tend to aggregate in order to reduce their surface energy, specially in this liquid phase preparation (Kim *et al.*, 2001). Thus, stabilization is required to conserve small sizes. Stabilizers adsorb to nanoparticle surface by coating with proteins (Xu *et al.*, 2006) starches (Kim *et al.*, 2003) non-ionic detergents (Soares *et al.*, 1996) or polyelectrolytes (Kim *et al.*, 2003). The main advantage of the coprecipitation methods is that a large amount of nanoparticles can be synthesized (Laurent *et al.*, 2008).

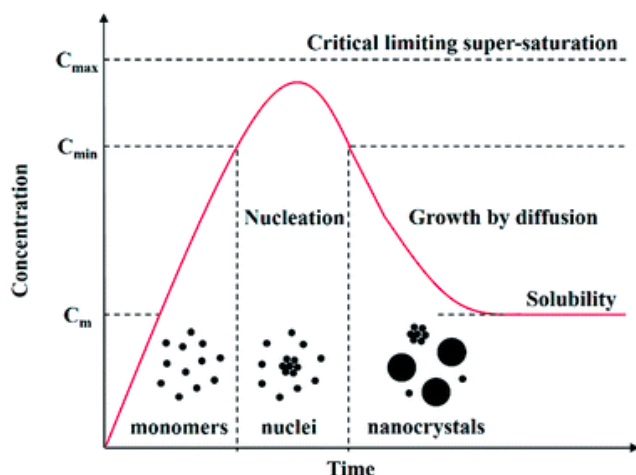


Figure 15: LaMer diagram formation of mono- dispersed nano- and microparticles with the nucleation and crystal growth mechanisms. Taken from: Chang & Waclawik (2014).

Figure 15 illustrate the nucleation and growth process of nanocrystals in solution. Monomers are initially formed due to the precursors dissolved in solvents, followed by nuclei generation and growth of nanocrystals via nuclei aggregation (Chang and Waclawik, 2014; Nguyen and Do 2011). Particle size must be controlled during short nucleation period, because particle number will be determined after nucleation and will not change during particle growth. Particle size in coprecipitation can be tailored by the addition of chelating organic anions or polymer agents, during the formation of nanoparticles, preventing nucleation which could lead to larger particles (Laurent *et al.*, 2008).

1.2.1.3 HYDROTHERMAL / HIGH TEMPERATURE REACTIONS

Iron oxides nanoparticles can be produced by thermal decomposition of organometallic precursors such as iron acetylacetonate (Roca *et al.*, 2006), iron carboxylate (Muraishi *et al.*, 1981), iron carbonyls (Majewski *et al.*, 2007) and iron cupferronates (Rockenberger *et al.*, 1999). While stabilization surfactants such as oleylamine, oleic acids and steric acids can be used for preventing agglomeration (Teja and Koh, 2009).

Synthesis can be performed in reactor or autoclaves, with really high pressures (higher than 2000 psi) and temperatures (above 200 °C). Two main methods are used for hydrothermal synthesis: Hydrolysis (where ferrous salts are used) and oxidation or neutralization of mixed metal hydroxides. Higher temperatures result in a faster nucleation than grain growth, and when reaction time is longer, it will favor grain growth (Laurent et al., 2008). Stefanescu et al (2011) studied iron oxide synthesis by thermal decomposition and found that maghemite is obtained at 250-300 °C and hematite at 400-500 °C. Rockenberger and colleagues (1999) produced maghemite of a size range of 6 to 7 nm by thermal decomposition at 300 °C reaction of iron cupferronates with trioctylamine. Hyeon and coauthors (2001) produced maghemite nanoparticles with size ranging of 4 -6 nm by the reaction of iron pentacarbonyl in octyl ether and oleic or lauric acid.

1.2.1.4 SOL-GEL REACTIONS AND POLYOL METHOD

The sol-gel reaction is about two crucial steps: hydroxylation and condensation of molecular precursors in a solution, originating nanoparticles (Laurent *et al.*, 2008). For the hydroxylation to take place, a basic or an acid solution can be utilized. If the acid solution is used, then the gel will be polymeric. On the other hand, if the solution for hydroxylation is a base, the gel will result in a colloidal (Lam *et al.*, 2008). Solution needs to be dried and then gelled by removing solvents for example (Teja & Koh, 2009). Good control of particle size, shape and homogeneity can be achieved by controlling several parameters such as agitation, temperature, concentration of salt precursors and pH just to name a few (Cannas *et al.*, 1998). Some of the precursors for iron oxide nanoparticles by sol-gel method can be: iron chloride, iron nitrate and iron acetylacetonate.

The molecular precursors hydrolyze and condensate in the solution, originating a solution of nanoparticles. Solinas and colleagues (2001), found gelation process determines the size and phase of the formed nanoparticles in a silica matrix, while Kim (2003) observed that rate of hydrolysis and condensation of gel precursors and other reactions occurring during gelling will determine the structural parameters and material porosity.

A disadvantage of the sol- gel method is the post-treatment needed because of the contamination derived of byproducts of reactions (Teja & Koh, 2009). Also, further heat treatment is needed to

acquire final crystalline state. For example, it has been reported than maghemite nanoparticles of 6 to 15 nm where obtained by sol-gel method after heating treatment of gels at 400 °C. Heat post treatment has several advantages in terms of an uniformity of size and good control of particle size (Laurent *et al.*, 2008).

Parameters that should be taking into account when synthesize by sol-gel are solvent, temperature, concentration of salt precursors, pH and agitation (Cannas *et al.*, 1998). For example Shah and coworkers (2015) synthesized iron oxide nanoparticles by sol-gel method using ferric chloride as a precursor ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and found a correlation of mixed phases of maghemite and magnetite at pH 5 and 7 highlighting the importance of pH during synthesis.

1.2.1.5 MICROWAVE-ASSISTED SYTHESIS

Electromagnetic radiation excites molecules. Microwave assisted is most of the cases ecofriendly, fast and very energy efficient (Kim and Lieber., 1999), waves can penetrate material and supply energy by generating heat. Microwave can be a technique used for complementing synthesis because of their extremely rapid kinetics, for example with the hydrothermal method. Morphology of nanoparticles can be affected by the heating method. Magnetite and hematite nanoparticles were prepared by microwave heating method by using FeCl_3 , polyethylene glycol and $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ by Wang and colleagues (2006).

Ramasahayam and colleagues (2012) reported a novel microwave-assisted synthesis of reduced iron oxide stabilized with pine wood shavings for P removal. They achieved a 100% P removal. The reduced iron oxide wood-impregnated still exhibited magnetism.

1.2.1.6 CONSTRAINED ENVIRONMENT REACTIONS

I) Reverse Micelle Or Emulsion Technology

Microemulsions are nanosized water molecules dispersed in an oil phase which stabilize by surfactants. Size of nanodroplets can be controlled by changing size of aqueous droplets core,

while the surfactant will surround the nanoparticles limits their growth and shape (Teja & Koh, 2009). In nanodroplet technology, iron salt solutions are encapsulated by a surfactant such as micelles (1-10 nm) or water-oil solutions (1-100 nm) (Pileni *et al.*, 2000). This nanoenvironment behaves as a nanoreactor which isolates the nanoparticles from surrounding organic solution. Growth and nucleation are limited by the surfactant-stabilized nanoreactor. Different kinds of nanoparticles can be produced by changing amount of surfactant and co surfactant, oil phase or reacting conditions.

Inouye and coauthors (1982) developed the first magnetic nanoparticles formed in micelles. They found that by changing the micelle temperature and surfactant concentration could control size of the magnetic particle. Later, another study conducted by Lee (2005) proved an inexpensive synthesis by using reverse micelles technology. Homogeneous and highly crystalline magnetite nanoparticles were able to form at high temperature. Size adjustment was correlated with an iron salts, surfactant and solvent proportions. The nanoemulsion can be lysed to recover particles from surfactant. Salazar-Alvarez (2004) use acetone to lyse iron oxide nanoparticles synthesized by the use of reverse emulsions.

Water-in-oil emulsions have been used to synthesize super paramagnetic iron oxide nanoparticles, with well defined nanodroplets of liquid phase, dispersed by the surfactant-molecules assembly in a continuous hydrocarbon phase (Lopez-Quintela *et al.*, 1997).

There are several ways to use microemulsions for nanoparticle synthesis, illustrated on *figure 16*:

- a) In one method, the reactants A and B are dissolved in a liquid phase of water and oil, then they form a AB compound which precipitates and is confined in nanodroplets which limit size and shape (Pillai *et al.*, 1995).
- b) Another method consists in adding a reducing agent to the microemulsion which contains water, oil, a surfactant and the reactant. The reducing agent could be presented in a gas or liquid phase.
- c) The production of oxide, hydroxide or carbonate precipitates, can be accomplished bubbling gases like CO₂, NH₃ or O₂ through a microemulsion containing a soluble salt

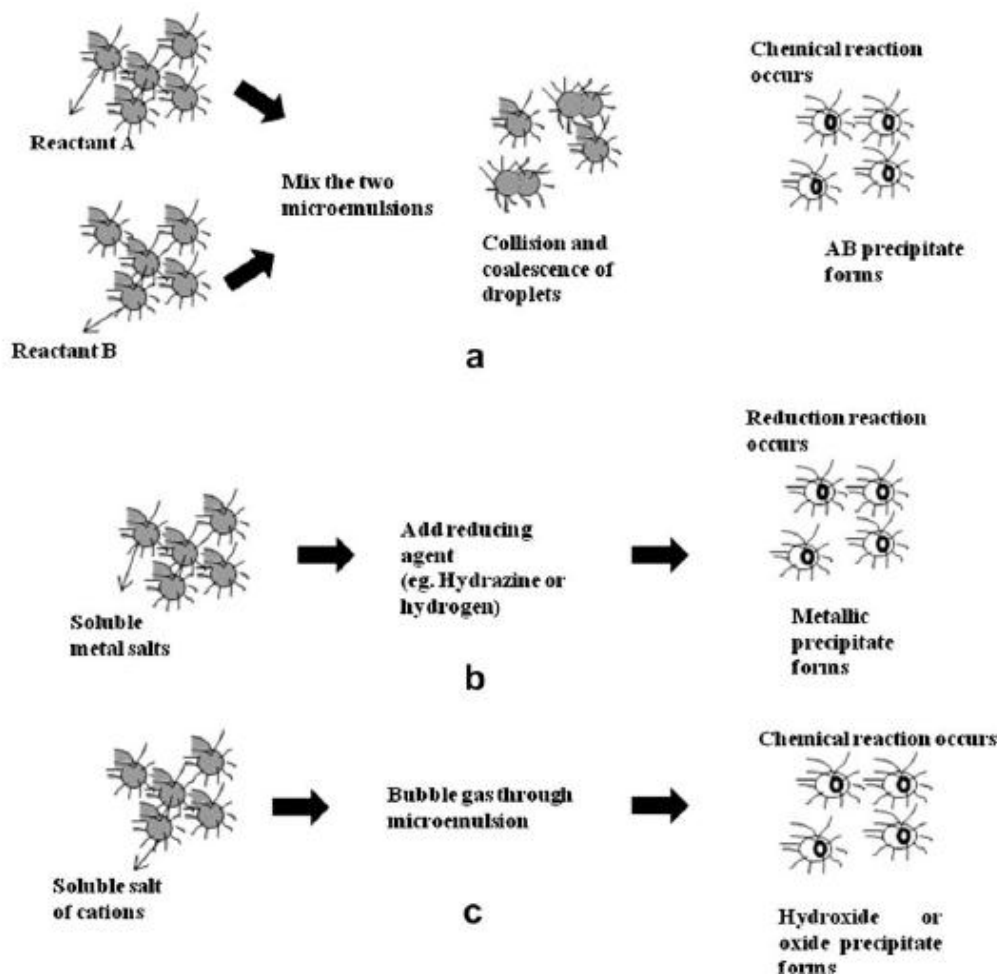


Figure 16: Graphic representation of nanoparticle synthesis in microemulsions a) by mixing microemulsions , b) by adding a reducing agent, and c) by bubbling gas trough the microemulsion. Image taken from: (Teja & Koh, 2009).

II) Magnetolipossomes

This coating approach consist in encapsulate iron oxide nanoparticles in liposomes to obtain a magnetic behavior: magnetoliposomes. The nanoparticles can be stabilized by a phospholipid bilayer (lipoparticles) or phospholipid vesicles which encapsulate iron oxide in aqueous compartment (magnetovesicles). Magnetovesicles can be produced by hydrate a lipidic field, followed by nanoreactor synthesis, congelation/decongelation, inverse phase evaporation or extrusion (Laurent *et al.*, 2008). Nanoparticles can be purified by centrifugation, magnetic sorting or gel-exclusion chromatography (GEC) (Man and Hannington., 1987)

III) Sonochemical or sonolysis method

Sonolysis uses acoustic cavitation in order to cause chemical effects. Nobel structures can be created by applying high intensity ultrasound. The acoustic waves can create bubbles which oscillate due to the alternating expansive nature of the waves. The bubbles are able to store ultrasonic energy and grow until collapse, releasing this concentrated energy in an instant, it happens transiently with a 5000 K temperature and a 1000 bar pressure (Suslick, 1990). The high temperature occurring by the rapid collapse leads to a conversion of ferrous salts into magnetic nanoparticles (Laurent *et al.*, 2008). Sonochemistry is the application of ultrasound to chemical reactions and processes. Bubbles grow and suffer a highly energetic collapse in a liquid. Such a miniemulsion droplet can be treated as a “nanoreactor” which may encapsulate specific particles (Teo *et al.*, 2009). Nevertheless, sonolysis is not recommended when fabricating nanoparticles with a established shape and dispersity (Wu *et al.*, 2015).

Iron oxide nanoparticles can be synthesized by sonication of aqueous ferro or ferrous salt solution, under ambient conditions (Qasem *et al.*, 2005). Zhu and coworkers (2013), recently utilized the sonolysis method to synthesize Fe_3O_4 nanoparticles of 30-40 nm, the nanocomposite was immobilized with hemoglobin to fabricate a biosensor for detecting H_2O_2 .

This procedure is innocuous and can produce a range of nanocomposites, with multiple combinations of different polymer and encapsulated materials (Wu *et al.*, 2015) which could be implemented for medical treatments. Zhu and coworkers (2013), recently utilized the sonolysis method to synthesize Fe_3O_4 nanoparticles of 30-40 nm, the nanocomposite was immobilized with hemoglobin to fabricate a biosensor for detecting H_2O_2 . Encapsulated methods generally involve multiple steps or non ambient preparation conditions, thus, Teo and colleagues (2009) developed a one step clean method for nanoencapsulation of magnetite nanoparticles within latex particles. Interestingly, the magnetite nanoparticles still preserved their magnetic behavior when encapsulated.

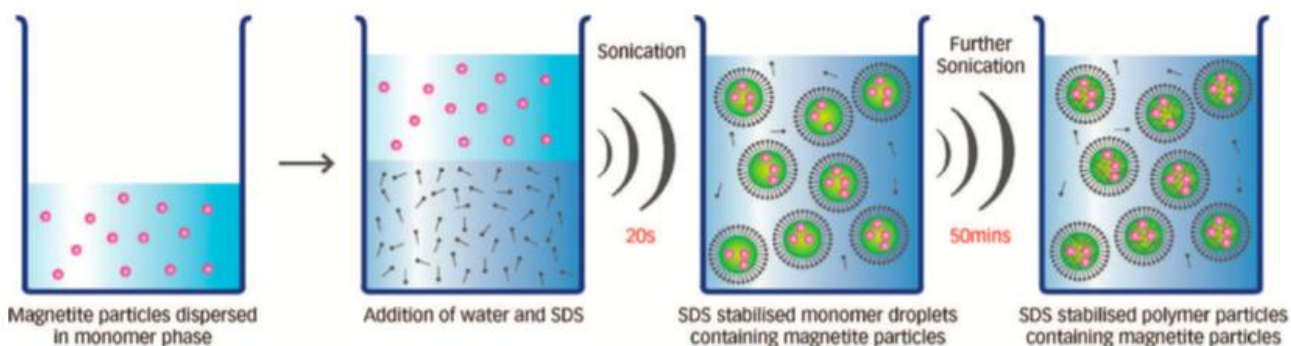


Figure 17: Schematic representation of the encapsulation of magnetite and monomer droplet to latex particle by sonochemical method divided miniemulsion polymerization pathway. Sodium dodecyl sulfate used as a stabilizer. Taken from Teo *et al.*, (2009).

Figure 17 illustrates the miniemulsion process where particle formation begins by a droplet nucleation mechanism. Authors refer to the miniemulsion droplet as a “nanoreactor”.

Organometallic precursors can decompose by sonolysis for iron oxide nanoparticles fabrication, together with polymers, organic capping agents or structural hosts, which limit nanoparticle growth (Laurent *et al.*, 2008).

1.2.1.7 AEROSOL / VAPOR TECHNIQUES

Synthesis of iron nanoparticles by aerosol technologies such as spray and laser pyrolysis are known to be effective for high rate production. The obtained nanoparticles precipitate as solid products from a gas phase.

As presented in *figure 18*, two routes are used for preparation of nanoscale particles. In gas-to-particle conversion, the source of the vapor is a liquid or solid evaporated, which will nucleate and condensate in the cooling state and solid particles are formed. While in the chemical vapor deposition, similarly, precursor solution is evaporated and thermally decomposed and solid particles are formed by nucleation, condensation and coagulation (Okuyama *et al.*, 2003; 2005). Gas-to-particle conversion can be achieved by reactors using flame, furnace, plasma, or laser.

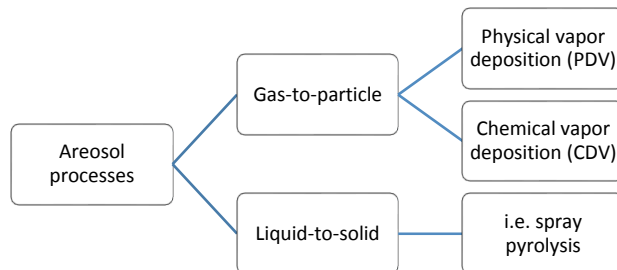


Figure 18: Aerosol process routes

I) Spray Pyrolysis

In this method, precursor (metal salt) and reducing agent are dissolved in an organic solvent. This initial solution is atomized from the initial solution and introduced to incinerator (atomization refers to the production of droplets and their dispersion into gas). Inside the heater, different processes can take place like evaporation of the solvent, diffusion of the solute, drying, precipitation, reaction between precursor and surrounding gas, pyrolysis or sintering (Okuyama & Lenggoro, 2003). Generally, solute condenses and the solvent evaporates (Pecharroman *et al.*, 1995).

Particle size depend on the initial droplets and the velocity of the droplet generated by atomization as shown in *figure 20*. In order to achieve a particle of 100 nm diameter from an dried initial droplet of 5 μm , solute must be less than 0.0008% of initial volume fraction (Okuyama & Lenggoro, 2003). Hence, preparation of nanomaterials with diameters above 100 nm with spray pyrolysis remains a challenge. The system used for spray methods consist of: (i) an atomizer (which converts the starting solution in droplets). (ii) carrier gas, (iii) tubular heater/reactor, (iv) sample or precipitator (Okuyama & Lenggoro, 2003).

For iron oxides, oxidation process can be controlled by varying fuel/air ratio during combustion and Fe precursor, as Strobel & Pratsinis (2009) did for obtaining Magnetic iron-oxide nanoparticles in the form of maghemite, magnetite and wustite.

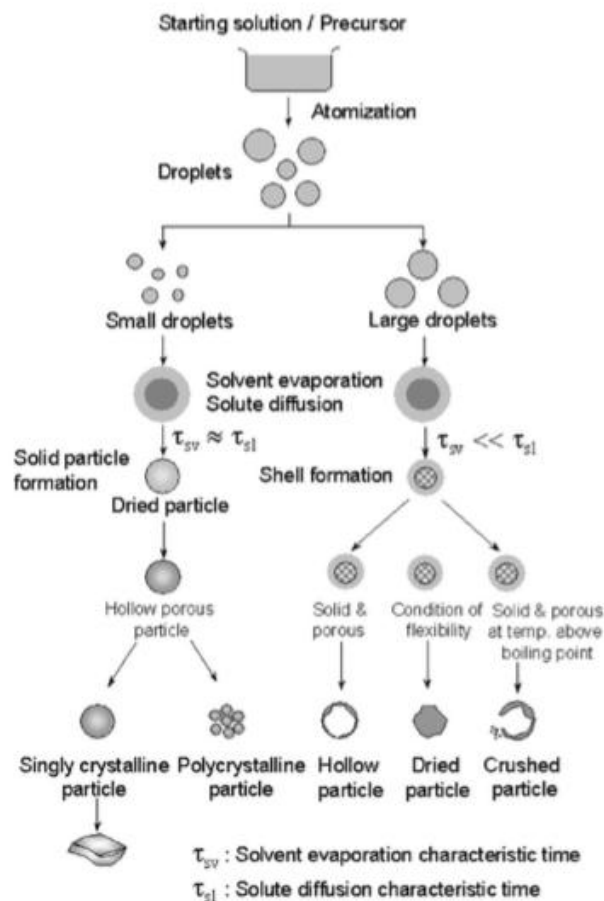


Figure 19: Morphology of particle preparing by spray method. Taken from: Okuyama and Lenggoro (2003).

II) Laser Pyrolysis

This method consists in heating a gaseous mixture of iron precursor and a flowing mixture of gas by a laser, producing particles at a nano-scale. Laser photons heat at least one gas species, which can be or a sensitizer or a reactant. A sensitizer is used to transfer energy to the reactant by absorbing CO₂ radiation. A continuous wave of CO₂ laser will initiate and sustain the chemical reaction together with the mixture of gas.

Veintemillas-Verdaguer and coworkers (2002) studied the effect of certain variables to determine how to control size of maghemite nanoparticles produced by laser pyrolysis. They found that oxygen present in gas phase has an enormous impact on size and crystallinity of the particles. As shown on figure 21, commonly iron pentacarbonyl is used as a precursor (Alexandrescu et al., 2005) and ethylene is utilized as a sensitizer which do not absorb radiation at the laser wavelength (Tartaj et al., 2003). Variables affecting the product, are oxygen concentration, gas phase

impurities, and the heating time, which need to be carefully controlled (Teja & Koh, 2009).

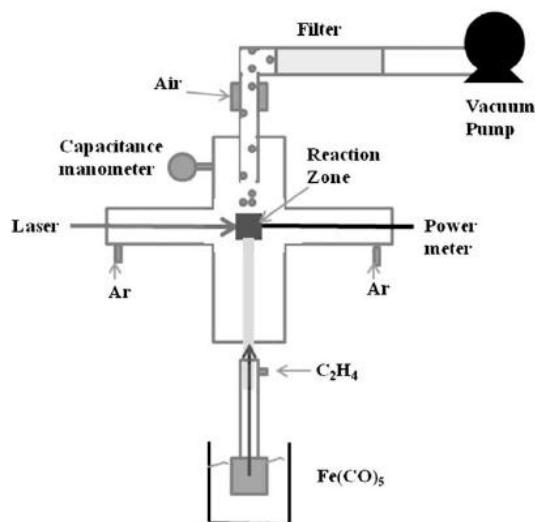


Figure 20: Representation of laser pyrolysis device (Dumitrache et al., 2005; Teja & Koh, 2009)

1.2.2 TOP DOWN METHODS FOR SYNTHESIS OF IONPS

Top down techniques for synthesis of nanoparticles involve beginning with a bulk solid and by structural decomposition to obtain a nanostructure (Koch, 2012).

An important drawback of top-down approach is the imperfection of the surface structure. Defects in surface structure may impact on the properties of nanoparticles. Of note that these methods are known to be not energetically efficient due to an enormous consumption of energy to maintain the high pressure and temperature required.

1.2.2.1 LASER ABLATION

Laser ablation is a physical method of synthesis, consisting in ablating or extracting electrons from a bulk material due to a strong electrical field generated by laser light within a penetrated volume (Hashida *et al.*, 2009). When laser flux is high enough, material is converted to plasma containing species of atoms, molecules, electrons, ions etc. which nucleate and grow on a substrate or a cool liquid medium (Zeng *et al.*, 2012). Moreover, laser ablation can take place in different environments: Gaseous, vacuum or in a liquid medium (Smith *et al.*, 1965). In the vacuum chamber environment (which is the most conventional medium) the material is vaporized by laser in ultrahigh vacuum or in the presence of a background gas to form a plasma plume which will then be deposited as a thin film on a substrate. High crystalline quality materials have been achieved (Chrissey *et al.*, 1994) specially in synthesis of 1D nanomaterials (Zhang *et al.*, 2011).

Laser ablation synthesis may be performed in an aqueous/liquid solution, where nanostructures form by two steps; first, the ultrafast quenching of a hot plasma and second, the interaction with the surrounding medium (Liu *et al.*, 2008). Amendola and colleagues (2011) have proved laser ablation synthesis in solution (LASiS) for obtaining FeOx magnetic nanoparticles which were stable in water even in the absence of stabilizers. LASiS is considered a green approach requiring limited manual operation and it is a one-step synthesis. Authors obtained FeOx by laser pulses at 1064 nm focused on a 99.99% pure iron plate placed at the bottom of a cell containing bidistilled water.

Zeng and coworkers (2005) synthesized iron oxide nanoparticles by laser ablation (*Figure 22*) by three steps; First, they induced Fe plasma by high-temperature and high-pressure above the iron target by one laser pulse. Then an adiabatic expansion of the hot plasma lead to the formation of Fe clusters (step II), which interacted with surrounding aqueous solution (step III), resulting in FeO nanoparticles.

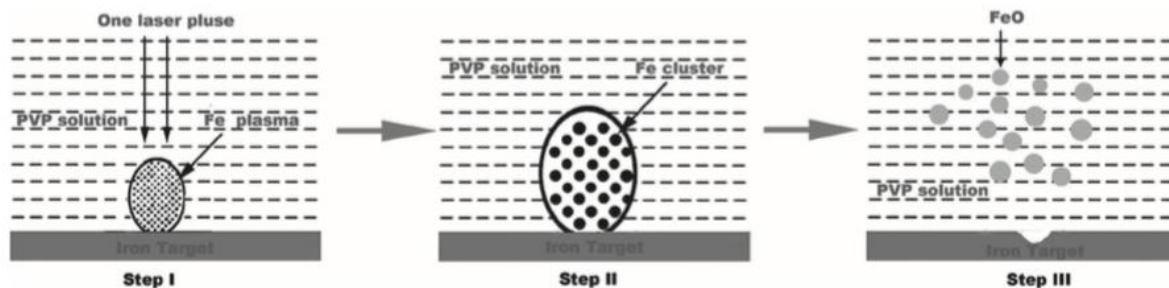
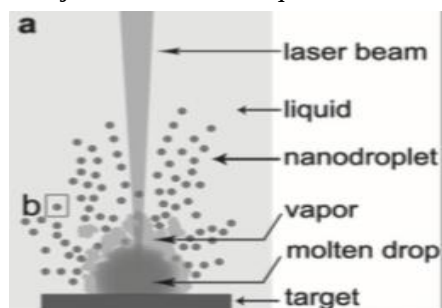


Figure 21: Illustration of formation of FeO nanoparticles by laser ablation. Taken from: Zeng *et al.*, 2005.

Final products can be controlled by selecting type and concentration of liquids, targets and laser parameters. Nanodroplets can be produced by using a low-energy millisecond laser and ejected into the liquid medium at high speed (Niu *et al.*, 2010) as shown in figure 23. Another



interesting example, is the work published by Swiathowska-Warkocka and colleagues (2011). They synthesized magnetite and wustite by pulsed laser irradiation in ethyl acetate and found Fe₃O₄/FeO phase ratio and magnetic properties of final products can be precisely controlled only by tuning laser fluency.

Figure 22: Formation of nanodroplets. Taken from: Niu *et al.*, 2010.

1.2.2.2 ELECTRO EXPLOSION

Electric explosion wires (EEW) is relatively simple to realize, the phenomenon was first observed and described in 1774 by Nairne (Chace, 1959). Explosion of metals in air, leads to oxide nanoparticles. Oxide metal nanoparticles are formed when high energy is injected in the metal, and is higher than the initial boiling point, which makes the wire to disintegrate, leaving a vaporous tail during the scatter, where the oxide nanoparticles are formed (Kotov, 2003). Oxygen concentration is important, to the complete process. Powder resulted in a mix of residual microparticles (from wire) and nanoparticles. For reactive metals, oxidation energy must exceed the sublimation energy. Korean scientists (Song *et al.*, 2012) prepared iron oxide nanoparticles by wire explosion method by exploding pure Fe in Ar-5%O₂, Ar-10%O₂ and Ar-

30% O₂ gas mixtures. Interestingly, they found that a variety of iron oxide species resulted by variation in oxygen partial pressure in the explosion chamber. As oxygen partial pressure increased, the phases in iron oxides were augmented in the order of FeO → magnetite Fe₃O₄ → maghemite (γ-Fe₂O₃) → hematite (α-Fe₂O₃).

1.2.2.3 ATTRITION MILLING

In attrition or milling, micro or macroscale particles are reduced by a physical mechanism of high energy collision from balls into smaller sized, nanoscale particles (Amendola *et al.*, 2011; Yadav *et al.*, 2012). Factors affecting nanoparticle composition and structure, include material and time of milling and the atmospheric medium. Different types of balls which impact upon the powder charge can be used for synthesis of nanoparticles, this balls are arranged in a layer by parallel and roll down the surface of a chamber in order to impact the powder and balls beneath them (Koch and Whittenberge, 1996; Janot and Guerard, 2005). High-energy mills used for nanoparticle synthesis include attrition jet, planetary, oscillating and vibration mills (Arbain *et al.*, 2010). Temperature has an effect of mechanical milling, higher temperatures are expected to result in phases which need higher atomic mobility, and lower temperature can promote the formation of nanocrystalline amorphous phases (Yadav, 2012). Drawback of milling is the potential for contamination from milling media (balls and vial) or atmosphere (Koch, 2003; Yadav *et al.*, 2012). Mechanical milling/grinding has the potential to be use for produce large quantities of nanoparticles or nanocrystalline powders. However, the size distribution is not always homogenic (which is one of the requirements for large production of nanoparticles) and many un-milled precursors remain at the end of the process (Tsuzuki, 2009). Apart from the size reduction, the high amount of energy induced by milling, induces a mechanochemical effect in particles, which may provoke structural defects, with a reduction of degree of crystallinity (Arbain *et al.*, 2011).

After 10 h of high-energy ball milling, Tung and colleagues (2016) demonstrate by XRD analysis the crystallite size of iron oxides decreased to 11 nm. Likewise, found after 12 h of milling, a coexistence of iron phases. Similarly, hematite powder was obtained by a planetary mill (Arbain *et al.*, 2011) after 10 h at 600 rpm with a minimum crystallite size of 17.1 nm.

Futhermore, magnetites particles of 20 nm were obtained by milling during 10 h (Carvalho *et al.*,2013).

1.3 IRON OXIDE NANOPARTICLES

Iron oxides are common compounds present in almost all forms in the global system (*Figure 24*): atmosphere, pedosphere, biosphere, hydrosphere and lithosphere (Cornell & Schwertmann, 2003).

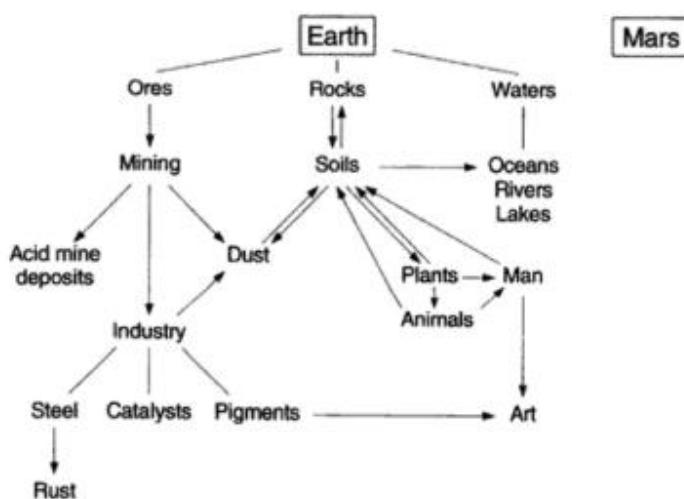


Figure 23:Iron oxides in the global system. Taken from: Cornell & Schwertmann, 2003.

There are two valence states of iron in environment, the water-soluble Fe (II) also known as ferrous iron and the highly water-insoluble Fe (III) or ferric iron. However, zero-valent or elemental/native iron (Fe(0)) can be found in very specific conditions as ultramafic rock, meteorites etc. It is, rarely formed at the surface of Earth, due to its high reactivity. In environment, the role of iron is important in contaminant mobility, due to the electron donor role and precipitant/sorbent substrate. Further, there are sixteen known iron oxides and oxyhydroxides (Cornell and Schwertmann., 2003). Magnetite (Fe₃O₄), maghemite (g-Fe₂O₃), and hematite (a-Fe₂O₃) are probably the most common forms of iron oxide in nature (Cornell *et al.*, 2003) (*Figure 25*).

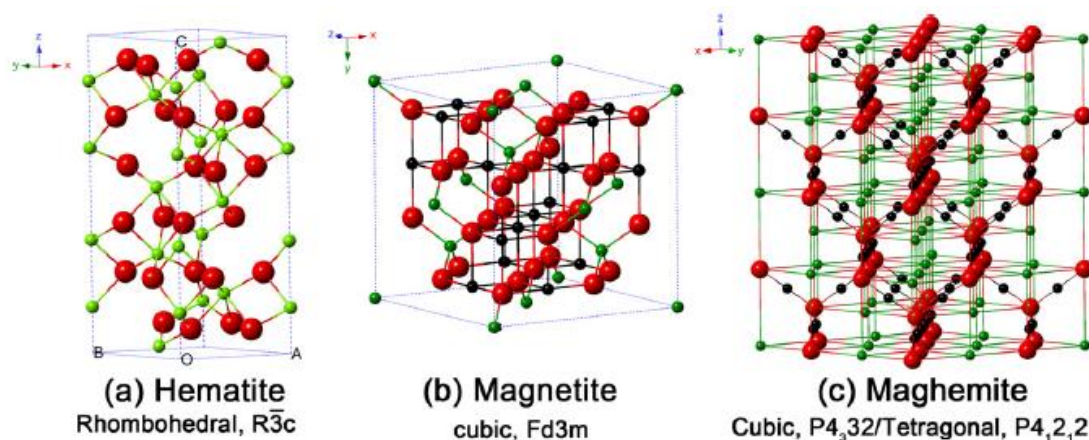


Figure 24: Crystallographic data of hematite, magnetite and maghemite the black ball is Fe^{2+} , the green ball is Fe^{3+} and the red ball is O^{2-} . Taken from: Wu *et al.*, 2015.

- Magnetite (Fe_3O_4) or black iron oxide, ferrous ferrite, loadstone, Hercules stone. Has the bigger magnetism of any transition metal oxide (Majewski *et al.*, 2007).
- Wüstite $Fe_{1-x}O$ ($x = 0.05-0.17$) is a transition metal monoxide, meaning their electronic structure is generated due to a competition between electrons in the narrow 3d bands. Stechiometric FeO does not exist at room temperature, it has a tendency to decay in two-phase mixtures of α -Fe and Fe_3O_4 (Schrettle *et al.*, 2012). The defect presented in this iron oxide specie, is “compensated” by a change in charges of other atoms in the solid.

Fe₂O₃

There are amorphous Fe_2O_3 and four polymorphs (alpha, beta, gamma and epsilon). Most frequent species are “alpha” (hematite) and “gamma” (maghemite).

- Hematite (α - Fe_2O_3) the oldest known of iron oxides, found in rocks and soils principally, is also known as ferric oxide, blood-red in color if divided, or black –grey if crystalline. Hematite is the end product of other iron oxides. In the structure of hematite, oxygen ions are in a hexagonal close-packed arrangement, with $Fe(III)$ ions occupying octahedral sites. It is known to be the most stable phase of iron oxide in air (Lu & Meng, 2010). At a temperature of $650^\circ C$, hematite turns into Fe_3O_4 with a high energy loss (Chirita and Grozescu, 2009). Hematite has semiconductor properties, and is extremely useful in solar energy conversion, photocatalyse and water splitting (Chirita and Grozescu, 2009).

- Maghemite ($\gamma\text{-Fe}_2\text{O}_3$) is the weathering product of magnetite or a product of heating other iron oxides (Teja *et al.*, 2009). Gamma Fe_2O_3 has a cubic spindle structure. In time, at room temperature, hematite turns into hematite crystalline structure (Chirita and Grozescu, 2009). Maghemite is biocompatible and is one of the most extensively used biomaterials specially for applications such as cell separation, drug delivery, hyperthermia and MRI contrast agent (Chirita and Grozescu, 2009).

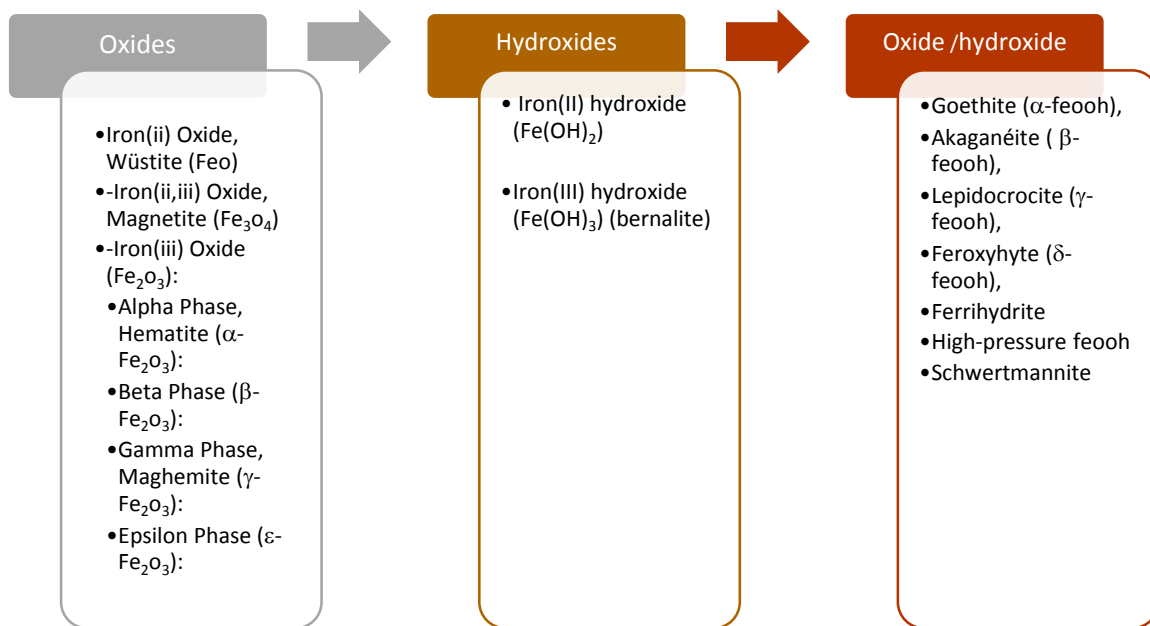


Figure 25: Different types of iron oxides and oxyhydroxides known so far. (Cornell and Schwertmann, 2003).

Each of the four Fe_2O_3 polymorphs (alpha, beta, gamma and epsilon) has unique magnetic, biochemical, catalytic properties, which can be targeted to a diverse range of applications. However, high temperature and high pressure will trigger polymorphous transformations which will lead to the formation of undesired mixtures of Fe_2O_3 . Alpha and gamma iron oxides generally occur in nature while beta and epsilon species are synthesized usually in laboratory (Machala *et al.*, 2011).

Property	Oxide		
	Hematite	Magnetite	Maghemite
Molecular formula	$\alpha\text{-Fe}_2\text{O}_3$	Fe_3O_4	$\gamma\text{-Fe}_2\text{O}_3$
Density (g/cm^3)	5.26	5.18	4.87
Melting point ($^\circ\text{C}$)	1350	1583–1597	—
Hardness	6.5	5.5	5
Type of magnetism	Weakly ferromagnetic or antiferromagnetic	Ferromagnetic	Ferrimagnetic
Curie temperature (K)	956	850	820–986
M_s at 300 K ($\text{A}\cdot\text{m}^2/\text{kg}$)	0.3	92–100	60–80
Standard free energy of formation ΔG_f° (kJ/mol)	–742.7	–1012.6	–711.1
Crystallographic system	Rhombohedral, hexagonal	Cubic	Cubic or tetrahedral
Structural type	Corundum	Inverse spinel	Defect spinel
Space group	R3c (hexagonal)	Fd3m	P4 ₃ 32 (cubic); P4 ₁ 2 ₁ 2 (tetragonal)
Lattice parameter (nm)	$a = 0.5034$, $c = 1.375$ (hexagonal) $a_{\text{Rh}} = 0.5427$, $\alpha = 55.3^\circ$ (rhombohedral)	$a = 0.8396$	$a = 0.83474$ (cubic); $a = 0.8347$, $c = 2.501$ (tetragonal)

Figure 26: Physical and magnetic properties of Iron oxides. Taken from: Teja and Pei-Yoong (2009)

1.3.1 MAGNETISM IN IRON OXIDES NANOPARTICLES

Magnetism is originated by the magnetic moment, a dipole moment in an atom which is determined by the spin of electrons, especially unpaired electrons in atoms orbitals. The magnetic behavior of iron oxides, is due to four unpaired electrons in its 3d orbital (Teja and Koh *et al.*, 2009). This magnetic states change when crystal formation. Iron oxide nanoparticle smaller than 20 nm are able to show superparamagnetic behavior at room temperature (Cornell *et al.*, 2003).

Magnetic moments arrangement is inverse to the temperature; If temperature increase, the magnetic moment decrease. Furthermore, T_c or Curie temperature is termed for determining the temperature at which ferromagnetic and ferrimagnetic materials loose their permanent magnetic properties, in order to be replaced by induced magnetism. On the other hand, Néel temperature or T_n is the thermal energy used for antiferromagnetic substances which become paramagnetic, destroying their magnetic order within the material (Spaldin, 2006).

Bulk materials are normally arranged in domains, each domain has their spins aligned, and the magnetic moment is the sum of all magnetic moments of atoms per unit of volume (*figure 28*). Moreover, iron atoms arranged in crystals, in a small length, number of domains decrease, until there is a single domain (Teja and Koh, 2009).

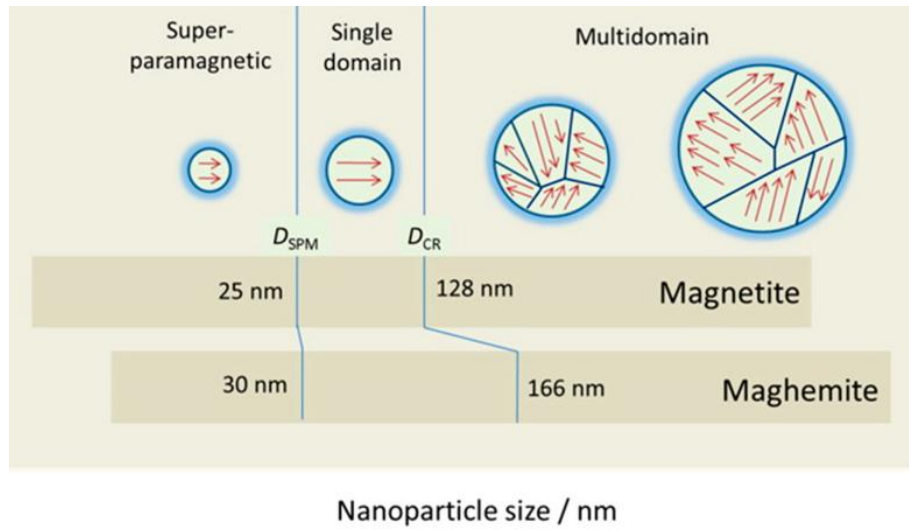


Figure 27: Magnetic domains in bulk material. Taken from: Estelrich (2015)

In paramagnetic state for example, the magnetic moments are randomly aligned, giving the crystal a zero-charge or zero net magnetic moment (*Figure 29*). Nevertheless, the crystal may have a small magnetic moment if is subjected to an external magnetic field, which will cause that some moment aligns.

On the other hand, according with Teja and Koh (2009) a ferromagnetic crystal, does not need the interference of an external magnetic field, as their magnetic moments are aligned by themselves and the domains do not return to their original orientations when an external magnetic field is applied and reaches a saturation magnetization value. However, a ferrimagnetic crystal has two types of atoms with different strengths and thus, two types of magnetic moments, arranged antiparallel order. It is important to notice that in case the different types of magnetic moments have the same strength or magnitude, the crystal will lose the magnetic moment, named antiferromagnetic (antiferromagnetic).

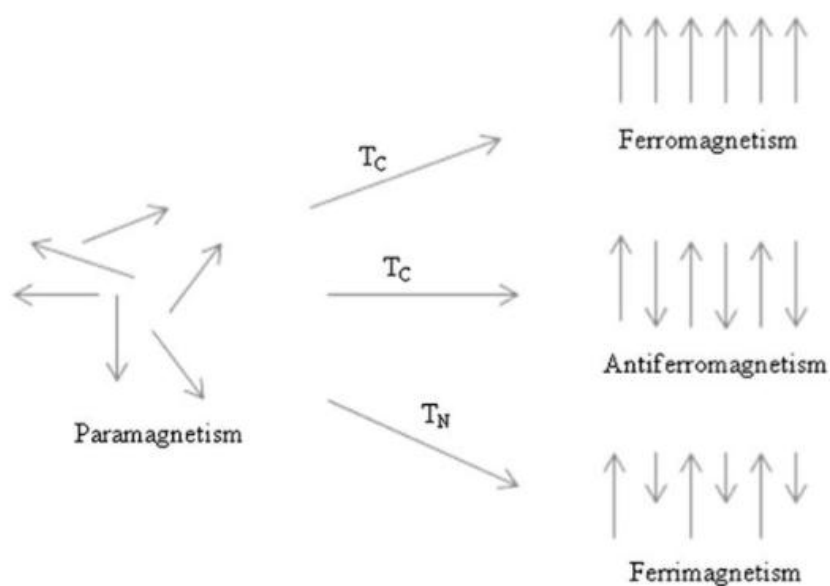


Figure 28: Alignment of individual atomic magnetic moments in different types of materials . T_c meaning Curie temperature and T_n meaning Neel temperature. Taken from Teja and Kho.,2009.

Bulk Hematite at room temperature, is weakly ferromagnetic. However, above $-13.15\text{ }^{\circ}\text{C}$ hematite exhibits a weak ferromagnetism and below this temperature shows an antiferromagnetism behavior, this is known as the Morin transition or Morin temperature (T_m) (Morrish and Eaton, 1971; Chririta & Grozescu, 2009). As size decrease, the Morin temperature will decrease until it vanishes for scales smaller than 8-20 nm and express a paramagnetic behavior above its $T_c = 682.85\text{ }^{\circ}\text{C}$ (Cornell and Schwermann , 2003; Zysler *et al.*, 2001).

Magnetite, is the oldest known ferrimagnetic material. Its Curie temperature is $576.85\text{ }^{\circ}\text{C}$ (Murad *et al.*,1985). Under ambient conditions, magnetite particles small than 6 nm are superparamagnetic (Teja and Koh, 2009). Magnetism depend strongly on changes in the crystal morphology (Margulies *et al.*,1996). Coercivity in magnetite (a measure referred to how much a ferromagnetic material withstand an external magnetic field without demagnetizing) is affected in the order: spheres < cubes < octahedral as the increase in the number of magnetic axes together with series of shapes (Teja and Koh, 2009).

Maghemite is also a ferrimagnetic material at room temperature (Dronskowski, 2001). It is unstable at high temperatures, for example it may transform irreversibly to hematite at approximately 126.85 °C, losing its magnetization. This is the main reason Curie temperature is not easily determined for this iron oxide species. When this transformation occurs, there is a loss of magnetization (Berry *et al.*, 1999). Moreover, maghemite smaller than 10 nm are superparamagnetic at room temperature (Neuberger *et al.*, 2005). There is an interesting property occurring when maghemite agglomerates: superferromagnetism. It occurs when particles begin to interact with each other and they order by magnetic moments. Gamma Fe₂O₃, has ten percent lower magnetic properties than magnetite Fe₃O₄ (Chirita and Grozescu, 2009).

Non-stoichiometric wüstite, with a cubic rock-salt structure, presents antiferromagnetic (AFM) order below Néel temperature of -73.13 °C (Schrettle *et al.*, 2012). Below Néel temperature, a rhombohedral structure results due to a magnetocrystalline distortion (Cornell and Schwertmann, 2003).

Magnetism in iron oxides is determined by the surface properties each phase will present (Tronc *et al.*, 2000). Hence, by variation of the synthesis methods, particles will present different properties which will directly affect magnetism behavior (Shen-Nan *et al.*, 2014). As the particle size decreases, the temperature will have a stronger effect in magnetization as compared with bulk material. Herein, when temperature increases, the magnetization will decrease in a faster rate in small particles (Hendriksen *et al.*, 1993).

1.3.2 MODIFICATION OF IRON OXIDES NANOPARTICLES.

“Naked” iron magnetic nanoparticles have high chemical activity, can be easily oxidized in air and are unstable in acidic solutions, leading to leaching and thus, reducing lifetime and limits reusability of the molecules (Xu *et al.*, 2014; Mahdavi *et al.*, 2008).

Agglomeration may happen due to strong magnetic attraction between particles and for reducing surface energies (Mahdavi *et al.*, 2008). Four forces may contribute to

aggregation: Electrostatic repulsive forces, Van der Waals forces, magnetic dipolar forces (in case of magnetic suspensions), and steric repulsion forces (Vincent *et al.*, 1986).

Stability of a magnetic suspension results from the equilibrium between repulsive and attractive forces (Laurent *et al.*, 2008).

Hence, several techniques have been employed in order to make iron nanoparticles (especially magnetic species) more stable. For example, surfactants such as oleic, lauric, alkylsulphonic and alkylphosphonic acids (Sahoo *et al.*, 2001; Xu *et al.*, 2014), polymers such as polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP) are also effective for coating materials in aqueous suspension (Lee *et al.*, 2006). Another option is the use of natural dispersants which have been extensively employed such as albumin (proteins), starch, chitosan (polysaccharide) just to name a few (Souza *et al.*, 2009).

However, according to Xu and coworkers (2014) surface silanization has been the most common used technique for stabilization of nanoparticles under acidic conditions. Silane molecules are hydrolysed leading to formation of a stable bond between the Si-OH groups of the silanol molecule and the OH groups at the surface as presented in *figure 30*.

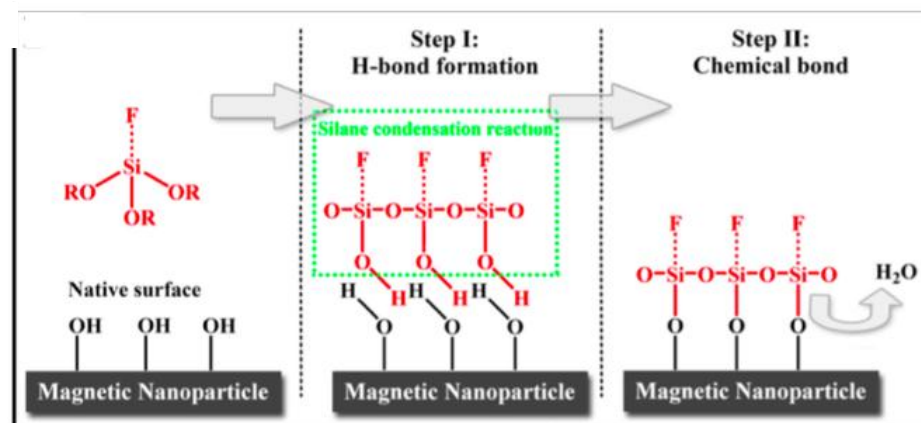


Figure 29: Silane for surface functionalization on magnetic nanoparticles. Taken from: Xu *et al* (2014).

1.3.3 APPLICATIONS OF IRON OXIDES

1.3.3.1 PROTEIN IMMOBILIZATION

Enzyme immobilization have potential applications in biotechnology, immunosensing and biomedical areas (Ansari & Husain, 2012). An advantage of using enzymes is their high specificity which can be used for industrial applications and do not produce a range of unwanted products.

Iron oxide nanoparticles have been intensively utilized for immobilization of proteins/enzymes because of the improvement in stability, reusability and ease to recover by a external magnetic field (Laurent *et al.*, 2009; Garcia-Galán *et al.*, 2011). Various mechanisms have been used for achieving immobilization such as physical absorption (Li *et al.*, 2003), covalent bonding (Ranjbakhsh *et al.*, 2012) and bioconjugation (Liu *et al.*, 2008). Functionalized supports can be implemented to achieve a better immobilization of enzymes. Coupling agents such as aldehyde groups are often utilized due to their interaction with both functional groups of the enzyme and the modified magnetic nanoparticle, producing a covalently cross-linked bound. Moreover immobilization can be achieved by multipoint covalent bonds between enzyme and modified magnetic nanoparticle. Glyoxyl was used as a funtionalized support and was effectively proved to generate a ultipoint covalent immobilization of several enzymes.

Chang and Tang (2014) immobilized horseradich peroxidase (HRP) with silica-coated Fe₃O₄ nanoparticles by using glutaraldehyde as a crosslinking agent. Further, authors used the HRP immobilized particles as an enzyme catalyst for activating H₂O₂ and degraded 2,4-dichlorophenol, indicating its potential for removal of organic pollutants.

Nanoparticles used for immobilize enzymes have several advantages for example stabilization in a broader working temperature and pH, as well as a higher thermal stability than the native enzymes (Ansari and Husain, 2012). Immobilization should reduce any conformational change which could lead to an enzyme inactivation (Mateo *et al.*, 2007). On the other hand, the surface

area of nanoparticles increases the loading of enzymes and improves the particle high mobility, thus reducing diffusion limitations (Xie *et al.*, 2009).

<u>Enzyme</u>	<u>Nanoparticle utilized</u>	<u>Applications</u>	<u>References</u>
Cholesterol oxidase	Fe ₃ O ₄	Analysis of total cholesterol in serum	Kouassi <i>et al.</i> ,2005
Keratinase	Fe ₃ O ₄	Synthesis of keratin	Konwarth <i>et al.</i> ,2009
Lipase	Fe ₃ O ₄	Hydrolysis of pNPP	Huang <i>et al.</i> ,2003
Invertase	Chitosan-coated γ -Fe ₂ O ₃	Conversion of sucrose into invert syrup	Suzuki <i>et al.</i> ,2015
α Amylase	Fe ₂ O ₃	Hydrolysis of starch and other polysaccharides	Khan <i>et al.</i> , 2012

Properties in enzymes are improved when supported on nanoparticles than when founded “free” in a solution. For example, Xia and coauthors (2014) immobilized glucose oxidase to Fe₃O₄/silicon dioxide nanoparticles and report that the enzyme retained 80% of its initial activity after 6 h at 45 °C compared to only 20% for the free enzyme.

Magnetite nanoparticles help as a matrix for immobilization because they do not interfere with the native structure of protein and can be easily recovered with external magnet and thus, reuse for further applications. Nanoparticles can be surface-modified via enzyme molecules attachments, or with organic (e.g. lauric acid) or inorganic (e.g. amino group) particles which will improve enzyme immobilization (Cao *et al.*, 2012). Many other supports for proteins have been implemented, for example porous supports which have diffusion problems making them more competitive for industrial solid-liquid systems (Xu *et al.*, 2014).

Once attached to the nanoparticles surface, the enzymes can be completely eluted by changing pH. For example, this change in solution, leads a decrease in the interaction of the protein and the magnetic nanoparticles, it is important to notice that the use of excessively high or low pH could impact significantly on the protein activity. Furthermore, another method utilized for desorption is a change in ion strength, which can be achieved by adding salts to the solution such as Na⁺, which reduce electrostatic reaction between nanoparticle and protein (Xu *et al.*,2014).

A common biology mediated protein immobilization is by using (strept)avidin-biotin technology which resumes on surface-modifying nanoparticles with streptavidin following by the biotin-

functionalized of the target protein or material, in order to bind nanoparticle with the biotinylated material (Nidumolu *et al.*, 2006). This modification ranks among the most frequently and extensively researched protein-mediated immobilization technique which present important advantages such as stability to extreme heat, denaturants, pH and proteolysis, emphasizing in the irreversible nature of this mechanism (Xu *et al.*, 2014).

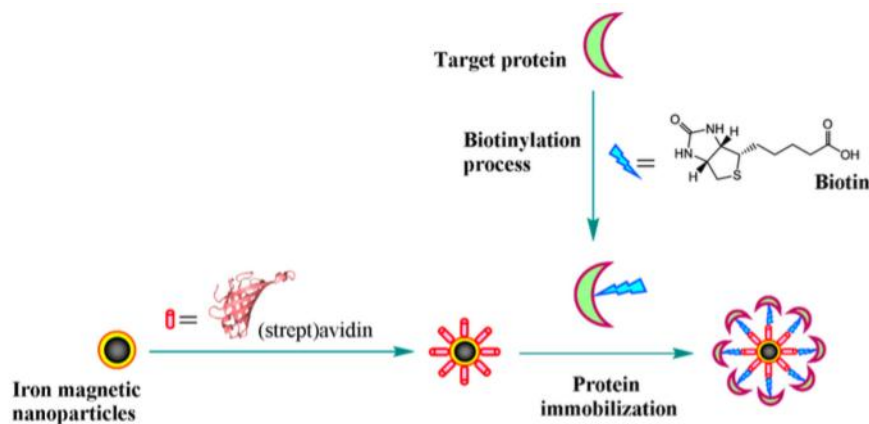


Figure 30: (Strept) avidin-biotin technology for protein immobilization onto iron magnetic nanoparticles.
Taken from: Xu *et al* (2014)

1.3.3.2 MEDICAL SCIENCE.

Iron nanoparticles have been used for several biomedical applications such as targeted drug delivery, biosensor, magnetic resonance imaging, hyperthermia, tissue engineering, magnetofection, etc (Xu *et al.*, 2014). Nanoparticles need to have several characteristics such as uniformity in size and shape, high dispersity and high magnetization values also they should be non-toxic and biocompatible. Magnetite and maghemite have been widely studied for applications in biomedical area due to their biocompatibility and low toxicity in the human body (Tartaj *et al.*, 2003). Both Fe(III) and Fe (II) ions are present in human body, presence of iron oxide nanoparticles are unlikely to cause any serious toxicological issues.

The phases of biocompatibility consist from in vitro evaluation by using cell cultures (1), following to in vivo test using animals (2) to finally, applications in human organisms (3) (figure 32) (Popescu *et al.*, 2014).

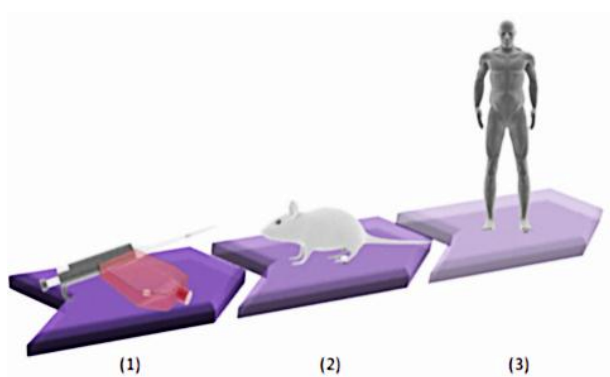


Figure 31: Required phases of biocompatibility for Fe_3O_4 magnetic nanoparticles for medical applications. Taken from Popescu *et al.*, 2014

Usually, for preparation of nanoparticles for biomedical applications, three steps are involved according with Mahdavi and colleagues (2013); First, a synthesis of nanoparticles with homogeneous size and shape, second an encapsulation of the nanoparticle using a polymer with active functional groups and lastly, an attachment of a desired functional group to bind a targeted drug or component for several medical applications.

1.3.3.2.1 Targeted Drug Delivery

Magnetic iron nanoparticles have become a major research focus on drug delivery because of their high specificity to target site and reduced adverse effects. Drugs physically attached to nanoparticle surface are released before reaching final destination. Instead, core-shell structures consisting of a magnetic core and a shell are preferred for delivering drugs, achieving a good transportation to target (Pan *et al.*, 2011). Hence, $\text{Fe}_3\text{O}_4@\text{SiO}_2$ core-shell nanoparticles have been prepared for carrying anticancer agent on their surface. Within 12 h, the nanoparticles released the drug in to the specified site for treatment (Chen *et al.*, 2008). Prerequisite for therapeutic efficacy is the prolonged exposure of high drug concentrations in the tumor lesion (Gutman *et al.*, 2000). Most of the administered drugs often results in subtherapeutic drug levels because of their poor selectivity toward the target tissue and could lead to a possible systemic toxicity (Heimberger *et al.*, 2000). Another interestingly application is focused on releasing drugs into the brain for treatment of several central nervous system diseases such as Alzheimer, Parkinson disease and brain tumors.

However, central nervous system is highly protected from xenobiotics and toxic substances by the blood-brain barrier (BBB) (Wong *et al.*, 2012). Almost 100% of the macromolecular drugs and around 98% of small-molecule drug carriers are unable to enter the brain (Pardridge, 2012). Nanotechnology development presents a novel and potential solution to overcome this problem (Gao, 2016). There are several kinds of strategies for overcoming BBB such as penetration by cellular internalization, opening it by physical and pharmacological methods and intranasal delivery (Gao *et al.*, 2013). For example, specific ligands which are bind with overexpressed receptors on BBB, can be functionalized onto nanoparticles and mediate their transport through the barrier for delivery drugs. Gao and colleagues (2012) reported the use of doxorubicin-loaded nanoemulsion and albumin nanoparticles which could passively target brain tumors and showed enhanced anti-brain tumor effect in comparison to tablets.

The ideal range of particle diameter for drug delivery and targeted chemotherapy is from 10 to 100 nm when injected via intravenous, having the most prolonged blood circulation time due to evasion of the reticuloendothelial system or macrophage system (Laurent *et al.*, 2008).

1.3.3.2.2 Gene Delivery

Gene therapy has the potential of mediating diseases by modifying cellular functions by transporting nucleic acid to target cells, which still remains a challenge. Iron oxide nanoparticles, having an important magnetic response can be utilized as a gene carrier and also enables a magnetic resonance imaging.

According with Kievit and coauthors (2009), in order to achieve an optimal transfection of DNA, nanoparticles must be cationic enough so they can condensate and form complexes with DNA molecules. Also they need to remain colloidal-stable after loading DNA which need to be protected from external molecules which could disrupt its functions such as nucleases. Finally, the complex nanoparticle-DNA should be detectable by MRI.

In order to protect nucleic acids Kievit and coauthors (2009) coated iron oxide nanoparticles with polymers of short chain, grafted to a natural and biocompatible polysaccharide (chitosan) and were able to tracking the nanoparticle in vivo with MRI. The nanoparticle was able to deliver intact

DNA into various sizes tumors as evidenced by the gene expression of an enhanced green fluorescent protein.

1.3.3.2.3 Hyperthermia

Magnetic nanoparticles have been used for hyperthermia in cancer therapy by exposing them to an alternating a magnetic field, producing heat around 41-45 °C (Neuberger *et al.*, 2005)

Since 1993, the hyperthermia treatment was studied due to the properties of superparamagnetic crystals to absorb energy from an oscillating magnetic field and converting it to heat (Hirshch *et al.*, 2000). Tumors cells are more sensitive to heat than healthy ones (Jordan *et al.*, 1993).

Two ways are usually implemented for achieving thermoablation of a tumor. First, an invasive method consists in implanting a radiofrequency electrode in the target pathological area, and second, a less invasive method lie on irradiating the pathological area with a external resonant microwave dipolar emitters (Paulides *et al.*, 2007).

A study published by Sneed and colleagues (1998) shown that hyperthermia is more effective in combination with radiation therapy. Authors report than after evaluating 112 patients with glioblastoma multiformae, and found the survival was doubled when applied radiation together with hyperthermia than compared to radiation therapy alone.

Specific adsorption rate (SAR) is utilize for measuring the electromagnetic energy absorbed by unit of mass on a biological material, expressed in calories per kilogram. SAR equation is expressed in the following equation (Laurent *et al.*, 2008):

Equation 1:

$$SAR = 4.1868 \frac{P}{m_e} = C_e \frac{\Delta T}{\Delta t}$$

Where P is electromagnetic wave power absorbed by the sample, m_e refers to mass of the sample and C_e specific heat capacity of the sample. Further, Rosenswein (2002) found that SAR is inversely proportional to the size of the crystal volume.

Is important to notice that, irradiation needs to be low enough so the intracellular ions remain unaltered. For a better control of irradiated energy on the pathological tissue doped by a ferrofluids, a low frequency magnetic wave is recommended, for 100 to 400 KHz, however, the more classical approach is to summit the patient to an electromagnetic wave of several 100 MHz frequency

(Laurent *et al.*, 2008). Superparamagnetic crystals should be encapsulated with a drug in a liposome ideally, in order for drug to be protected and be able to release in the targeted area submitted to the magnetic field (Babincova *et al.*, 2002).

1.3.3.2.4 Magnetic Resonance Imaging

MRI is a non-invasive technique to visualize structure and function of tissues, based on behavior, alignment and interaction of protons in the presence of an external applied magnetic field (Xu *et al.*, 2014). MRI has been used for visualization of various bio-events, for example gene expression and metastasis at cellular and subcellular levels (Oghabian and Farahbakhsh, 2010). A radio wave is applied in short pulses, perpendicular to an applied magnetic field, the short pulses disrupt the orientation of the magnetic moment. Therefore when the magnetic moment reestablishes, the voltage shifts in proton resonance frequency are recorded and arranged in a white and black image which is further analyzed (Auckland, 2006)

There are different relaxation times for different types of tissues in the body, which can be differentiated from each other in MRI results (Xing *et al.*, 2012). Relaxation rate means the amount of time required for a disrupted atom by a radio wave to reestablish its prior orientation in the magnetic field (Softways, 2014). Contrast agents in MRI, increase relaxation rate of hydrogen spins per concentration of agent (Xing *et al.*, 2012) the unpaired electrons in a material can be used for contrast agents. Iron oxide, for example magnetite, has 5 unpaired electrons in its valence shell.

Iron oxides have been used as a contrast agent (Azhar *et al.*, 2008; Coelho *et al.*, 2008) due to their tendency to accumulate by different densities in different tissue compositions (Indira and Lakshmi, 2010). These nanoparticles are strong enhancers of proton spin–spin relaxation, being this ability a pre-requisite to MRI visibility (Weissleder *et al.*, 1995). It is easier to differentiate between tissues when relaxation rates of hydrogen atoms increase (Carter, 2015). Moreover, several investigators have reported that magnetic nanoparticles can be retained at tumor site. The administration is local, can be intravenous, and is combined with an external magnetic field in order for the nanoparticles to respond magnetically and enable magnetic targeting (Widder *et al.*, 1983). Chertok and coworkers (2009) reported that iron oxide core and starch-coating nanoparticles may be used for

delivery of chemotherapeutic agents to brain tumors is highly effective by intravenous administration along with magnetic targeting. The results presented shown a 5-fold increase in the tumors exposure to drugs and an effective monitor of the iron oxide magnetic nanoparticles distribution by MRI methods (*Figure 33*).

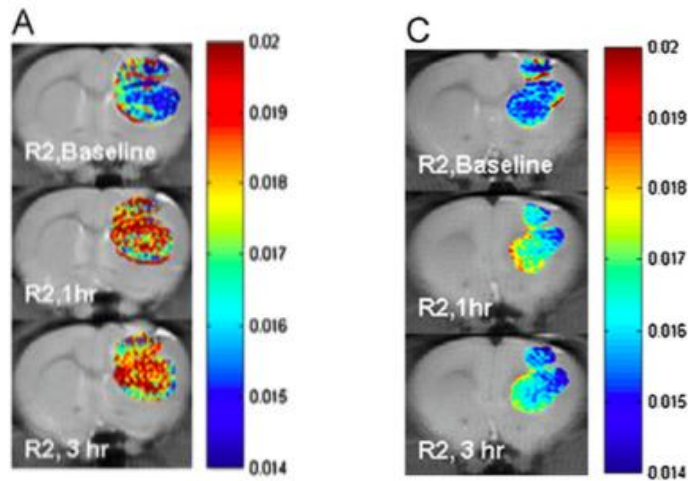


Figure 32: Kinetics of nanoparticle accumulation monitored by MRI in targeted groups of animals. Taken from : Chertok et al.,(2008). (A) targeted and (C) non targeted

In *figure 33*, Chertok and coworkers present a kinetic series of MRI scans demonstrate the nanoparticle accumulation in a 9L gliosarcoma. (A) with and (B) without magnetic targeting. The tumor location is observed as a hyper-intense lesion by spin echo T2-weighted baseline images. GE baseline images were obtained before nanoparticle injection and GE images 1 and 3 hours after nanoparticle administration. Authors conclude that chemotherapeutic drugs to brain tumor by intravenous administration and along with magnetic targeting is 5-fold increase in the total glioma exposure.

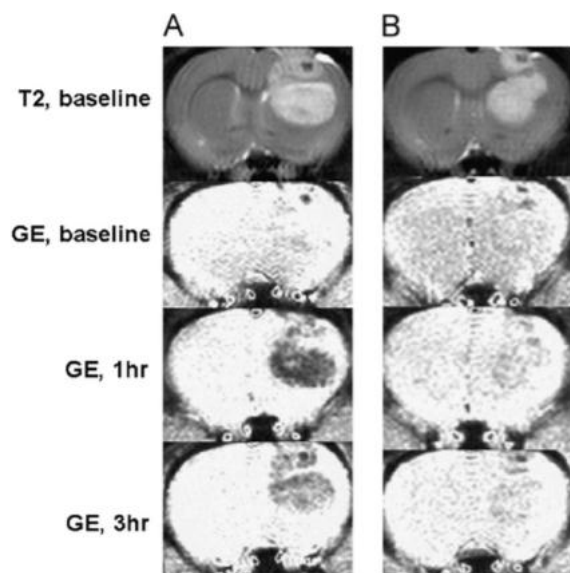


Figure 33: Kinetic series of Magnetic resonance imaging scans of nanoparticle accumulation in 9L gliosacome. Taken from: Chertok *et al.*,(2008).

1.3.3.3 DATA STORAGE APPLICATIONS

Because of the high surface area of the particles in the nano-scale, it is expected that by using FePt nanoparticles, data storage density capacity will increase from 35 gigabytes per square centimeter which is the storage of the most advanced computers to several terabytes per square centimeter (Reiss & Hütten., 2005). FePt nanoparticles for data storage applications was triggered by the discovery of their spontaneous self-organization on a surface, and their stabilization or remained magnetization after removing a magnet.

Particles must have a switchable, stable magnetic state when exposed by temperature fluctuations (Tartaj *et al.*, 2003) as well as resistance to corrosion. Maghemite is chemical and physically stable which makes it a promising material for recording and data storage applications (Sharrock *et al.*, 1985). Iron nanoparticles have been coated with cobalt for improving the storage capacity and achieving greater thermal stability (Cornell and Schwertmann., 2003). Maghemite, on the other hand due to its high magneto resistance, is useful in magnetic recording heads (Camley *et al.*,1993) and when coating with cobalt, the predominant material for its use are video tapes, audio tapes and magnetic discs (Sharrock and Bodnar.,1985).

1.3.3.4 PIGMENTS

Hematite, maghemite and magnetite can be used for pigments industry possessing a variety of colors and presenting a stable and high resistant to acids and alkalis (Teja and Koh, 2009). Hematite-based pigments are red, whereas for maghemite are brown and black for magnetite (Lam *et al.*, 2008). Fouda and colleaues (2012) synthesised hematite by microemulsion method, and evaluated sucesfully the properties of this iron oxide for its use as a pigment. They found that the best concentration for optimal pigments properties is 0.20 M of precursor ferric salt. Properties such as high scattering and reflection efficiency were atributed to their small size which reflected the highest tinting.

1.3.3.5 CATALYST

In industry, catalyst play an important role in reactions. Iron oxides are used as a base catalyst (Shi *et al.*, 2007). Magnetite and hematite are semiconductors which generate reduction/oxidation reactions leading to its use as a catalyst (Al-Sayari *et al.*, 2007). For example, hematite was used as a gold-supported material in order to oxidize carbon monoxide (Zhong *et al.*, 2007) and as a photocatalyst for degradating clorophenol dyes (Bandara *et al.*, 2007). Khali and Munawar (2015) evaluated the properties of hematite nanoparticles to catalyze desulphurization reaction and found a positive catalytic activity for decomposing highly stable aromatic organosulphur compounds such as thiophene. This catalytic ativity was investigated in order to improvement of oil production with is associated with reduction of heavy oils viscosity originated by the degratation of large hydrocarbon molecules which is the result of desulphurization of organosulphur compounds. Hematite has been used as a photocatalyst. First, because it absorbed light in a visible range (photoactive) and second because of its electrical characteristics (d orbital).

1.3.3.6 IRON NANOPARTICLES AND ITS ROLE FOR ENVIRONMENTAL REMEDIATION

Metal oxide nanoparticles are known to have effective removal rate for organic and inorganic contaminants from water and wastewater (Ehrampoush *et al.*, 2015; Ngmosik *et al.*, 2005). Magnetic NPs are formed by a magnetic core which lead to a notorious advantage due to its easily recover with an external magnet (*figure 35*). It is known that magnetite particles even without a surface coating are able to retain elements such as Ba^{2+} and Eu^3 .

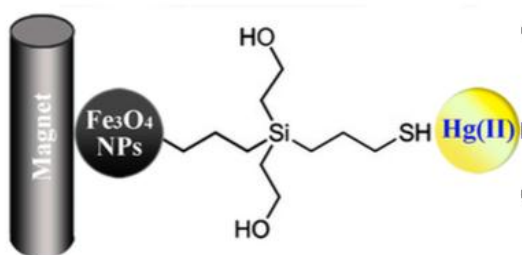


Figure 34: Recovery of Hg(II) using thiol-functionalized mesoporous silica-coated magnetite nanoparticles taken from: Hakami et al., 2012.

Even though nanoparticles tend to form aggregates, unlike microparticles have the characteristic of remain in suspension, this provides an advantage for inject them into contaminated soils, sediments and aquifers (Zhang *et al.*, 2013).

According with Cundy et al (2008), Iron-based remediation can be divided into two groups “...based on the chemistry involved in the remediation process: technologies which use iron as a sorbent, (co-) precipitant or contaminant immobilizing agent (referred to here as sorptive/stabilization technologies); and those which use iron as an electron donor to break down or to convert contaminants into a less toxic or mobile form (reductive technologies)”. There are plenty of environmental pollutants which have been transformed by iron nanoscale technologies (*Figure 36*) including inorganic anions such as Arsenic. However there is another route for degrading pollutants: photo catalysis.

Chlorinated methanes	Trihalomethanes
Carbon tetrachloride (CCl_4)	Bromoform (CHBr_3)
Chloroform (CHCl_3)	Dibromochloromethane (CHBr_2Cl)
Dichloromethane (CH_2Cl_2)	Dichlorobromomethane (CHBrCl_2)
Chloromethane (CH_3Cl)	Chlorinated ethenes
Chlorinated benzenes	Tetrachloroethene (C_2Cl_4)
Hexachlorobenzene (C_6Cl_6)	Trichloroethene (C_2HCl_3)
Pentachlorobenzene (C_6HCl_5)	<i>cis</i> -Dichloroethene ($\text{C}_2\text{H}_2\text{Cl}_2$)
Tetrachlorobenzenes ($\text{C}_6\text{H}_2\text{Cl}_4$)	<i>trans</i> -Dichloroethene ($\text{C}_2\text{H}_2\text{Cl}_2$)
Trichlorobenzenes ($\text{C}_6\text{H}_3\text{Cl}_3$)	1,1-Dichloroethene ($\text{C}_2\text{H}_2\text{Cl}_2$)
Dichlorobenzenes ($\text{C}_6\text{H}_4\text{Cl}_2$)	Vinyl chloride ($\text{C}_2\text{H}_3\text{Cl}$)
Chlorobenzene ($\text{C}_6\text{H}_5\text{Cl}$)	Other polychlorinated hydrocarbons
Pesticides	PCBs
DDT ($\text{C}_{14}\text{H}_9\text{Cl}_5$)	Dioxins
Lindane ($\text{C}_6\text{H}_6\text{Cl}_6$)	Pentachlorophenol ($\text{C}_6\text{HCl}_5\text{O}$)
Organic dyes	Other organic contaminants
Orange II ($\text{C}_{16}\text{H}_{11}\text{N}_2\text{NaO}_4\text{S}$)	N-nitrosodimethylamine (NDMA) ($\text{C}_4\text{H}_{10}\text{N}_2\text{O}$)
Chrysoidine ($\text{C}_{12}\text{H}_{13}\text{ClN}_4$)	TNT ($\text{C}_7\text{H}_5\text{N}_3\text{O}_6$)
Tropaeolin O ($\text{C}_{12}\text{H}_9\text{N}_2\text{NaO}_5\text{S}$)	Inorganic anions
Acid Orange	Dichromate ($\text{Cr}_2\text{O}_7^{2-}$)
Acid Red	Arsenic (AsO_4^{3-})
Heavy metal ions	Perchlorate (ClO_4^-)
Mercury (Hg^{2+})	Nitrate (NO_3^-)
Nickel (Ni^{2+})	
Silver (Ag^+)	
Cadmium (Cd^{2+})	

Figure 35: Environmental contaminants that can be transformed by iron nanoparticles. Taken from: Zhang (2003).

Iron is only one of the most widespread elements in the planet and its ease in synthesis render nanoscale ferric oxide to be a low-cost toxic metal adsorbent.

According to the Environmental Protection Agency (EPA), developing cost-effective groundwater in situ treatment methods and technologies could save billions of dollars in cleanup cost. Unlike the pump and treat method for remediation, the in situ remediation is performed belowground, currently used to remediate contaminated groundwater. The pump and treat was used frequently as a water remediate system until 1998 (Yunus *et al.*, 2012). Further, the permeable reactive barrier (PBR) have been used instead as an *in situ* method for treatment of active zones which degrade or immobilize pollutants as they pass through with water flow. PBR is effective for clean up pollutants such as chlorinated hydrocarbons, immobilization or transforming of pesticides and fertilizes, aromatic nitro compounds and polychlorinated biphenyls (PCBs) as shown in *figure 38* (Zhang *et al.*, 2003; Yunus *et al.*, 2012). IONPs can actually be pumped directly to contaminated sites with no risk of secondary contamination (Deliyanni *et al.*, 2004). Nano zero valent iron (nZVI) has been proposed as a filter material due to its capacity to reduce dangerous contaminants since early 1990 (Uyttebroek *et al.*, 2010). This nanoiron specie can be used by injection into soil,

sediment or solid waste. Once injected, the nanoparticles remain in suspension creating a remediation area or treatment zone (Yunus *et al.*, 2013). Moreover, particles can be attached to a solid matrix such as zeolites, or activated carbon. The reduction by micro scale iron particles, is in comparison negligibly slow (Theron *et al.*, 2008). Core-shell model of nZVI exhibit characteristics of both iron oxides (as a sorbent) and metallic iron (as a reductant) the core is formed of mainly metallic iron, whilst the shell is composed by iron oxides and hydroxides as presented on figure 37 (Theron *et al.*, 2008).

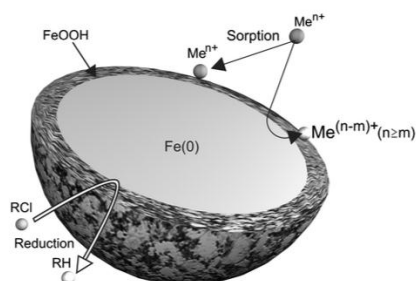


Figure 36: Core-shell model of zero-valent iron nanoparticles. Taken from: Theron *et al.*, 2008

Zhang (2003), demonstrated that iron nanoparticles remain active in water and soil, in a period of more than 4 to 8 weeks, and the field test showed the injection method of nanoparticles into aquifer is relatively simple and can flow with groundwater over 20 m distance. Chlorinated organic solvents such as trichloroethene (TCE) was reduced in situ up to 99% in just few days.

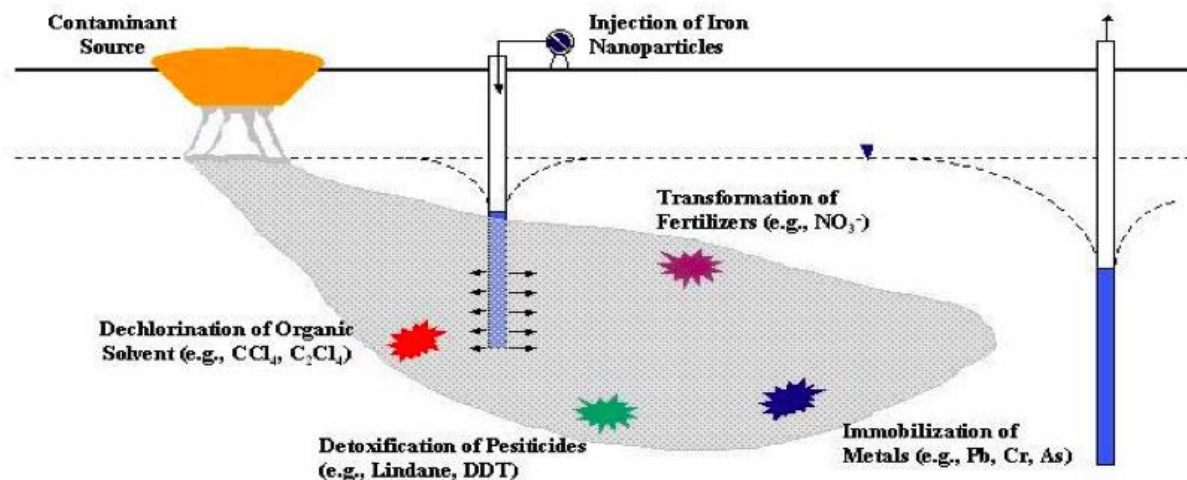


Figure 37: Potential remediation of groundwater, using nZVI . Source: Zhang (2003).

Magnetic iron oxide has several advantages as it leads to chemical stability, low toxicity, excellent recyclability of adsorbent and high capacity to remove pollutants (Guo *et al.*, 2008). Different nanoscale materials have been exploited for remediation like metal oxides, carbon nanotubes, enzymes, and various noble metals, however, the most common material for water remediation are Zero-Valent Iron nanoparticles (Figure 39).

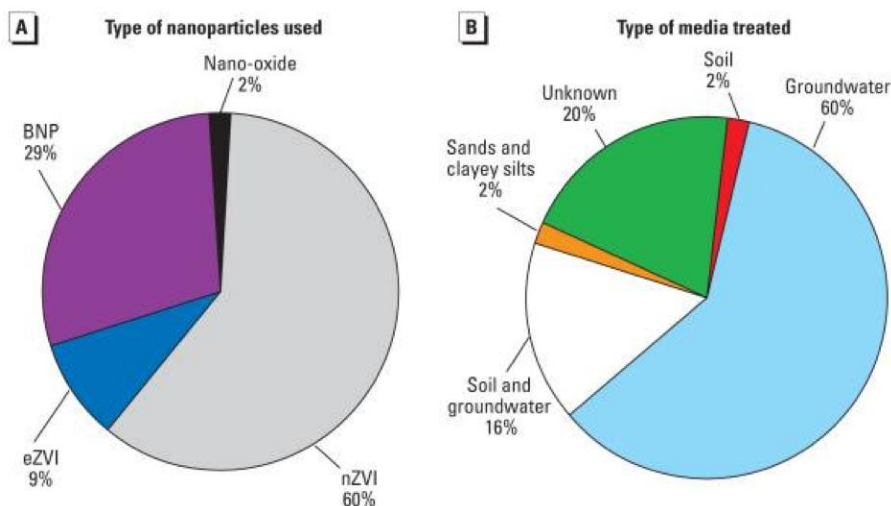


Figure 38: Types of nanoparticles used for environmental remediation and type of media treated. Taken from Khan *et al.*, 2009

IONPs smaller than 20 nm, often show Superparamagnetic behavior at room temperature (Cornell *et al.*, 2003), which facilitates their recovery along with the absorbed pollutants from aquatic environment with magnets (Kakavadi *et al.*, 2013).

Adsorbate	Adsorbent	Synthesis method	Reference
Aniline	Fe ₃ O ₄ activated carbon	Chemical co-precipitation	Kakavadi <i>et al.</i> , 2013
Cadmium	Iron oxide	Biosynthesis Tangerine peel	Ehrampoush <i>et al.</i> , 2015
Cu(VI)	Graphene-Based multifunctional Iron oxide nanosheets	Growth of iron oxide on a surface	Koo <i>et al.</i> , 2011

Ni (II), Cu (II), Cd(II), Cr (VI)	Fe ₃ O ₄	Co-precipitation	Sheng <i>et al.</i> , 2009
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Another interesting method for water remediation is the use of ferritin. Ferritin is a protein found in animals and plants, formed when 24 polypeptides developed a cage conformation, allowing he iron to enter this “cage” and by mineralization, transform iron molecules into ferrihydrite nanoparticles. The main function to ferritin is to store iron. Moretz and colleagues (2004) discovered the ability of ferritin to remediate pollutants such as toxic metals and chlorocarbon components under visible light or solar radiation. Ferritin was proved to reduce Cr(VI) which is carcinogenic into Cr(III) (Watlington *et al.*, 2005) which is less poisonous.

1.3.3.6.1 Mechanisms of Adsorption of IONPs

Two terms are commonly used for describing adsorption mechanisms depending on the force or bonds formed; chemisorption, where adsorption is held by chemical bonds and physisorption where forces involved are Van der Waals. Some authors argue that adsorption of pollutants is due to physical interaction happens with oxide surface (Nassar *et al.*, 2010) Despite the different explanations of adsorption found in literature (generally in aqueous solution) protonation/ deprotonation are the main mechanisms nanoadsorbents presents. H⁺ and OH⁻ are the potential determining ions. This allows iron oxide nanoadsorbents to remove certain compounds such as cations, anions and organic pollutants. Generally, the negative electrostatic surface of iron oxide allows adsorption of metal ions (Nassar, 2012).

Adsorption capacity hinges on an accessible surface area. This surface area will increase while reducing particle size and maximizing particle concentration (Nassar *et al.*, 2007). Many authors have demonstrated an increase in the adsorption capacities of arsenic species while reducing particle size of iron oxides (Yean *et al.*, 2005; Mayo *et al.*, 2007). For example, when magnetite particle size decreased from 300 to 12 nm, adsorption of arsenite and arsenate increased 200 times (Mayo *et al.*, 2007).

On the other hand, not only a small particle size is related to a high adsorption capacity, other factors including pH, temperature, amount of adsorbent and contact time are crucial (Shen *et al.*, 2009).

Although the sixteen phases of iron oxides and hydroxides, all the reported iron oxide based nanoabsorbents have shown successfully removal of wastewater pollutants in a very short time with magnetite having the highest adsorption capacity among the other adsorbents (Nassar, 2012).

1.3.3.6.2 Mechanisms Of Desorption Of IONPs

In chemisorption mechanism may not be reversible due to the changes in the absorptive(s) chemical nature which can be affected by surface dissociation, also desorption of the original species cannot be recovered. In contrast with chemisorption, physisorption or physical adsorption is a reversible mechanism which does not alter natural chemical composition (IUPAC, 2002).

1.3.3.6.3 Effect of pH in Adsorption

pH conditions are the most important factor in adsorption process. It seems that in acidic conditions, adsorption decreases due to the higher concentrations of hydrogen ions which interact with adsorbent sites of metal oxide nanoparticles (Salmani *et al.*, 2014). However, it varies according to the kind of metal implemented. For example, zinc oxide nanoparticles used for cadmium removal the optimal pH was 7, while for alumina nanoparticles used to remove heavy metals, the removal efficiency increases at pH 5 (Afkhami *et al.*, 2010).

On the other hand, Nashaat (2012) argued that due to electrostatic attraction between negatively charges of the iron oxides surface and metal ions, if conditions of medium are acidic, the number of negative charged sites decrease and the positively charged sites increase, which will not favor metal ions adsorption on iron oxides due to electrostatic repulsion. Again, this will depend of the adsorbent. In case of anionic pollutants, an acidic medium ($\text{pH} < 7$) will increase adsorption (Nassar *et al.*, 2010).

1.3.4 TOXICITY OF IRON OXIDES NANOPARTICLES

As stated before, the IONPs, can be used for a wide range of applications, including magnetic resonance imaging (MRI) contrast agents, targeted drug delivery vehicles, magnetic hyperthermia and development of immunoassays just to name a few (Teja and Koh, 2009). However, these

applications required a superparamagnetic behavior of nanoparticles at room temperature, otherwise the remnant magnetization would lead to agglomeration and blockage of blood vessels and stability (Majewski and Thierry, 2007).

Iron is found mainly in four protein or iron-protein complexes in the human body; hemoglobin, myoglobin, transferrin and ferritin in different percentages (Ali *et al*; 2016).

Furthermore, it has been assumed that iron oxide nanoparticles could be degraded in the same way as the iron ions at a molecular level. Ferritin and transferrin being the principal iron protein complexes responsible for store and shuttle iron ions (Ruíz *et al.*, 2013).

In a research conducted by Jain and colleagues (2008) it was found that more than 50% of iron nanoparticles were found in a mouse liver after 6 hours of injection. The phagocytic system in the body is believed to act as degradation/eliminator of nanoparticles by opsonization through macrophages stored in hepatocytes. Some of the concerns regarding implications for human health due to IONPs are for example the speeding up of reactions by using nanoparticles as catalyst, which could lead to illness or death or toxic effects by ingestion or inhalation which could kill useful microbes (Ali *et al.*, 2016).

According with Laurent and coworkers (2008), nanoparticles with less than 10 nm can be removed by extravasations and renal clearance. On the other hand, particles from 10 to 100 nm when delivered by intravenous injection for example, have the most prolonged blood circulation times. The reason behind this, is that they are small enough to evade the reticuloendothelial system (responsible for the blood cell formation and destruction, storage of fats and metabolism of iron and pigment, having an important role in immunity) and to penetrate small capillaries of tissues. Moreover, particles with a diameter higher than 200 nm are easily sequestered by the spleen and removed by the phagocyte system.

1.4 MANUEL ÁVILA CAMACHO DAM

The Valsequillo dam (which is popular name) was built in 1946 by the Irrigation National Commission. It has a storage space of 405,000,000 m³. Its runoff area comprises 22 Municipalities of Puebla and 48 Municipalities of Tlaxcala, and it retains suspended solids for the Attoyac-Sahuapan and Alseseca rivers in the Puebla and Tlaxcala states (Rodríguez-Espinoza *et al.*, 2011).

Plants such as water hyacinth (*E. crassipes*) and other macrophytes acts as biofilters which decrease concentration of COD (209,000 Ton/year) and BOD₅ (64,000 Ton/year) (Rodriguez-Espinozsa *et al.*, 2011). It is estimated that the Atoyac river contributes 69 tons/day of pollutants, the Zahuapan river 8 ton/day, San Francisco 21. 5 ton/day and 28 ton/day from the Alseseca river, it is clear that the dam faces a serious pollution issue from domestic and industrial waste which generates bad odors and water aspect (Díaz *et al.*,2008).

The water of Valsequillo dam is utilized tor irrigation of the Distrito de Riego Valley and the Atlixco-Izucar de Matamoros zone, which has a restricted risk of pollution water (Dominguez *et al.*,2009). Yet, it irrigates 33,820 ha of crops.

The function of the dam is to control the flow of the Atoyac River and irrigation activities. This dam is localized at 5 km south of Puebla city, located between 18°53' and 18°57' North latitude and 98°06' with 98°15' West longitude. At 2,100msnm above sea level.

Water fom the Valsequillo dam was analyzed by atomic adsorption spectroscopy (Celdeño-Valdéz *et al.*,2008).

Metal	Concentration, mg/L
Cd	< 0.02
Zn	0.035
Ni	0.034
Fe	0.18
Mn	0.44
Cr	< 0.04
Pb	0.086
Cu	0.033

Figure 39: Current metals in Valsequillo Dam, Puebla. Taken from: Celdeño-Valdéz *et al.*, (2008)

1.5 CHARACTERIZATION OF NANOMATERIALS

As applications of nanoparticles strongly depend of their size, shape, distribution, agglomeration and stability, several methods in different modalities can be implemented in order to obtain particles characteristics.

Table 3 : Analytical techniques for assessment of physicochemical properties of nanomaterials.

Adapted from: Ali et al.,2016

Modality	Physical and Chemical properties analyzed
Dynamic light scattering (DLS)	Analyzes the size distribution based on the hydrodynamic behavior of the material.
Fluorescence correlation spectroscopy (FCS)	Dimension and the kinetics of hydrodynamics
Surface-enhanced Raman scattering (SERS)	Size distribution and hydrodynamic size
Raman scattering (RS)	Size distribution and hydrodynamic size
Tip-enhanced Raman Spectroscopy (TERS)	Variations in structural, chemical, conjugate and electronic characteristics
Zeta-Potential	Stability concerning to charge on surface
Near-field scanning optical microscopy (NSOM)	Shape and size of nanomaterials
Circular dichlorism (CD)	For biomolecules. Conformational variations and structure for example for DNA and protein
Mass spectroscopy (MS)	Molecular weight, composition, structure, surface properties
Infrared (IR)	Bioconjugate
Attenuated total reflection with Fourier transform infrared (ATR-FTIR)	Structure, conformation and surface properties
Scanning electron microscopy (SEM)	Size and distribution
Environmental scanning electron microscopy (ESEM)	Shape, aggregation, dispersion
Transmission electron microscopy (TEM)	Shape heterogeneity, size, dispersion, accumulation
Scanning optical microscopy (STM)	Shape heterogeneity
Atomic force microscopy (AFM)	Shape heterogeneity, dispersion

Nuclear magnetic resonance (NMR)	Indirect analysis of size, structure purity and concentration.
X-ray diffraction analysis (XRD)	Crystalline materials, shape, size and structure determination
Small-angle X-ray scattering (SAXS)	Shape, structure and size

1.5.1 ULTRAVIOLET-VISIBLE LIGHT

This technique is implemented in analytical chemistry for characterize materials. The ranges of light emitted are over ultraviolet and visible light spectra (200-800 nm) (RSC,1998). The incident light (I_0) which is the maximum intensity of light, is absorbed when pass through the sample. Concentration can be obtained by solving Beer-Lambert Law, together with the intensity of the transmitted light (I) (Equation 2):

Equation 2: Beer Lambert Law
$$\text{Absorbance} = -\log_{10}\left(\frac{I}{I_0}\right) = \text{Concentration} \cdot \epsilon \cdot l$$

The molar extinction coefficient ϵ and the knowledge of the path length (l) which commonly is 1 cm are required.

Transition metals and organic compounds can be efficiently measured by UV-Vis spectroscopy. Organic compounds can be identified by their bond structure and functional groups which present a specific peak in a given spectrum (RSC, 2015).

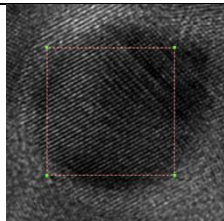
1.5.2 TRANSMISSION ELECTRON MICROSCOPY

TEM is a microscopy technique whereby electrons are transmitted from a filament and pass through an ultrathin sample. In order to form an image, the electrons transmitted through the

specimen are magnified and focused by lens, the image will form in a fluorescent screen (in most TEMs).

The TEM can yield information such as size distribution, particle size and morphology of nanoparticles. Microscopy is the only method which the individual particles are directly observe and measured (Akbari et al., 2011).

This technique has similar features to the light microscope. Instead of light, the TEMs utilize electrons as a “light source” in order to create high resolution images. Light microscope has focus lenses, while the TEM uses electromagnetic lens in order to direct the electrons into a thin beam. Electrons are send through a vacuum column by a tungsten filament until reaching the sample (AZoNano, 2006). One of the features of TEM that allows high resolution or subnanometer resolution is because of the short wavelength of the electron beam, which is 100,000-fold shorter than photons in visible light (Winey *et al.*, 2014).

Optical microscope	Transmission electron microscope (TEM)	High Resolution TEM (HRTEM)
		
Resolution limit: ~200 nm	Resolution limit: ~ 0.25 nm	Resolution limit: <20 nm

The brighter areas of the obtained image, correspond to an area of less density and higher density regions of the sample will be shown as darker areas in the images because fewer electrons will pass through (in bright field microscopy, which is the most common). The diameter of the sphere of the projected image results in the size of particle. TEM images can be useful to judge if sample has good dispersion or whether agglomeration is present in system (Akbari *et al.*,2011). Some advantages of using TEM are: the small amount of sample needed, sample do not need to be

multiphase only, and the composition of the sample can be further determined by EDS or EELS (Shah, 2012).

High-resolution TEM (HR-TEM) image the atomic arrangement of crystalline nanoparticles (Laurent *et al.*, 2008). In HRTEM, there is a sample thickness requirement of 500 nm. Main difference between TEM and HRTEM is that HR uses both the transmitted and the scattered beams to create an interference image, and TEM in bright field block scattered electrons to form an image. Diffraction pattern obtained is the Fourier transform where all the diffracted and the primary beam are brought together. Picture is seen on the screen at magnifications of typically 10^6 .

In order to have a reliable mean size data, hundreds of particles should be measured to present statically reliable mean size data. Some drawbacks of this technique, are that the analyzed sample is not always representative of the total sample. Also ultrathin samples are needed, and the pretreatment can damage or varies the sample composition specially if its composition is mainly organic.

Three models have been used to characterize nanoparticles in biological matrices: bright field, dark field and angle annular dark field scanning transmission electron microscopy (HAADF-STEM). The main different among these techniques is the electron population used to construct the image (Klein *et al.*, 2015) as presented in figure 41..

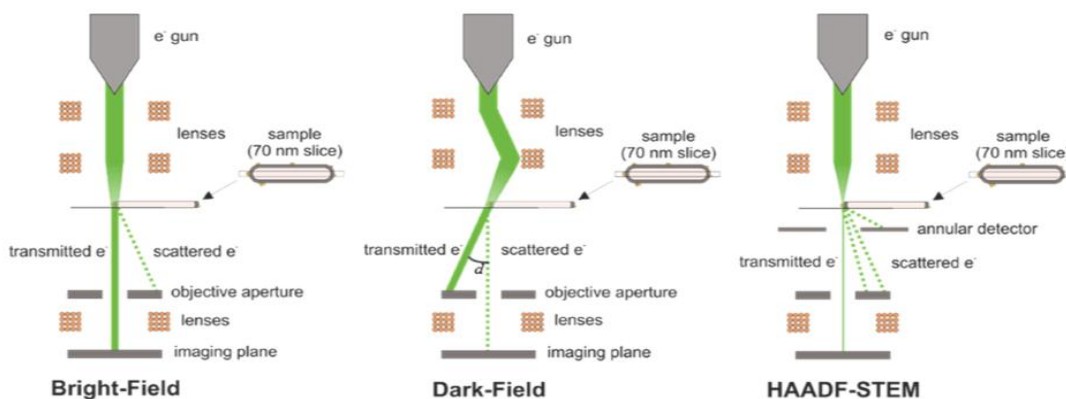


Figure 40: Comparison of bright field TEM, dark field TEM and HAADF-STEM. Taken from: Klein *et al* (2015).

The bright field is the traditional imaging mode where electrons are transmitted through the sample for constructing the image, allowing nm-scale detail (Mayhew *et al.*,2009). Where the objective aperture is often used to increase the contrast by blocking scattered electrons. However, high mass and inorganic particles will appear dark when the aperture is inserted. In order to create an image of this particles, the use high-angle annular dark field imaging (HAADF-STEM) decreases the noise by using an annular detector which will image only high scattered electrons passing through the sample. The image will result in a strong bright signal for high mass materials. A drawback of the HAADF-STEM is the specialized annular detector required to acquire signals from scattered electrons. The HAADF detector, due to its circular nature, can gather scattered electrons simultaneously from all directions. Hence, high mass noncrystalline areas will gain same contrast as high mass crystallinity areas (Klein *et al.*, 2015).

In traditional dark field mode, the incident beam of electrons is tilted at an angle (θ) (radially or angularly) to the sample. This angular beam will change the orientation of the electrons. Dark field allows bright crystalline contrast in order to easily identify nanoparticles in low concentration samples (Klein *et al.*,2015).

The standard pretreatment of samples for further analyzing in TEM are; fixation, taining, dehydration, infiltration with polymer resin, oven curing and slicing via microtome (Klein *et al.*,2015). However, pretreatment may differ fro sample to sample.

1.5.3 X-RAY DIFFRACTION

This technique is used to obtain the crystalline structure of the particles. The randomly oriented crystals cause broadening of diffraction peaks (Akbari *et al.*,2011).

Interatomic distances in crystals and molecules are in the range of 0.15 -0.4 nm. In the electromagnetic spectrum, wavelength of x-rays ranges from 0.01 to 10 nm, thus interatomic distances can be obtained.

Diffraction occur when electromagnetic radiation impinges on structures with geometrical variations on the length scale of the wavelength of radiation.

The correlation between XRD and TEM is that TEM values are usually higher or equals than the XRD (*Figure 42*) As the crystallite size becomes similar to the particle size, the nanoparticle will denominate single-crystal (Akbari *et al.*, 2011).

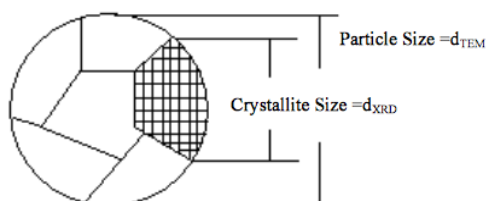


Figure 41: Size obtained by TEM and x-ray diffraction peak broadening analysis. Taken from: Akbari et al., 2010

X ray diffractometers has three basic elements: A X ray tube, a sample holder and a detector of X-Rays. Electrons are produced by heating a filament in a cathode ray tube. Electrons are accelerated by applying voltage toward target. If electrons have enough energy, they dislodge inner shell electrons of the material and produce X-Ray spectra. The detector is mounted on an arm to collect the X-Rays and rotates at an angle of 2θ , whereas the sample rotates in an θ angle. The instrument used to maintain an angle and rotate the sample is termed goniometer (Dutrow & Clark, 2016).

X rays are generated by a cathode ray tube, then filtered to produce monochromatic radiation, collected to concentrate and finally directed towards the sample (Dutrow & Clark, 2016). The wavelength of the incident X-rays, the angle of incidence and the spacing between the crystal lattice planes of atoms are correlated in the Bragg's Law, expressed as:

$$n \lambda = 2d \sin \theta$$

Equation 3: Bragg's law

Where n is the order of reflection, λ is the wavelength of the incident X-rays, d is the interplanar spacing of the crystal and θ is the angle of incidence (Henry et al., 2016). As presented on *figure 43*.

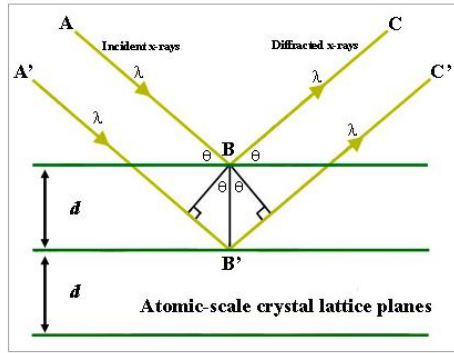


Figure 42: Bragg's law reflection. Taken from: (Dutrow & Clark, 2016)

By solving Bragg's Law, the interplanar spacing (d-spacing) between crystal lattice planes of atoms can be found. In a crystal, each plane of atoms will undergo refraction at a unique single angle when X-rays have a fixed wavelength. Peaks in intensity are produced when the geometry of the incident X-rays impinging satisfied the Bragg's equation and a constructive interference takes place (Dutrow & Clark, 2016).

CHAPTER TWO: METHODOLOGY

2.1 MATERIALS, REAGENTS AND EQUIPMENT USED

NO.	REAGENT	MODEL	COMPOSITION
1	HCL	SIGMA-ALDRICH	-
2	SOL. BUFFER 10 (BORATE)	J.T. BAKER	BORATE/ POTASSIUM
3	SOL. BUFFER 7	J.T. BAKER	PHOSPHATE, POTASSIUM, SODIUM.
4	SOL. BUFFER 5 (BORATE)	MERK	CITRIC ACID /SODIUM HYDROXIDE
5	SOL. BUFFER 3 (BORATE)	MERK	CITRIC ACID /SODIUM HYDROXIDE/HYDROGEN CHLORIDE
6	FECL3	SIGMA-ALDRICH	IRON (III) CHLORIDE, 97%
EQUIPMENT	MODEL		INSTITUTE
UV-VIS SPECTROPHOTOMETER	JENWAY/GENOVA		UNIVERSIDAD POPULAR AUTÓNOMA DEL ESTADO DE PUEBLA
ULTRASONIC CLEANER	MODEL 275T		UNIVERSIDAD POPULAR AUTÓNOMA DEL ESTADO DE PUEBLA
CENTRIFUGE	HERMLE TYPE Z326		UNIVERSIDAD POPULAR AUTÓNOMA DEL ESTADO DE PUEBLA
PLASMA CLEANER	SPI SUPPLIES		UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
X-RAY DIFFRACTOMETER	POWDER DIFFRACTOMETER BUKER D8 ADVANCE		BENEMÉRITA UNIVERSIDAD AUTÓNOMA DE PUEBLA
TRANSMISSION ELECTRON MICROSCOPY (TEM)	JEOL JEM-2010F FASTEM (1.9 Å)		UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

2.2 PROCESSING OF BIOMASS

Water hyacinth was collected from the Valsequillo dam in Puebla, México, it was washed with common water until the water was clear. In order to ease the drying process, the water hyacinth was cut into small pieces (approx. 3cm). Drying process was carried out in a oven at $80 \pm 5^\circ\text{C}$ for over a week. Furthermore, the pieces of water hyacinth were crushed in a blender until fine powder was obtained. The the powder was submitted to a .01N HCl solution in distilled water in a relation of 500mL for every 100g of biomass, leaving the solution rest for 30 min. This HCl washing was repeated and finally it washed out with common water until the pH was close to neutral values. In order to remove the HCl solution, biomass powder was filtrated with a cotton cloth and dehydrated in aluminium container for $80 \pm 5^\circ\text{C}$

2.3 BIOSYNTHESIS OF IRON OXIDE NANOPARTICLES

Silva-Gómez (2015) reported, by employing a method developed by Isaza et al (2007) the tannin concentration of *E. crassipes* per plant section (root, stem and leaf) and found that leaf has a higher concentration of tannins. Further, it was found a relation between size of the biosynthesize nanoparticle and the tannin concentration. Size distribution were closer to zero when concentration of tannins was higher. Thus, the section of the plant used, was the leaf.

In order to reduce the metal salt in less than 10 nm aggregates, the following method developed by Rosano-Ortega (2006) was implemented.

1. A metal solution of $3 \times 10^{-4}\text{M}$ FeCl_3 was prepared with deionized water.ⁱ
2. A biomass solution was prepared in a 5mg/mL relation of dry biomass and deionized water and placed in polyethylene tubes of 25 mL.
3. Tubes were placed in an ultrasonic bath for 15 minutes. Then, centrifuged at 5000 rpm for 15 minutes, leaving 5 minutes' lap in between.
4. Buffer of the target pH (pH: 5,7,10) was added to each sample in a relation of 1mL per 5mL of biomass solution.
5. Again, samples were placed in an ultrasonic bath for 15 minutes. Then, centrifuged at 5000 rpm for 15 minutes, leaving 5 minutes' lap in between.
6. Metal solution was added to each sample to obtain a 1:1 relation with the biomass solution. (1 mL of metal solution per 1 mL of biomass solution).
7. Then, the samples were subjected in an ultrasonic bath for 20 minutes, leaving them rest for 5 minutes and centrifuged 20 min at 5000 rpm.
8. Biomass was separated from solution by vacuum filtration.

*Ultrasound is used to induce a mechanical stress on biomass cells by the production of cavitation in the sample. Is a cellular break down that generates a solubilisation of metabolites in the solvent (Segneanu *et al.*, 2013).

2.4 CHARACTERIZATION OF NANOPARTICLES

2.4.1 HRTEM

High resolution transmission electron microscopy (HRTEM) determines the elemental composition and internal structure of the nanoparticles, it allows for direct imaging of atomic structure of the sample.

For preparation of the solution, a drop of solution of biosynthesis was placed on a micro copper grid covered with carbon and allowed to dry at ambient. This sample was subjected to an argon plasma bath for 3 minutes in order to remove a percentage of biomass and to obtain better images of the nanoparticles. The pressure of the plasma cleaning was ~30 millitor.

The analysis by HRTEM was performed by a electron transmission microscope JEOL JEM-2010F FasTem (1.9 Å) at the Physics Institute in UNAM. High resolution images were obtained using a CCD camera Sensys, and processed using filters in Fourier space. HRTEM images obtained together with the Fourier Transform and size measurement was further analysed by *Digital-micrograph* software.



Figure 43: Transmission electron microscope (TEM) JEOL JEM-2010F , physics institute, UNAM.

2.4.2 X-RAY DIFFRACTION (XRD)

XRD is a technique used to obtain characteristics such as structure and shape of crystals.

For preparation, the biosynthesis sample was dried for 96 hours at 40 ± 5 °C in order to obtain powder. The analysis was performed in the Benemérita Universidad Autónoma de Puebla BUAP.

2.4.3 ULTRAVIOLET VISIBLE SPECTROSCOPY (UV-VIS)

UV-Vis quantify the absorbed and scattered light by a sample. The intensity of the beam was measured before and after passing through the sample.

The sample was read after 24 hours of biosynthesis in an UV-Vis Jenway/ Genova spectrophotometer. The blanked used was FeCl_3 solution alone and the wavelength range was 200 to 700. This analysis was carried out in Universidad Popular Autónoma del Estado de Puebla (UPAEP).

2.4.4 ENERGY-DISPERSIVE X-RAY SPECTROSCOPY (EDS)

EDS is an analytical technique used for characterization of a sample and obtaining its chemical composition. An X-ray source excites the sample and allows a unique set of peaks corresponding of each element with a unique atomic structure. Particles used could be charged electrons or protons.

2.4.5 RECOVERY OF POWDER FROM FEO BIOSYNTHESIS SUSPENSION

In order to obtain the production rate in grams from the synthesis, final samples were centrifuged at 6000 rpm for 20 minutes, and the suspension was left to dry for 96 hours at 40 ± 5 °C in plastic containers (the recovery is difficult in glass containers).

2.4.6 SIZE DISTRIBUTION

In order to measure with accuracy, the nanoparticles, several images of TEM were analyzed by *Digital Micrograph* software by measuring the length in a precise and accurate way. In all the measurements, the higher length of the nanoparticle was considered. More than 200 nanoparticles were measured to generate histograms of length vs. % frequency.

2.4.7 STRUCTURE AND PHASE

Crystalline structure and specie of iron oxide (wustite, hematite, magnetite, maghemite) were determined by measuring interplanar distances presented by IONPs. The process for measuring interplanar distances was the follow: An image obtained by TEM was opened in the *Digital Micrograph* software. From this image, a section which presented a similar pattern was selected and a FFT of this section was generated. The length of the vectors in the reciprocal space was measured and its inverse was calculated. Further, the value was compared to crystallographic tables in order to find the match or closer d(A) and identify the iron oxide specie (*Figure 45*). Lattice plan has a Bragg diffraction indice (hkl) .

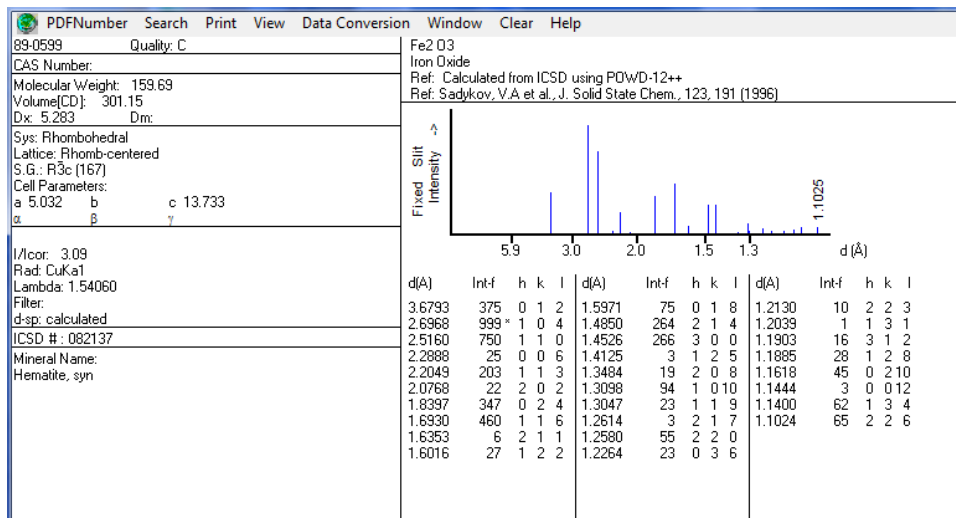


Figure 44: Example of a crystallographic table for rhombohedral hematite

CHAPTER 3 RESULTS AND DISCUSSION

3.1 BIOSYNTHESIS

The use of plant biomass for biosynthesis can be really effective and simple, requiring no toxic chemicals, no sophisticated laboratory equipment and in this particularly case, the plant is highly abundant. It is advantageous to work with a plague, which not only cause a lot of problems in the biota but for the communities living near. Thus, implementing this plant for a specific use in this case as the source of a reductant for producing nanoparticles is effective because the resource is almost unlimited and it is an auspicious way to remove the plant from the dam, instead the conventional methods which only make the pollution in dam worst as the plant sediment, once triturated in the bottom with all of the heavy metals and pollutants concentrated making the dam more toxic.

Biosynthesis have been already performed with *E. crassipes* for Mn, Cr, Fe, Co (Rosano-Ortega, 2006), Ag (Munive, 2014; Silva, 2014; Prusty and Parida, 2014; Thombre *et al.*, 2014) and Au (Jannathul and Lalitha,2013) nanoparticles, proving its efficiency as a bioreductor agent.

The present biosynthesis method has been succesful in the production of nanoparticles. However, in the obtained powder, biomass components are present as well. It is well known that biomass contain natural products such as: waxes, fatty acids, polyacetylenes, terpenoids (monoterpenoids, iridids, sesquiterpenoids, diterpenoids, triterpenoids etc.), steroids, essential oils, phenolics, flavonoids, tannins, anthocyanins, quinones, coumarins, ligans, alkaloids, and glycosidic derivatives (saponins, glycosides, flavonoid glycosides) (Segneanu *et al.*, 2013) that need to be taken in consideration when focusing in to certain future applications such as medical.

CHARACTERIZATION

3.2 UV – VIS SPECTROSCOPY

The UV- Vis spectroscopy measured the absorbance of solution in a wavelength range from 200 to 700. In this region of the electromagnetic spectrum, atoms and molecules will experience electronic transitions. Charge transfer transitions which involve FeIII-O or FeII-FeIII are responsible for absorption of visible light (Cornel and Schwertmann; 2003).

Typical UV-vis spectra for iron oxides nanoparticles (magnetite and gamma Fe₂O₃) present a decreasing graph from 400 nm to 600 nm (Wang *et al.*, 2005; Zhang *et al.*, 2010), which is highly visible in the obtained results. This is because red and yellow iron oxides absorb strongly in the UV region (15 nm to 400 nm) and are weakly absorbing in the visible/near IR region (700nm – 1000000 nm) (Cornel and Schwertmann; 2003).

When evaluating pH5, the graph demonstrates an unusual behavior due to the negative values. However, three peaks are highly distinguishing, at ~288, 414 and 668 nm.

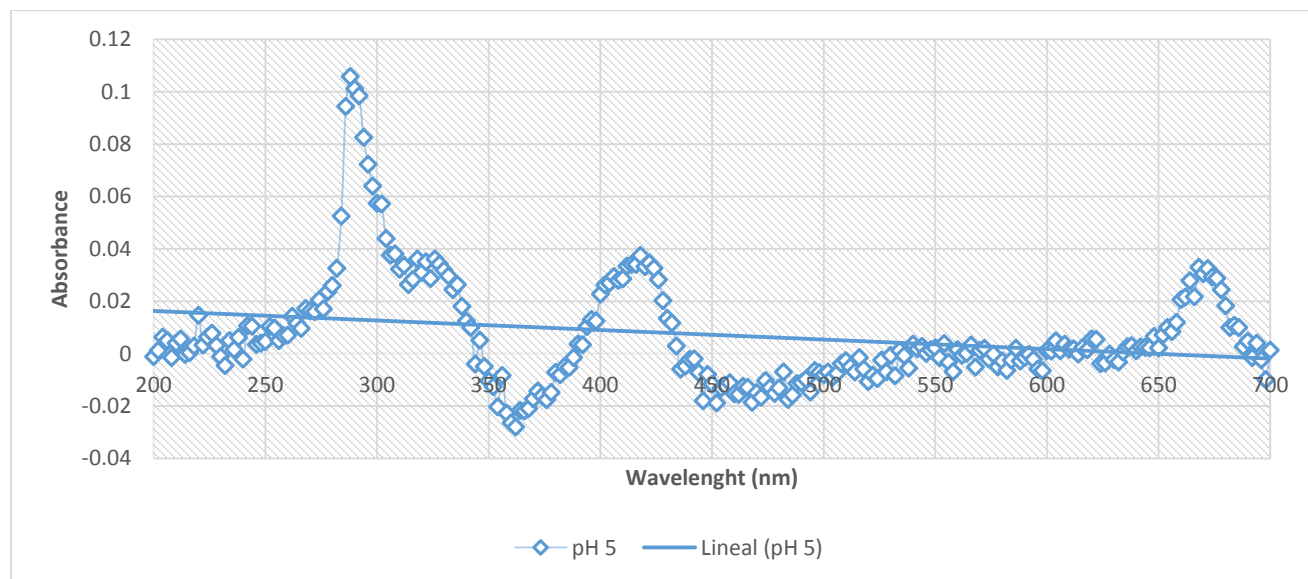


Figure 45: UV-Vis absorption graphic of pH 5 of iron oxides nanoparticles.

For the neutral sample (pH 7), there are 3 peaks which are highly noticed; around 288, 412 and 668 nm. Similar to the graph of pH5.

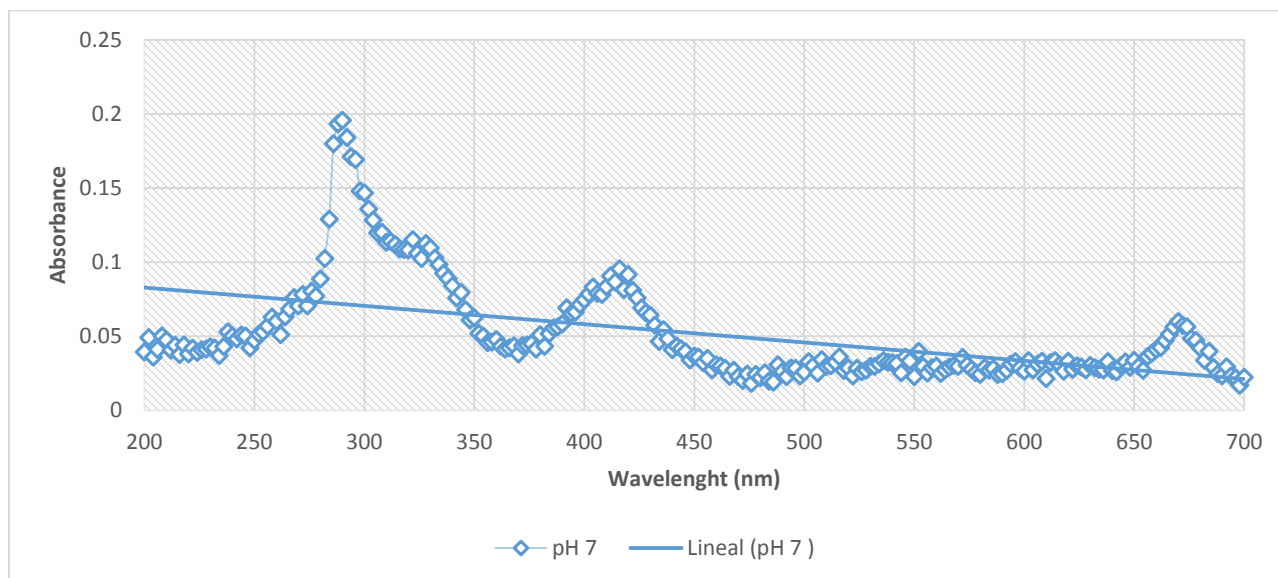


Figure 46: UV-Vis absorption graphic of pH 7 of iron oxides nanoparticles.

The graph of the alkaline sample (pH10) has three main peaks at 288 nm, 402 nm and 668 nm, with an absorbance of 0.3696 nm, 0.264 and 0.0764 nm respectively (figure 48)

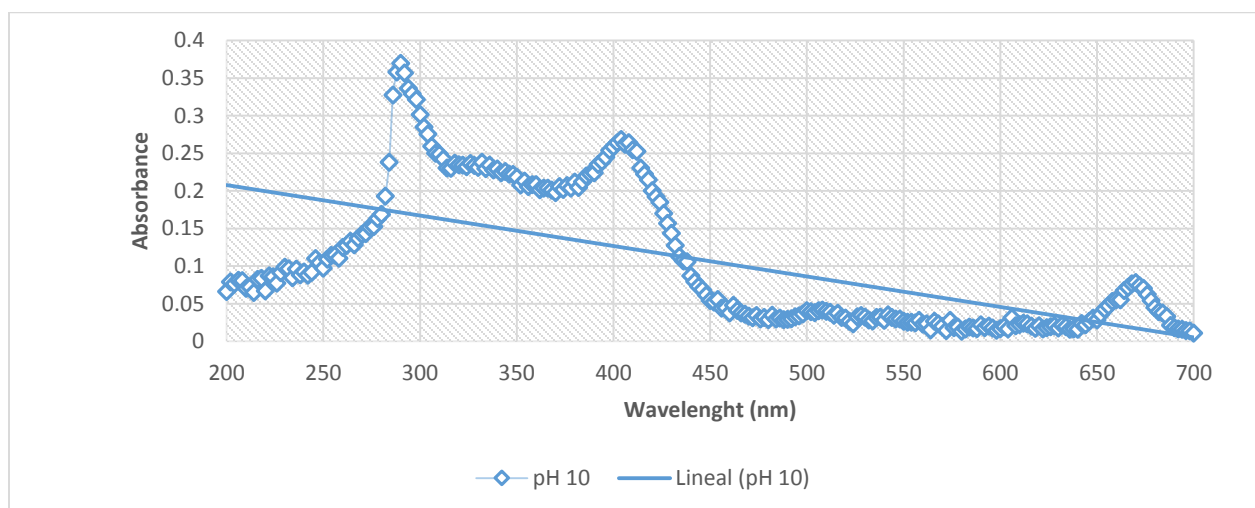


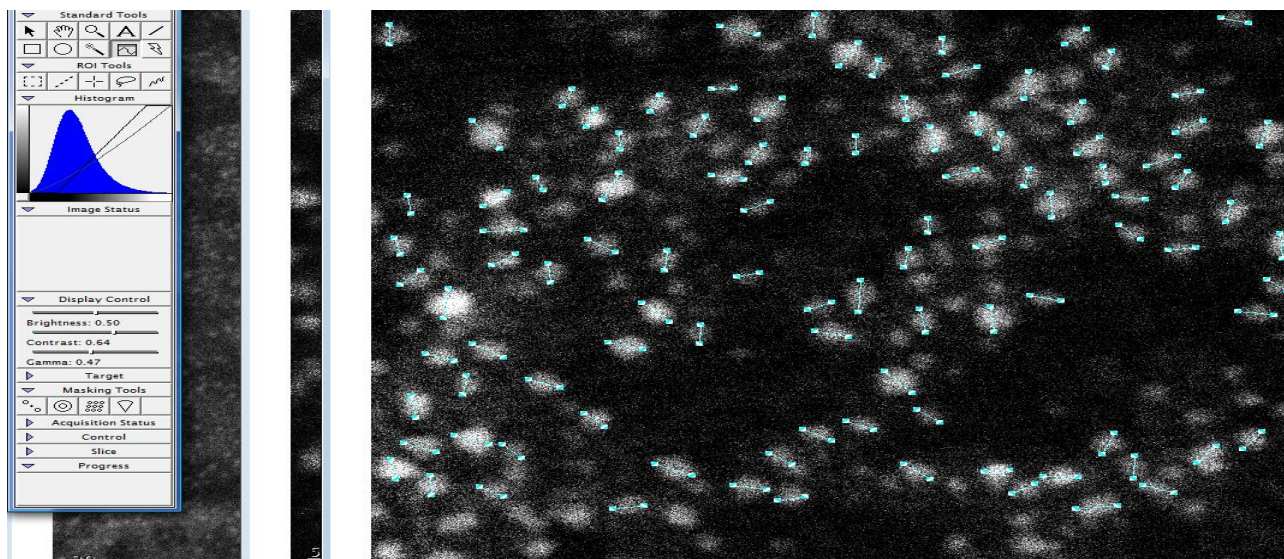
Figure 47: UV-Vis absorption graphic of pH 10 of iron oxides nanoparticles.

In the samples (pH 5, 7 and 10) there are three highly distinguished peaks, first ~288 nm, second ~400 and 450, and a last around 650 nm. According with Levy (1997) hematite has an absorption spectrum of 200, 230, 285 and 340 extending to 560 nm. On the other hand, it has been identified that maghemite has absorption spectra of 200 and 285 nm (Qian *et al.*,2000).

When analyzing in a visible range between 200 to 800 nm wavelength, the graphics of UV-Vis pH10 presented a strong peak around the 404 nm band which is related to a low dimensional Fe₂O₃ phase, presenting reddish colors (Mohammed *et al.*, 2011; Cornel and Schwertmann, 2003; Luna *et al.*, 2015). On the other hand, there are several peaks in wavelengths such as 288nm and around 326 nm which might be due to plant extracts. Studies in chromatography and spectroscopy have shown that macrophytes releases fatty acids, steroids, polyphenols and tannins (Sanaa *et al.*, 2010). Band around 270 might represent presence of polyphenols in sample (Anouar *et al.*,2012). However this band can be correlated with the maghemite spectra (Qian *et al.*,2000)

3.3 SIZE OF NANOPARTICLES

The average size of nanoparticles was obtained by measuring 274 nanoparticles of several dark-field images of pH10 from TEM with the software *Digital-micrograph* (Figure 49). It was possible only to measure particles at pH 10, because for samples of 7 and 5 pH it was difficult to find particles and they were highly dispersed or presented agglomeration, thus the counting was irrelevant due to the extremely low number of nanoparticles found (figure 50 and 51).



- *Figure 48: Dark field image for measuring size of particles for pH10..*

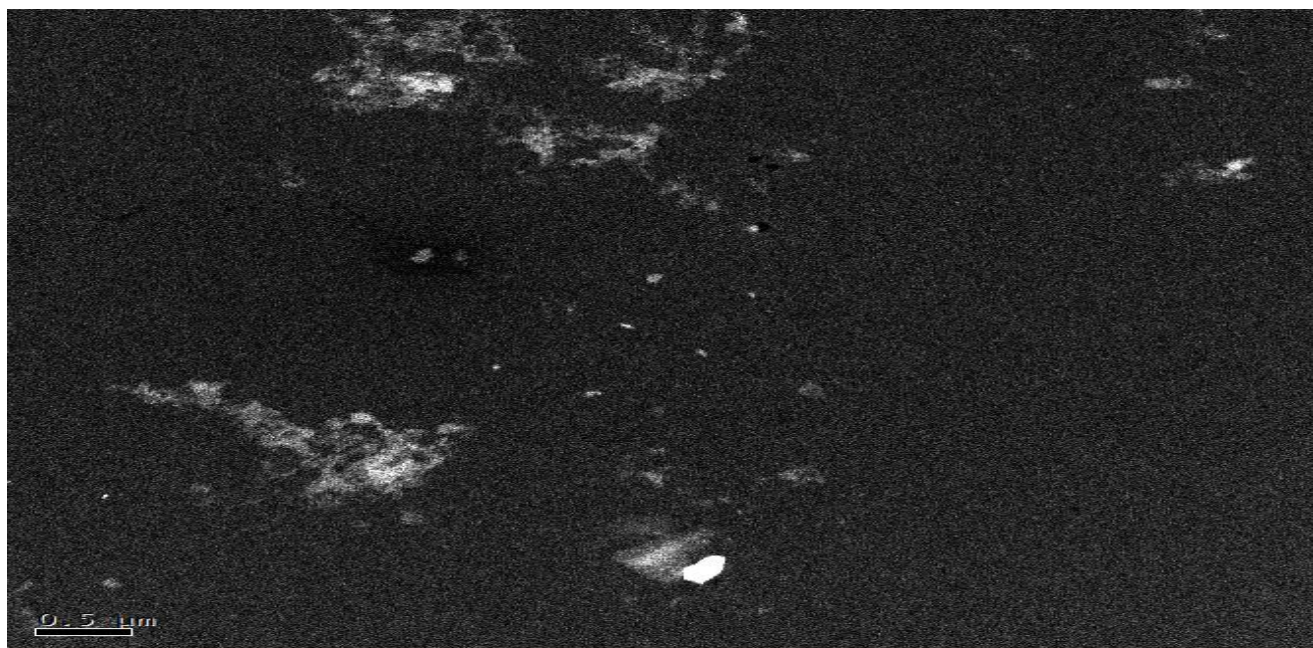


Figure 49: pH 7 sample

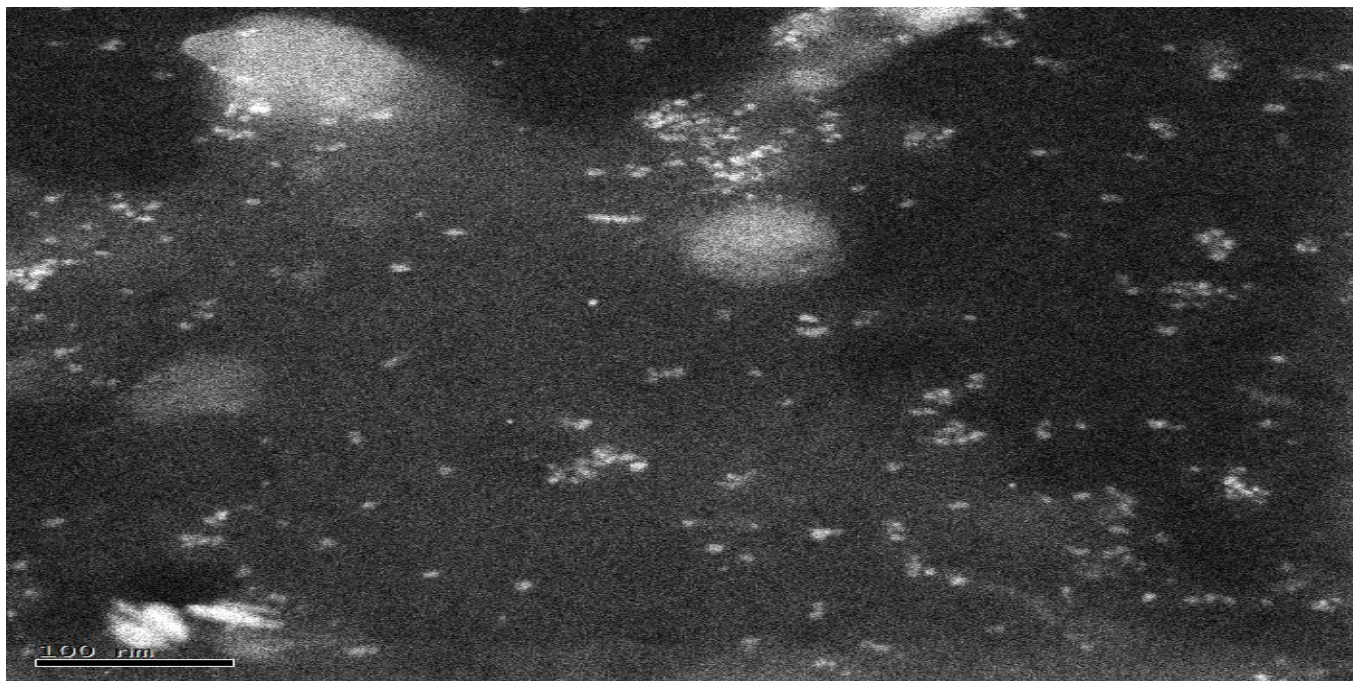


Figure 50: Image from sample pH5

By the biosynthesis method using *Eichhornia crassipes* biomass at 10 pH, the size distribution of nanoparticles obtained was between 1 and 3.5 nm. It has been reported that by the biosynthesis method, using a pH between 3, 5 and 10 the size distribution range below 4 nm (Rosano-Ortega, 2009). Similarly, tannins and alfalfa were reported to produce IONPs with sizes smaller than 10 nm, at a pH of 10 (Herrera-Becerra *et al.*, 2009).

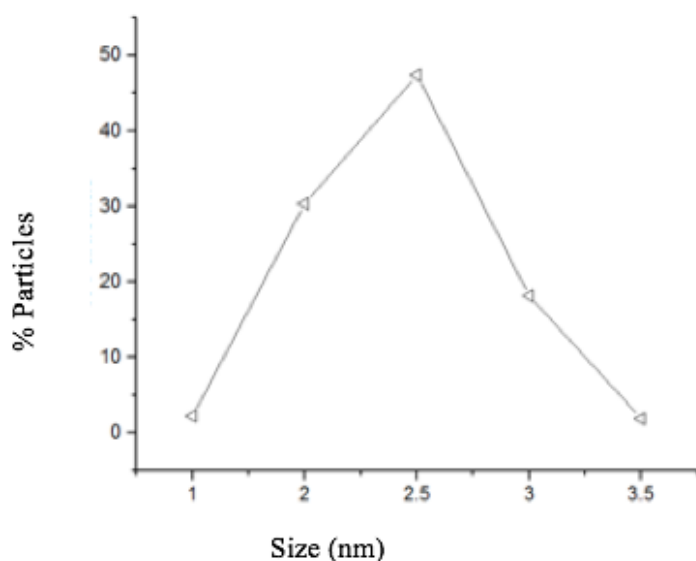


Figure 51: Histogram of iron oxide nanoparticles distribution size of pH 10.

A histogram was constructed from the higher values (in length) of the measured nanoparticle, presenting the percentage of particles vs size in nanometers.

Distribution extends from ~ 1 nm to ~ 3.5 nm. As histogram shows, 50% of the nanoparticles are below 2.5 nm.

<i>NANOPARTICLES COUNTED</i>	<i>STANDARD DEVIATION</i>	<i>MEDIAN</i>	<i>MAX VALUE</i>	<i>MIN VALUE</i>
270	0.361430839	2.159	3.291	1.241

3.4 PRODUCTION RATE

In order to obtain the production rate of the nanoparticles, after the last centrifuged step in biosynthesis, the suspension alone was taken. The smaller nanoparticles will not sediment, rather they will remain in suspension. After 96 hours of drying the suspension in an oven, a very thin, dark-green layer was recovered from the plastic containers with a spatula and weighted to get the production rate in grams. It is important to notice that the powder recovered from the plates do not contained the iron oxide nanoparticles alone but also small particles from biomass which remained suspended as well. Production rate obtained was 0.718 mg/ml.

3.5 PLASMA CLEANING

In order to achieve optimum results during TEM, it is necessary to have a clean specimen. To prepare specimens, pretreatments should be carefully elected so the composition and structure remained. Pretreatments such as mechanical polishing, electrolytic thinning or ion beam milling might cause amorphous damage layers of sample (Isabell & Fischione, 1998). Previous work (Rosano-Ortega, 2009; Herrera- Becerra *et al.*,2009) stated that at pH10, the observation of nanoparticles by TEM was difficult due to the formation of a dense film of biomass.

During characterization, excess of *E. crassipes* biomass interfered when trying to find nanoparticles of FeO in TEM. Also, if excess biomass is not removed in a pretreatment, when analyzing, the incident electron beam constantly burned up the biomass and vapors were released preventing accuracy and making analysis difficult and time consuming.

Hence, a “bath” of argon plasma was implemented as pretreatment, in order to remove excess biomass. The removal of carbon contamination in surfaces by plasma cleaning with argon or helium-oxygen has been address in early research papers. For example, it has been published that mild plasma surface cleaning is soft enough to preserve magnetic properties in a Fe-Co film (Okane & Mittal, 1974; Grundmeier & Stratmann, 1993).

Plasma, knowing to be the fourth state of matter can be used for cleaning surfaces by dissociate molecular bonds, formed at very high temperatures and high frequency voltages (from kHz to >MHz) in order to ionize low pressure gas. Plasma might be produced by various gases such as

O₂, Ar, or He. Argon- Oxygen plasma is effective for breaking most organic bonds. A second cleaning step will take place when oxygen species created in plasma react with organic contaminants to form lower-molecular-weight materials which are subsequently removed by the vacuum (Pizzi & Mittal, 2003).

Several intervals of time for cleaning with argon plasma were proved. When leaving the sample in the plasma cleaner for less than 3 minutes, the sample still contained biomass, which was a problem regarding the analysis by TEM and HRTEM. Furthermore, when the sample was subjected into the plasma cleaning for more than 3 minutes, it broke forming fissures on its surface which were seen with the electron microscope. Thus, the optimal time for leaving the sample in the plasma cleaner was 3 minutes. After this time, nanoparticles were found easier and further analyzed. Three main advantages can be taking into consideration when working with Argon plasma. First, is an inert gas so it will not change chemic composition of sample. Second, Argon cleans organics but is not capable of surface modification. Third, it will not oxidize the surface, altering the expected results (Plasma etch, 2016). By using plasma cleaning, the need of using chemical solvents is eliminated.

3.6 TEM AND HRTEM

In the following figures, the FFT of some sections of the images obtained by HRTEM are presented for three different pH solutions: 5, 7 and 10. From the FFT, the interplanar distances were measured and analyzed.

Images from pH5

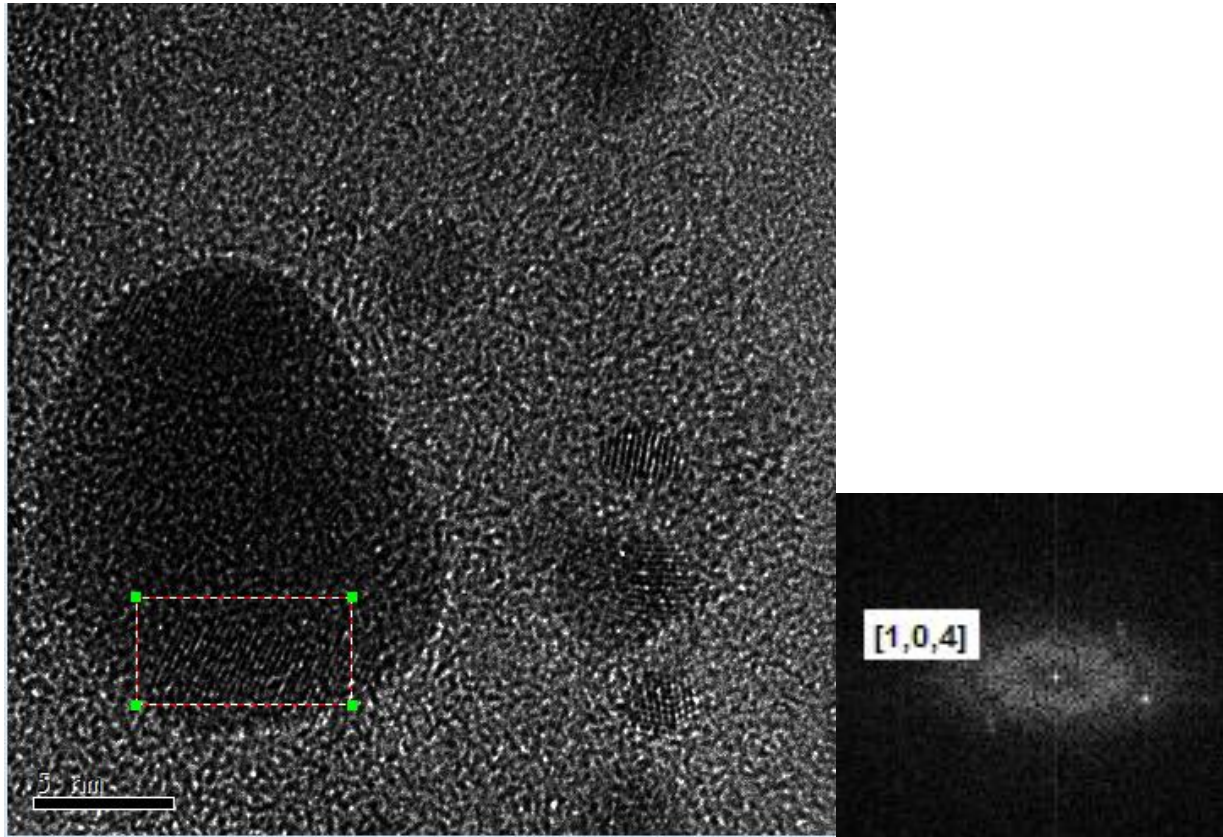


Figure 52: image one from sample Ph5_0042

MEASUREMENT	VALUE IN TABLES D(A)	INT-F	H	K	L
2.61	2.6968	999	1	0	4
2.98	2.953	35	2	2	0
MEASUREMENTS CORRESPOND TO HEMATITE FE_2O_3 AND Γ - FE_2O_3 MAGHEMITE					

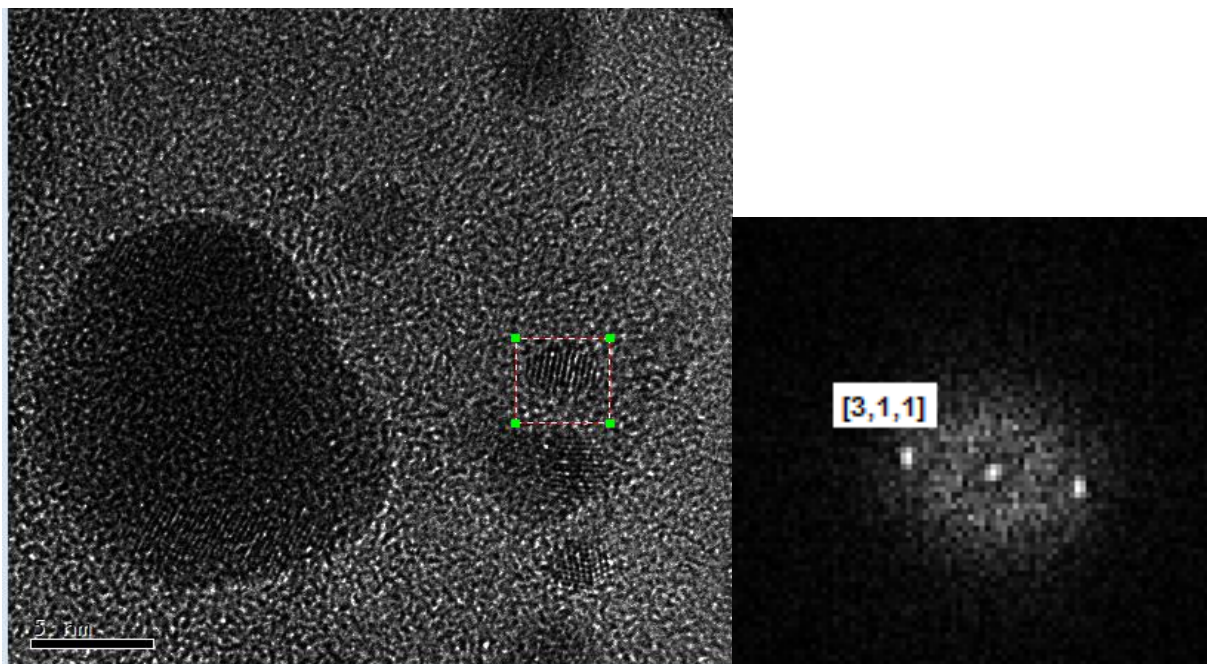


Figure 53 : Image two from sample ph5_0042

MEASUREMENT	VALUE IN TABLES D(A)	INT-F	H	K	L
2.54	2.5177	100	3	1	1
MEASUREMENTS CORRESPOND TO HEMATITE Fe_2O_3					

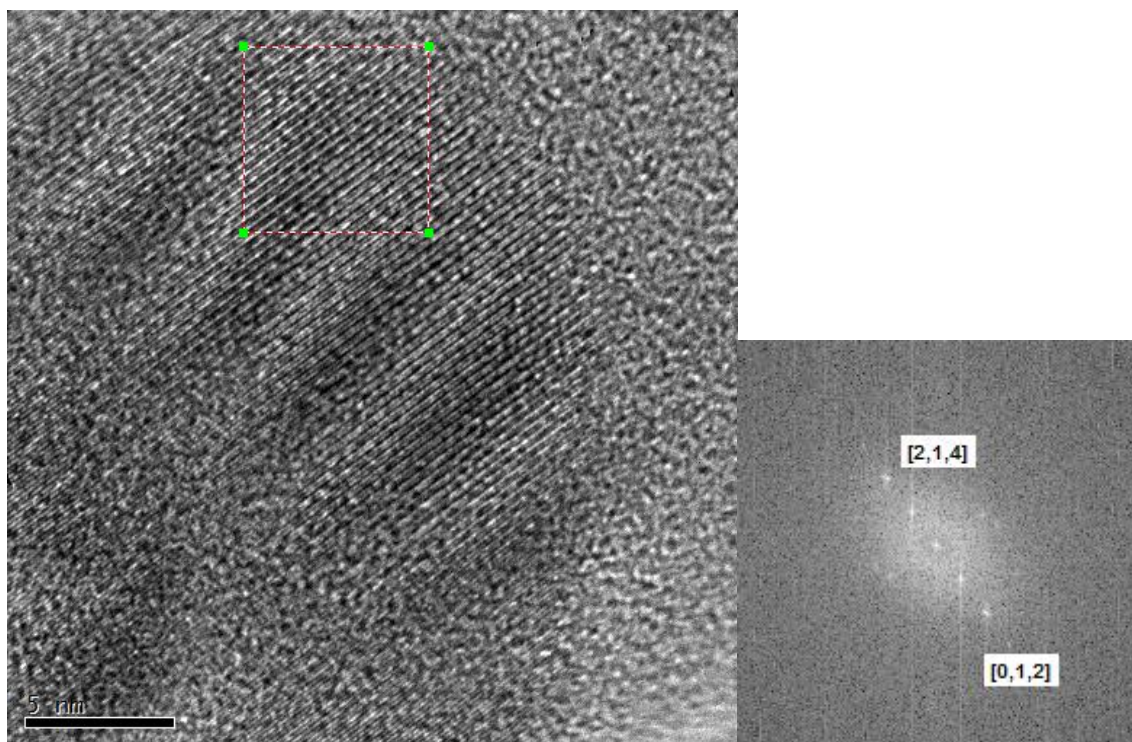


Figure 54: Image two from sample pH5_009

MEASUREMENT	VALUE IN TABLES D(A)	INT-F	H	K	L
2.54	2.5177	100	3	1	1
MEASUREMENTS CORRESPOND TO MAGHEMITE γ - Fe_2O_3					

In pH 5, there were only few images analyzed due to the difficulty to observe nanoparticles. In this case, the biomass was not the main problem but where the nanoparticles themselves. It was time consuming to find them. The size of particles at this pH were higher in comparison with the obtained in alkaline solution. Sizes ranged from ~ 30 nm to 4 nm. Moreover, TEM and HRTEM analysis can be not that accurate. Sometimes the analyzed sample is not a representative of the hole or some gratings of the copper grid may have received more sample than others and generally only a few of gratings are focused for observation in the microscope. Nevertheless, it is a little unclear if the particles founded at this particularly pH are representative because it was reported previously (Rosano-Ortega, 2009) that when using a pH5 for synthesis, 65% of the particles of FeO had a size range of 2 nm.

Images from pH7

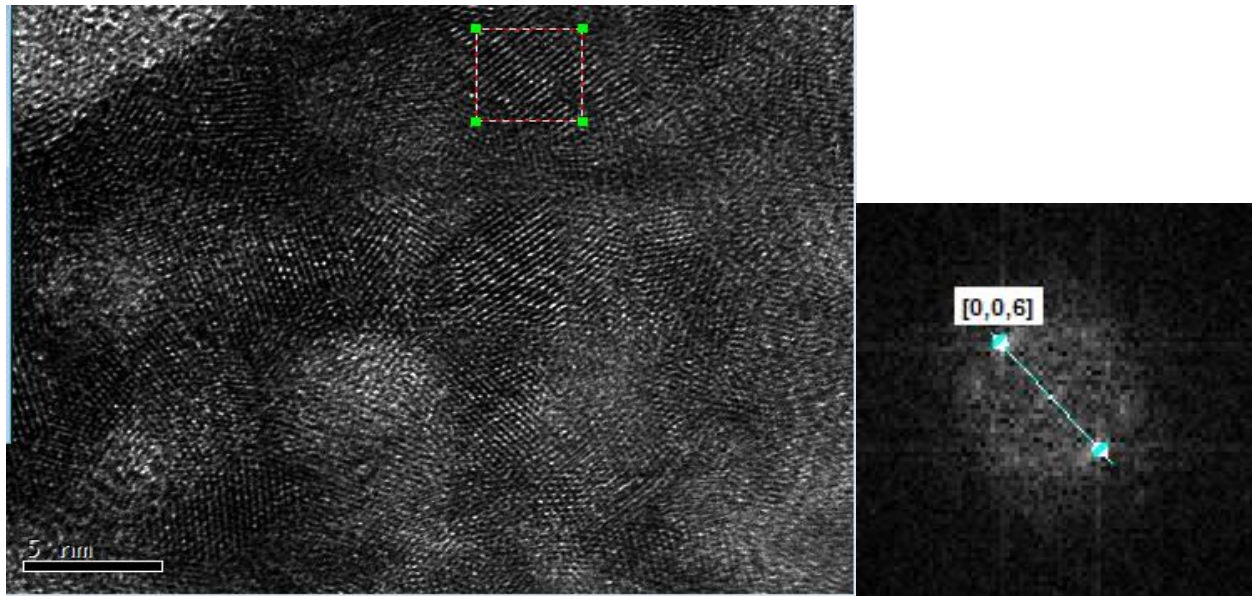


Figure 55: Image one from pH7_002 in HRTEM and FFT together with interplanar distances (right).

MEASUREMENT	VALUE IN TABLES D(A)	INT-F	H	K	L
2.30	2.288826	25	0	0	6
MEASUREMENTS CORRESPOND TO HEMATITE Fe_2O_3					

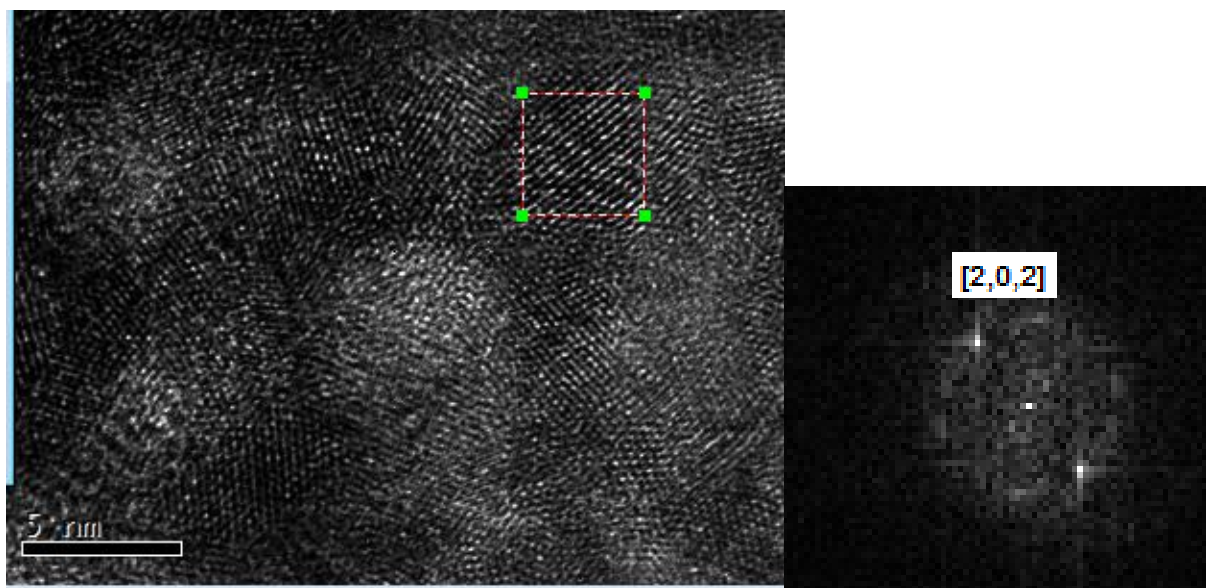


Figure 56: Image two from pH7_002

MEASUREMENT	VALUE IN TABLES $D(A)$	INF-T	H	K	L
2.03	2.0768	22	2	0	2
MEASUREMENTS CORRESPOND TO HEMATITE FE_2O_3					

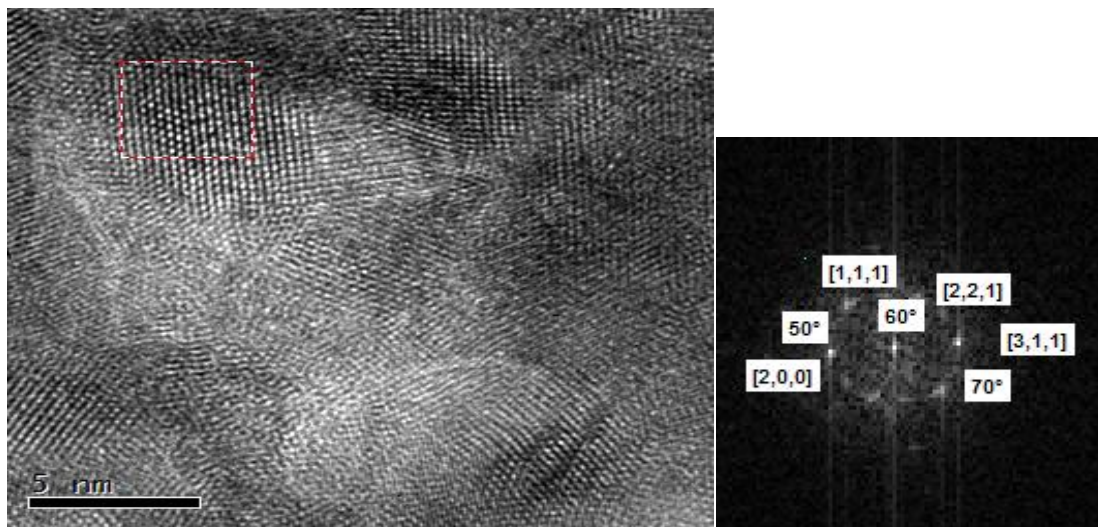


Figure 57: Image from sample pH7_004

MEASUREMENT	VALUE IN TABLES $D(A)$	INT-F	H	K	L
2.15	2.153	100	2	0	0
2.59	2.5177	100	3	1	1
2.34	2.49	80	1	1	1
2.77	2.784	2	2	2	1
VALUES CORRESPOND TO A MIXTURE OF WUSTITE AND MAGHEMITE Γ - Fe_2O_3					

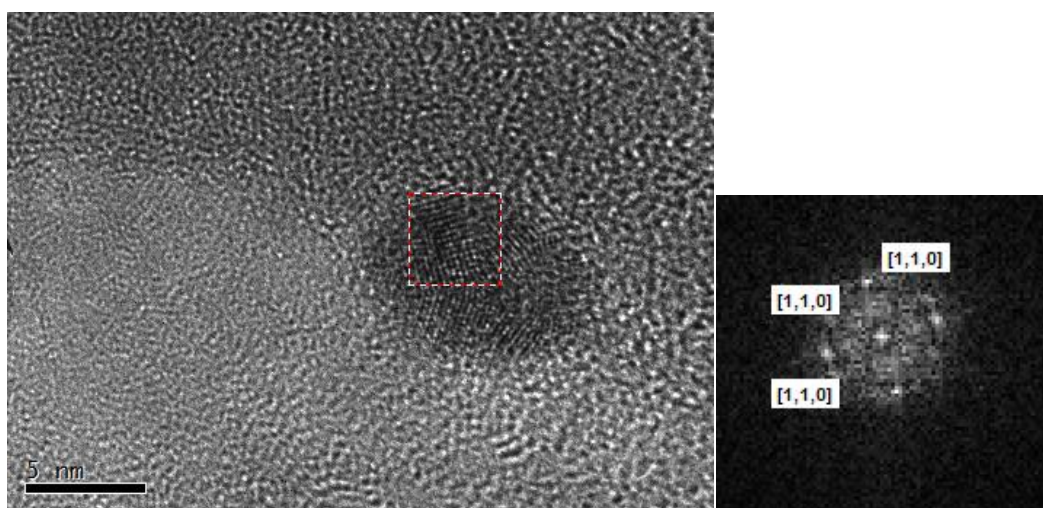


Figure 58: Image from sample pH7_e

MEASUREME NT	VALUE IN TABLES D(A)	H	K	L
2.55	2.516	1	1	0
2.47	2.516	1	1	0
2.50	2.516	1	1	0
VALUES CORRESPOND TO HEMATITE				

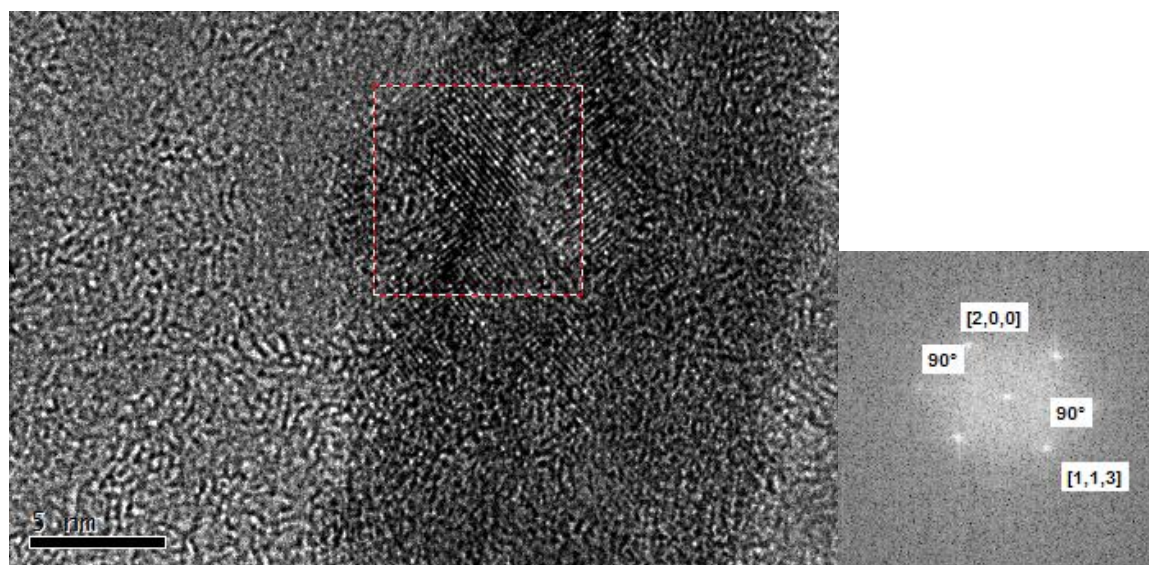


Figure 59: Image from sample pH7_f HRTEM

MEASUREMENT	VALUE IN TABLES D(A)	INT-F	H	K	L
2.16	2.153	100	2	0	0
2.18	2.2049	203	1	1	3
MEASURED VALUES CORRESPOND TO A MIXTURE OF WUSTITE AND HEMATITE					

In neutral solution (pH7) the images of high resolution presented agglomeration and resulted in a combination of phases due to the superposition of particles. Furthermore, size distribution was not

uniform. Sizes were in the range from ~ 5 to 15 nm it was difficult to determine the size of single nanoparticles due to the high agglomeration and superposition they presented.

It has been reported that extensively aggregation of nanoparticles began at approximately pH values of 4 to 5, reached a maximum at 8.5 for iron oxides nanoparticles (Baalousha *et al.*, 2008). Aggregation is explained mainly by charge neutralization.

Images from pH10

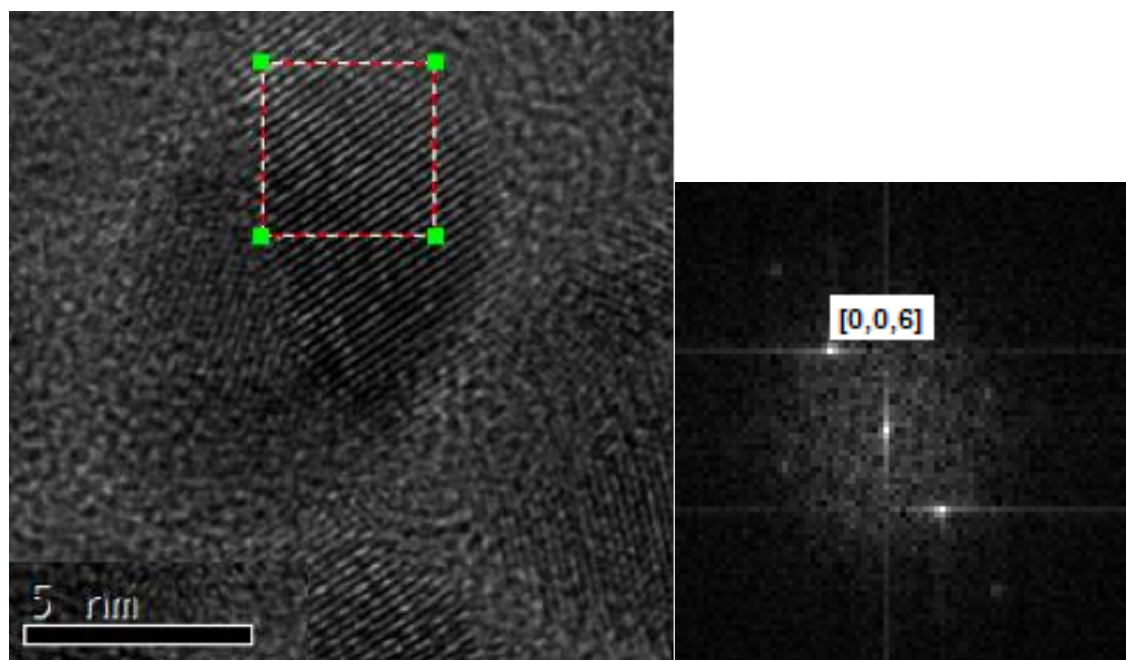


Figure 60: Image from sample ph10_0025 in HRTEM (left)and FFT together with the interplanar distances (right)

MEASUREMENT	VALUE IN TABLES D(A)	INT-F	H	K	L
2.41	2.4119	3	2	2	2
IRON OXIDE : MAGHEMITE γ - Fe_2O_3					

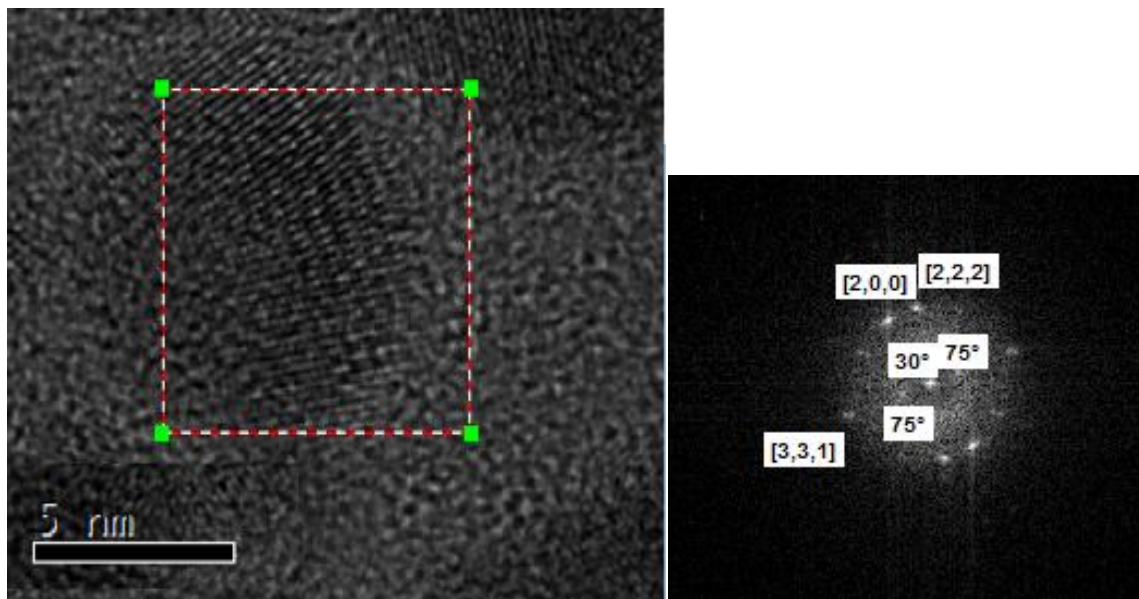


Figure 61: Image from sample ph10_0023 in HRTEM (left) and FFT together with the interplanar distances (right)

MEASUREMENT	VALUE	IN	INT-F	H	K	L
	TABLES					
2.42	2.4511		28	2	2	2
2.15	2.153		100	2	0	0
1.93	1.9479		3	3	3	1
IRON OXIDE: WUSTITE FEO AND MAGHEMITE γ -FE ₂ O ₃						

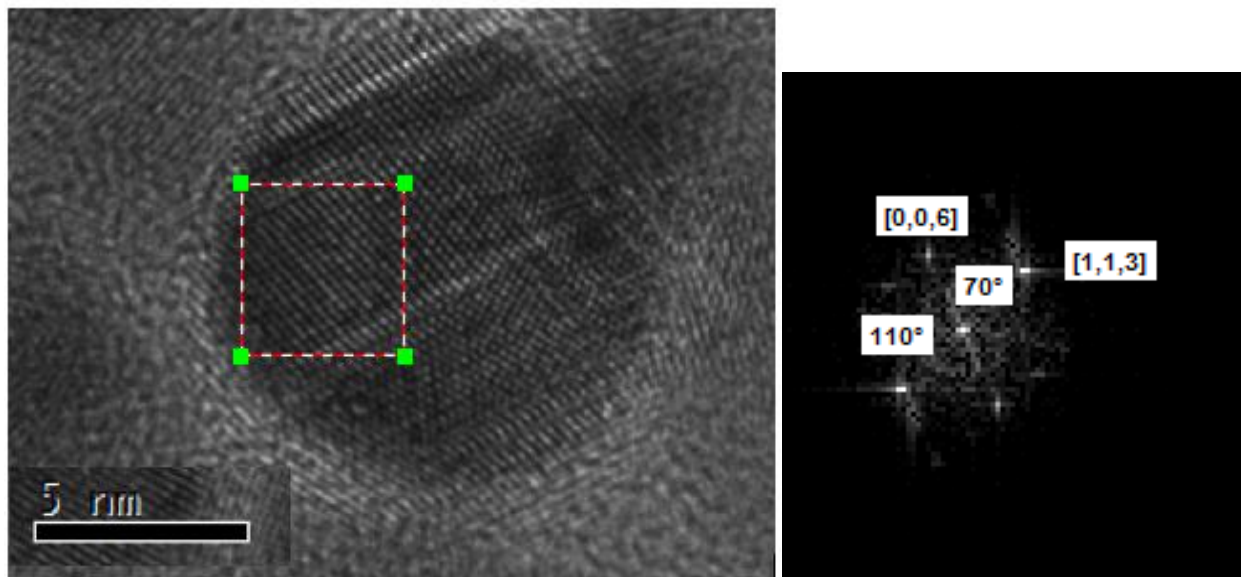


Figure 62: Image from sample ph10_0028 in HRTEM (left) and FFT together with the interplanar distances (right)

MEASUREMENT	VALUE	IN	INT-F	H	K	L
	TABLES					
2.31	2.2888		25	0	0	6
2.19	2.2049		203	1	1	3
IRON OXIDE: HEMATITE Fe_2O_3						

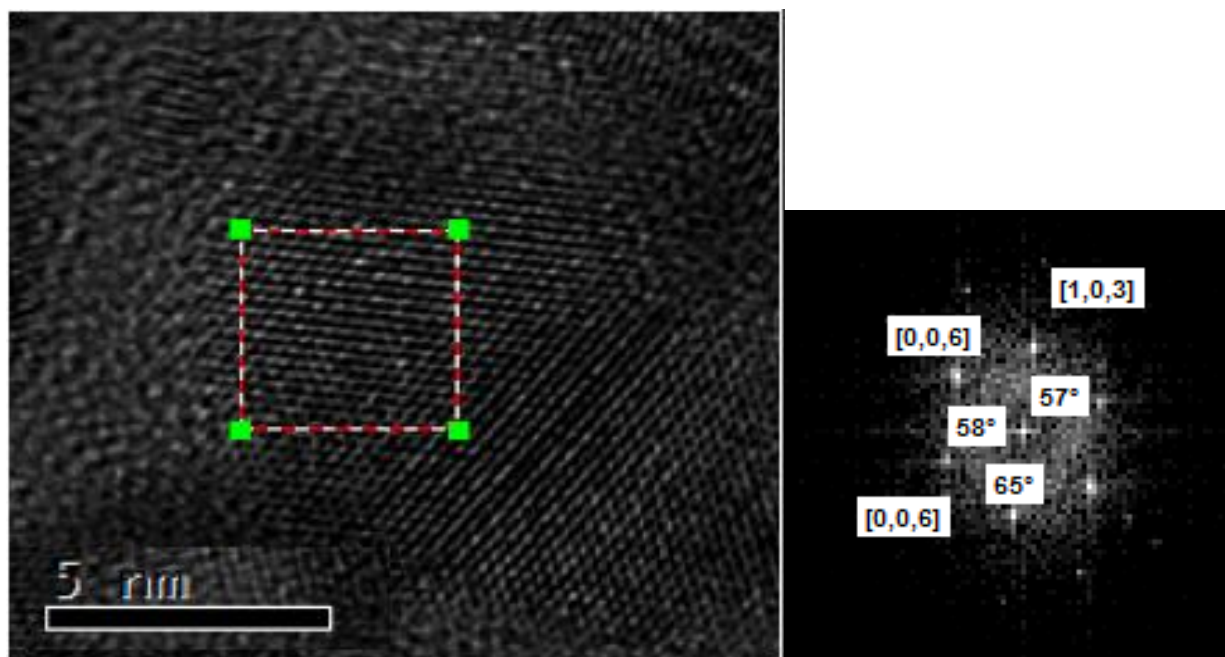


Figure 63: Image from sample ph10_0022 in HRTEM (left) and FFT together with the interplanar distances (right)

MEASUREMENT	VALUE IN TABLES	INT-F	H	K	L
2.21	2.2049	203	1	0	3
2.28	2.2888	25	0	0	6
2.31	2.2888	25	0	0	6
IRON OXIDE: HEMATITE Fe_2O_3					

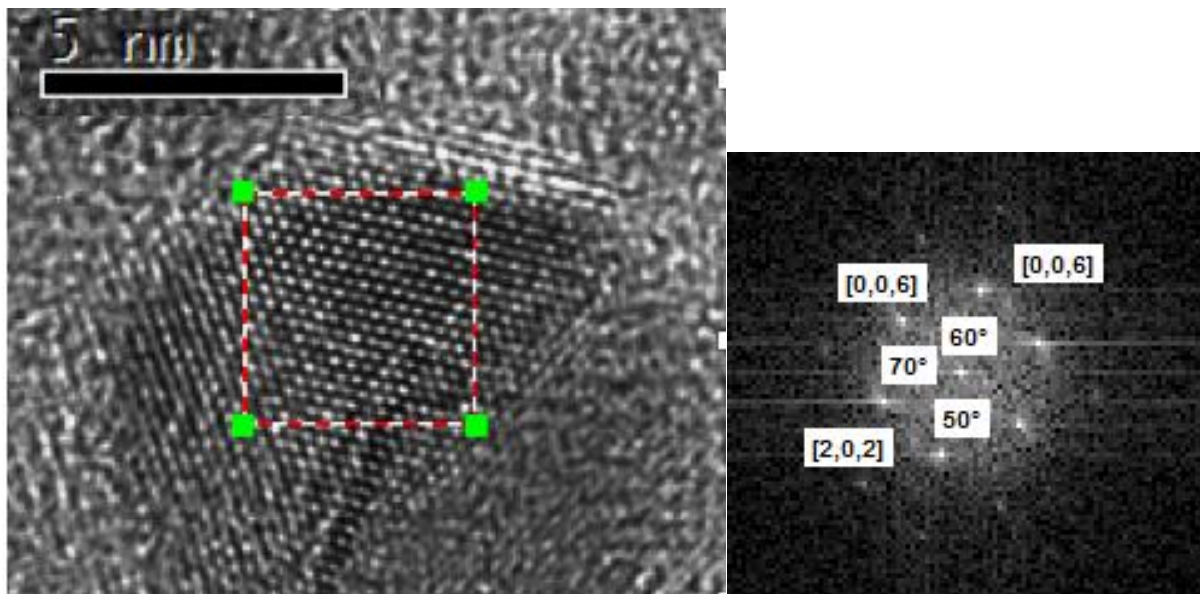


Figure 64: Image 1 from sample ph10_0019 in HRTEM (left) and FFT together with the interplanar distances (right)

MEASUREMENT	VALUE	IN	INT-F	H	K	L
	TABLES					
2.33	2.2888	25	0	0	6	
2.27	2.2888	25	0	0	6	
2.09	2.0768	22	2	0	2	
IRON OXIDE : HEMATITE FE2O3						

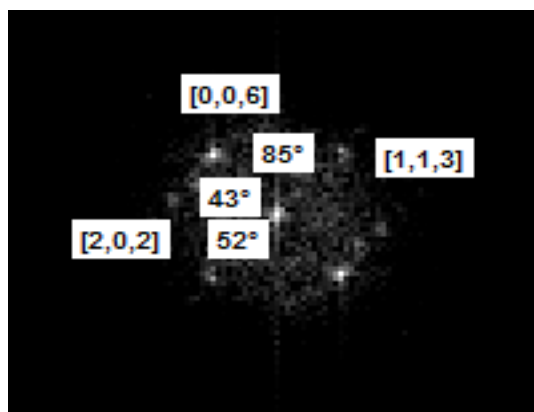




Figure 65: Image 2 from sample ph10_0019 in HRTEM (left) and FFT together with the interplanar distances (right)

MEASUREMENT	VALUE	IN	INT-F	H	K	L
	TABLES					
2.20	2.20		203	1	1	3
2.27	2.2888		25	0	0	6
2.03	2.0768		22	2	0	2
MEASUREMENTS CORRESPOND TO HEMATITE Fe_2O_3						

Figure 61 to 66 shown the interplanar spaces by HRTEM micrographs in pH10. According with crystallographic tables, three different structures were found, corresponding to three different Fe_xO_y phases. Maghemite was found in sample 0023 and 0025. Hematite was found in sample 0019, 0022 and 0028. Wustite was found in sample 0023.

			FeO phase			
Analyzed nano-particles			Hematite (Fe ₂ O ₃)	Maghemite (γFe ₂ O ₃)	Magnetite (Fe ₃ O ₄)	Wustite Fe _x O _y
pH	5	4	50%	50%	-	-
	7	7	57.14%	14.28%	-	28.57%
	10	7	57.14%	28.57%	-	14.28%
Total		18	55.55%	27.77%	-	16.66%

Table 4: Iron oxide phases found by percentage

Samples of pH10 were highly defined by their lattices and their size was more uniform, below 10 nm. It is important to notice that in order to obtain the size distribution of nanoparticles, image from dark field TEM was analyzed, instead of the HRTEM images.

When measuring interplanar distances of the HRTEM images, and comparing data to crystallographic tables of iron oxides, three species were found of iron oxides nanoparticles in three pH samples: hematite, wustite and maghemite. Hematite was the most frequent specie.

According with the Bravais lattices, and with values measured, hematite is rhombohedrally rhomb-centered, having lattice points on the corner of cell with two additional points along the longest body diagonal (B), whereas wustite has a cubic, face centered structure (A) and maghemite a primitive cubic structure (C) (Figure 67).

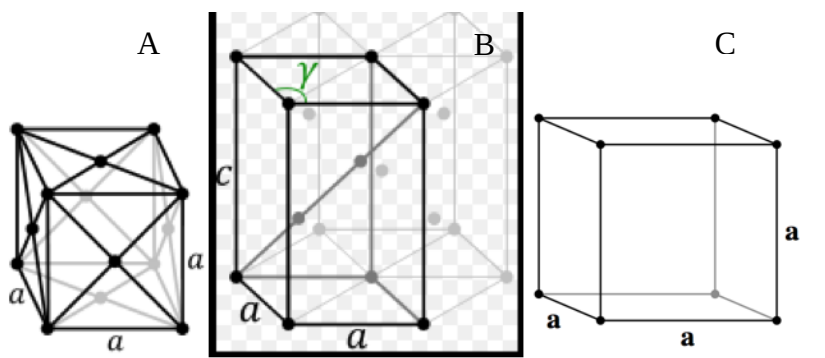


Figure 66: Crystal structures of (A) Wustite, (B) Hematite and (C) Maghemite

Rhombohedral tri-iron oxide (Fe_2O_3) and cubic $\text{Fe}_{0.902}\text{O}$ structure have been reported previously when synthesizing with *E. crassipes* biomass (Rosano-Ortega 2009), alfalfa biomass and tannic acid (Herrera-Becerra *et al.*, 2009).

Iron oxide NPs change their stability and aggregation behavior in response to changes in pH and organic matter (Baalousha *et al.*, 2008). Specialty in environmental applications, aggregation need to be carefully controled because it could affect the movility and transportation in water column in order to retain/remove target pollutants.

3.7 X-RAY DIFFRACTION

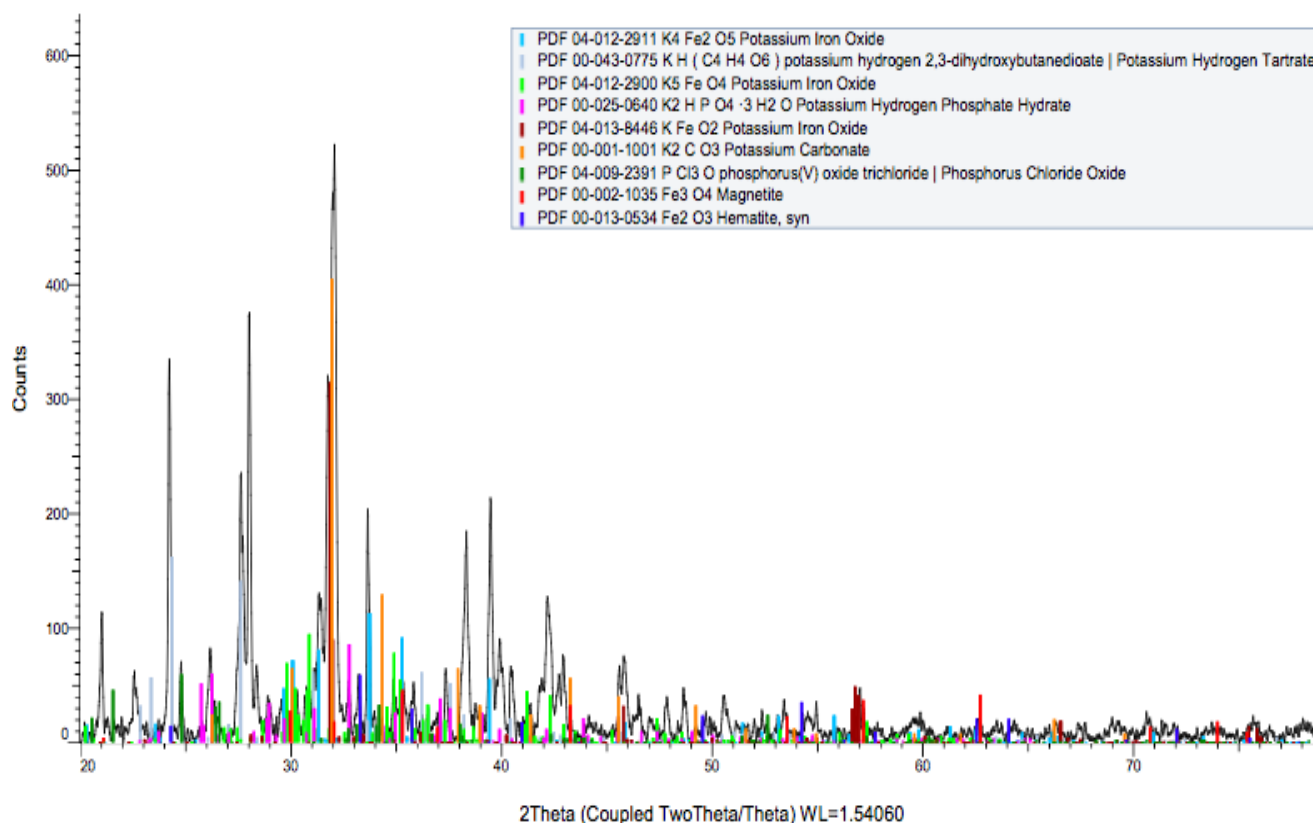


Figure 67: DRX analysis with various components identified.

Although there are iron oxides peaks in the analyzed sample (red band representing magnetite and dark blue representing hematite), many others species are present such as potassium iron oxide (light blue and light green band), potassium hydrogen tartrate (grey band), potassium carbonate (orange band) and phosphorous chloride oxide (dark green).

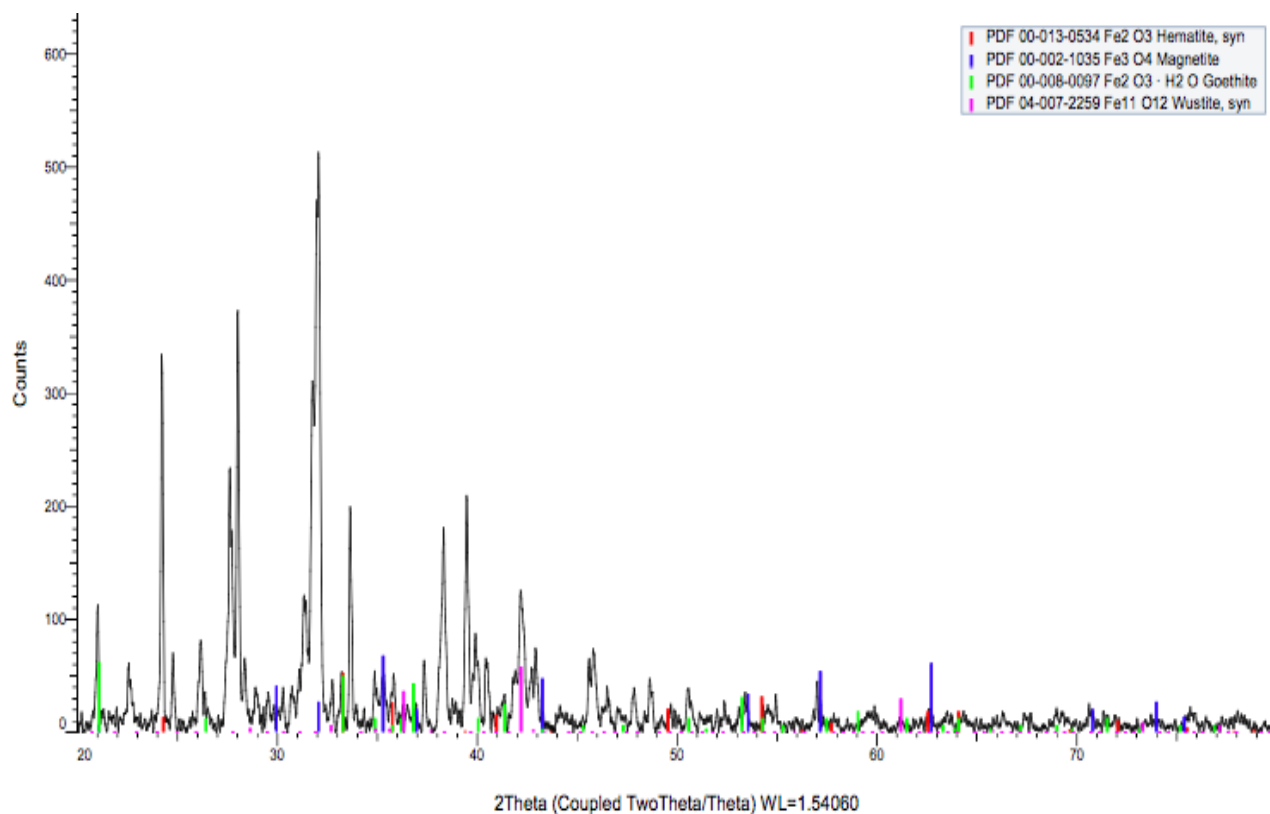


Figure 68: DRX analysis representing four different iron oxides species.

Figure 69 represents the matched iron oxide species found in sample; red bands indicate presence of hematite, blue bands represent maghemite, green goethite and purple bands wustite.

X-Ray diffraction analysis confirmed the presence of various iron oxides phases, such as hematite, magnetite, goethite and wustite. The oxidation in crystalline-phase magnetite, is common due to the reactivity of iron oxide when is in contact with air. This way, different phases are form in the core and in the superficial layers, which has been reported in previous studies (Shkylyny *et al.*,2010).

3.8 ENERGY- DISPERSIVE X-RAY SPECTROSCOPY (EDS)

Energy dispersive X-ray spectroscopy confirmed that samples contained iron oxide. In the analyzed sample, there are other elements present such as Cl, P, C, Si and K in higher quantities as shown in table 1. The analysis was taken from a section Spectrum 3 from a SEM image (*Figure 67*).

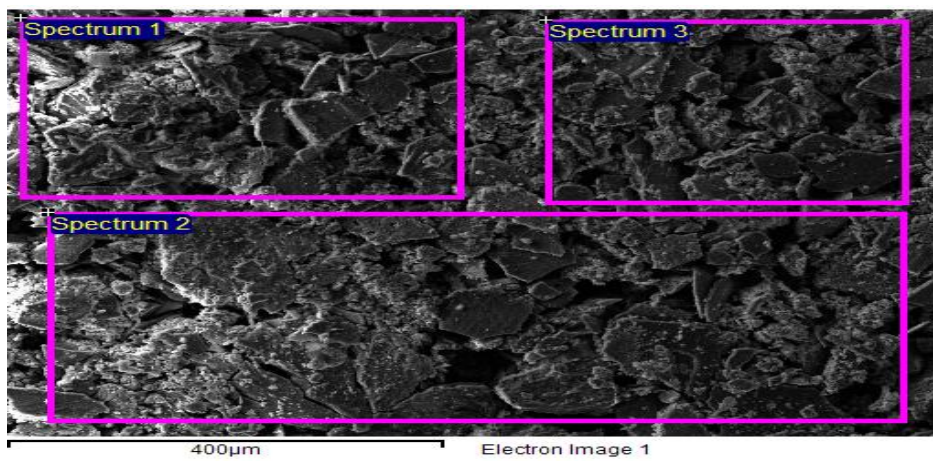


Figure 69: SEM image

Spectrum	In stats.	C	O	Si	P	Cl	K	Fe	Total
Spectrum 1	Yes	8.14	60.47	0.19	0.20	1.83	28.22	0.95	100.00
Spectrum 2	Yes	8.59	59.84	0.21	0.27	1.66	28.67	0.77	100.00
Spectrum 3	Yes	6.62	59.08	0.15	0.20	2.06	30.93	0.95	100.00
Mean		7.78	59.80	0.19	0.22	1.85	29.27	0.89	100.00
Std. deviation		1.03	0.69	0.03	0.04	0.20	1.45	0.10	
Max.		8.59	60.47	0.21	0.27	2.06	30.93	0.95	
Min.		6.62	59.08	0.15	0.20	1.66	28.22	0.77	

Table 5: Main elements found in sample from a SEM image

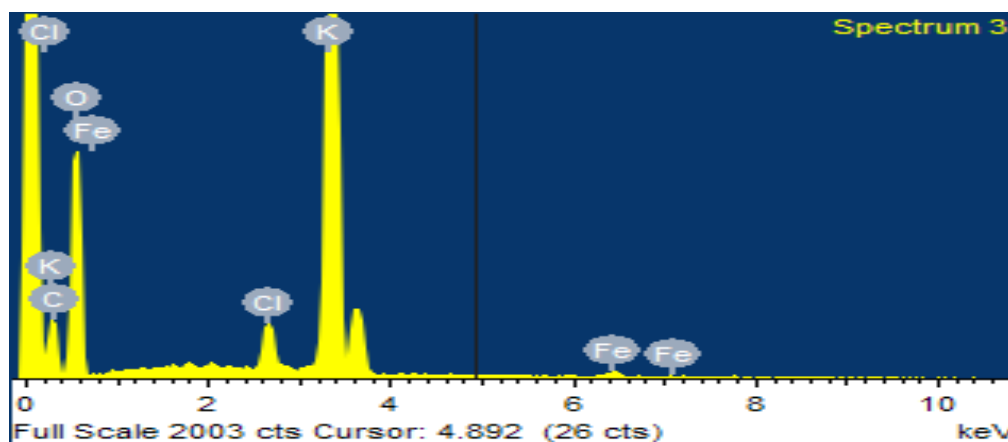


Figure 70: EDS graphic

In EDS, these components are presented in following quantities O>K> C> Cl>Fe> P> Si. And in XRD analysis, six species of potassium were detected, three of them containing iron oxides.

This results correspond to biomass components. Rosano-Ortega (2009) evaluated the main elements in roots of *E. crassipes* and reported K is found at 533 mg/Kg and Cl at 4200 mg/Kg. Similarly, Zhang and colleagues (2014) addressed that water hyacinth had concentrated mineral nutrients (K, Mg, Ca, Na and P) when evaluating the plant for its role in environmental remediation.

The amount of iron used for the iron solution was 0.0003 M which is reflected on the results of EDS as a very small proportion in comparison with the other elements.

3.9 MAGNETIC PROPERTIES AND POSSIBLE APPLICATIONS OF THE OBTAINED IRON OXIDES SPECIES.

- Hematite

α -Fe₂O₃ nanoparticles are known to be the most stable iron oxide in air under ambient conditions. Early works in the field have reported that the coercivity (a measure of a ferromagnetic material to withstand a external magnetic field without demagnetized) of α -Fe₂O₃ samples depend on the size, shape and synthetic conditions . However, at nano-scales, the increased surface to volume ratio, is correlated to an enhance of magnetization due to an increase number of uncompensated surface spins which will tend to interact ferromagnetically. As the particle size decrease, the surface spins

increase entailing an increase in magnetization (Kopanja *et al.*, 2016). As the obtained particles in this work are less than 5 nm of size, it is expected that they have ferromagnetic behavior. For rhombohedral shape hematite, the coercivity H_c is 99.21 Oe (Jing and Wu, 2004)

The currently obtained hematite nanoparticles, could be used for environmental applications as long as the components found in the biomass (K, Cl, P, Si) do not compete with the target.

Similarly, due to the components founded in *E. crassipes* biomass, it will be not possible to use the obtained nanoparticles for biomedical applications, unless the biomass could be removed almost completely. Nanoparticles produced for medical applications need to be carefully controlled in terms of quality and uniformity, with not remnants of production such as biomass. However, a feasible way to implement the current nanoparticles, it could be to extract or isolate the active compounds responsible for the reducing activity (tannins and polyphenols) from water hyacinth.

On the other hand, in not highly controlled environments, hematite nanoparticles could be implemented as antibacterial (Rufus *et al.*, 2016), or environmental applications such as removal for a variety of compounds for example as Arsenate (As (V)) which has been proved to have an effective removal of almost 100% (Singh *et al.*, 1996). The capacity of absorption increases as the size of hematite nanoparticle decrease according with Gao and colleagues, (2007). The obtained nanoparticles in this work, range below 10 nm in size thus making them feasible for retaining pollutants.

Fenton reaction is a solution of H_2O_2 with iron ions which produce a hydroxyl radical, responsible for oxidizing organic pollutants from water resulting into primarily carbon dioxide and water. According with this mechanism, reaction is initiated by the formation of a precursor surface complex H_2O_2 on the iron oxide surface. It has been reported that nanoscale iron oxides such as hematite are effective catalyst of Fenton reaction almost 35 times higher than bulk. Properties of the current obtained hematite could be advantageous due to the high surface area to be implemented for Fenton reactions (Duke, Zhao, & Semiat, 2013).

- Wustite

Wustite is antiferromagnet with a Néel temperature of ~ 200 K, below the spins are antiparallel.

The structure is known to be complex due to a defect structure and defect-related physical properties due to a formation of vacancies due to a deficiency of iron (Chen *et al.*, 2010). Also, it has been studied because of their interesting phase transformation to multiphase nanostructures.

Currently, FeO NPs are prepared by high energy milling (Chen *et al.*,2010). The mixture used is $\text{Fe}_2\text{O}_3 + \text{Fe}$ or $\text{Fe}_3\text{O}_4 + \text{Fe}$, under high temperatures ($>570^\circ\text{C}$) with high or not high pressure. Although there are no toxic solvents used in this physical method of synthesis, a lot of energy is required and thus, cost is elevated.

Wustite can be used in thin films together with maghemite for filtrate solar radiation (Chavez-Galan and Almanza, 2007). Currently there are not enough and recent sources in the literature supporting the use of wustite nanoparticles for diverse applications. The obtained nanoparticles could be used for semiconductors. Wustite can be stabilize in order to controlled stoichiometry, by organic ligands, thus biomass of water hyacinth could be use for stabilization.

- *Maghemite*

Some of the most common applications of maghemite are for magnetic resonance imaging (Fiorani *et al.*,2002) and magnetic recording media (Itoh and Sugimoto, 2003). This iron oxide is known to be ferrimagnetic and less stable than hematite, however, as size decrease, the stability increase (Chen *et al.*,2005).

CHAPTER 5: CONCLUSIONS

In the production of nanoparticles, it is highly important to explore sustainable processes for synthesis. Taking into account economic viability, environmental balance and social adaptability. By finding this equilibrium, prices of nanotech-based products would be affordable to consumers and the production of toxic compounds will be avoided or diminished. The current biosynthesis method used in this thesis, demonstrate it is effective for producing iron oxide nanoparticles in an *environmental-friendly* way.

Eichhornia crassipes plague, affects biota and cause eutrophication among other issues world wide, including the Valsequillo dam in Puebla, México. Its biomass, rich in polyphenols content can be successfully used for the production of nanoparticles due to the reductive behavior of tannins.

Three different iron oxide species were produced by biosynthesis; Fe_xO_y , (Wustite), Fe_2O_3 (Hematite) and $\gamma\text{-Fe}_2\text{O}_3$ (Maghemite). The structure found for each phase was face-centered cubic, cubic primitive and rhombohedral, respectively. Hematite was the most frequently identified oxide.

Quantum dots were obtained when using a solution of 10 pH. Moreover, for acidic and neutral conditions, the nanoparticles tended to form aggregates and presented more heterogeneous magnitudes. Acidic pH lead to an increment in the proton concentration, thus reducing the ability of tannins to oxidize. As a consequence, the size of particles was higher at a lower pHs, probably due to growth of smaller particles by coalescing.

TEM and HRTEM analysis were crucial to demonstrate the effect of pH in formation and stabilization of nanoparticles, besides for the identification of phases by using fast Fourier Transform. However, it was common to observe interference from the biomass. in order to ease the observation of the nanoparticles, Argon plasma cleaner was implemented (for 3 minutes). This constitutes a novel pretreatment for removing biomass for further applications.

Future perspectives for the project

Production of Nanoparticles involves, as a priority, the exploration of sustainable processes for synthesis, taking into consideration further vibrating sample magnetometry (VSM) and superconducting quantum interference device magnetometer (SQUID) studies for hematite, wustite and maghemite, could be performed. VSM studies determine size and shape dependence of magnetic properties of specie which will be useful for determining future applications and SQUID magnetometers are very sensitive, measuring extremely small magnetic fields.

Biomass is an integral part of the process, however, biomass components present drawbacks. First, the difficulty to observe nanoparticles in TEM. Second, the limiting medical applications due to the interference and possible adverse effects of this high quantities of unwanted elements such as Si or P for example. Third, tannins may vary from plant to plant, as stated before, these polyphenols are thought to be a plants mechanism against microorganisms and environment (reducing activity) thus, it may be possible that depending of the environment, the tannin content will differ, and thus the production of nanoparticles.

It is proposed to extract the tannins from the water hyacinth plant in order to developed a more controlled production of nanoparticles and taking into account that biomass is irrelevant for the biosynthesis. It is known that polyphenols act as capping agents (Herrera-Becerra *et al.*,2009), thus, they will prevent agglomeration and maintain nanoparticles size. Procedure for extracting tannins differ, for example, Segneanu and coworkers (2013) successfully obtained polyphenols by microwave-assisted ultrasound extraction together with a pretreatment of saponification. Solvents such as methanol, ethanol, acetone and ethyl acetate have been used for polyphenols extraction from plant materials (Dai & Mumper, 2010). Also, polyphenols have been recently investigated due to their effects in prevention of oxidative stress associated diseases (Manach *et al.*,2004) such as cardiovascular, neurodegenerative diseases and cancer which have been deduced from *in vitro* and *in vivo* studies (Rasmussen *et al.*,2005; Arts and Hollman, 2005; Hertog *et al.*,1994)

Another future project that could as complement of the current, is the evaluation of tannin concentration between *E. crassipes* of different locations. As one of the functions of tannins are a

defense mechanism against pathogens and microorganisms, it may vary depending of the threats the plant could receive from different environment.

As three species of iron oxides have been obtained by the biosynthesis method using water hyacinth, and each have been related to particular applications, a future research could be performed in order to achieve a separation technique to individually isolate the species.

A recent review (Herlekar *et al.*, 2014) focused on iron nanoparticles biosynthesized, and mentioned 18 plants extracts which have been extensively used for synthesizing iron nanoparticles, in comparison with only 3 plant biomass used for same purpose. This data encourages to investigate opportunities and advantages that biomass had over extracts.

Magnetite is one of the most studied and wanted nanoparticles due to their magnetic properties for a variety of applications. However, by this synthesis method magnetite was not obtained. Some of the biological entities which have been reported for producing this particular specie are *Fusarium oxysporum*, *verticillium* sp. (Bharde *et al.*, 2006) and magnetotactic bacteria (Roh *et al.*, 2001). It would be interesting to establish a method for production of magnetite by the use of *Eichhornia crassipes* biomass, and by changing certain parameters which could be crucial in producing this specie of iron oxide.

Finally, although iron oxides are known to be unarmful, we propose the use of toxicology test to ascertain that these iron oxide species obtained by the bioreduction method using water hyacinth are not toxic.

Appendix

- Distribution of size

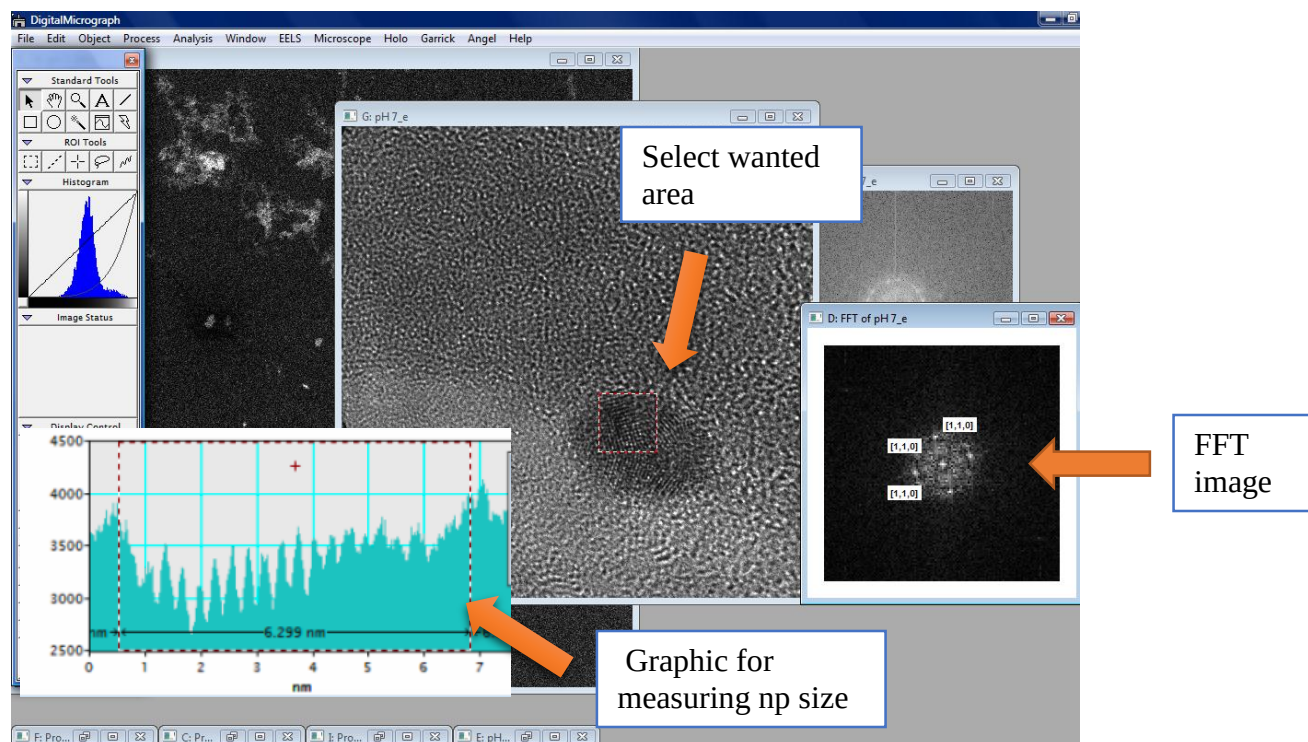
In order to obtain an average size of the biosynthesized nanoparticles, Digital Micrograph software was utilized.

Procedure

1. Open Digital Software in WINDOWS
2. Open the wanted image from the FILE menu and select from the STANDARD TOOLS a Line Profile tool.
3. In order to measure, make a line along the nanoparticle diameter.
4. After making a line, a graph will appear instantaneously. However, for obtaining a precise measurement, it is necessary to position the cursor on one of the peaks of the graph and drag it to a peak of the same distance. In other words, adjust the endpoints by dragging the handles on the line profile.
5. By adjusting the endpoints by double clicking on the line profile, a change profile info dialog will emerge.

Fourier transform

1. Open the wanted image from FILE menu and choose with the square form ROI tools the area of interest. You can select this area by clicking CTRL+ ALT key while drawing a square region of interest as well.
2. Choose FFT(CTRL+F) from PROCESS menu. A new window will open instantly, and a FFT image will emerge.
3. In order to obtain a higher quality picture for further analysis, from the display control, you can modify contrast, brightness etc.
4. To measure the inter-planar spacings, select from Standard Tools the line profile option and drag the cursor from side to side and adjust the endpoints.
5. Measure the inter-planar distances.
6. Compared with crystallographic tables of the wanted specie.



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