RAPID STOPPED FLOW ASSEMBLY OF MULTICORE IRON OXIDE NANOPARTICLE

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RAPID STOPPED FLOW ASSEMBLY OF MULTICORE IRON OXIDE NANOPARTICLE

by

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Harivindren Asokan

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LIST OF ABBREVIATIONs

DLVO	**Derjaguin-Landau-Verwey-Overbeak**
HCl	**Hydrochloric Acid**
IONPs	**Iron Oxide Nanoparticles**
PSS	**Poly(sodium(4)styrenesulfonate)**

ASEMBLAJ NANOPARTIKEL BESI OKSIDA (IONP) MELALUI KAEDAH PERHENTIAN ALIRAN

ABSTRAK

Formasi nanopartikel besi oksida (IONP) melalui kaedah perhentian aliran pantas dijalankan menggunakan RX2000 Rapid Mixing Unit. IONP multiteras telah distabilkan menggunakan polimer PSS 70k dengan pelbagai kepekatan IONP dari 10ppm hingga 50ppm. Dengan masa yang ditetapkan pada satu minit, kinetik penjerapan telah diperolehi. Keputusal kinetik penjerapan tidak stabil dan bising, dan membawa kepada kesimpulan bahawa penyalutan polimer pada IONP tidak berlaku. Oleh sebab sifat IONP untuk menggumpal and memendap, kluster telah terbentuk sebelum kerja eksperimen bermula, dan telah mewakili gerakan penyebaran, bukannya kinetik penjerapan yang dikehendaki. Dengan ini, dapat disimpulkan bahawa kaedah perhentian aliran pantas tidak boleh menghasilkan multiteras zarah nano besi oksida.

RAPID STOPPED FLOW ASSEMBLY OF MULTICORE IRON OXIDE NANOPARTICLE

ABSTRACT

The rapid stopped-flow assembly of multicore iron oxide nanoparticle (IONP) was performed using the RX2000 Rapid Mixing Unit. IONP was to be stabilized using polymer PSS (Poly(sodium(4)styrenesulfonate)70k with varying concentrations of IONP from 10ppm to 50ppm. With a fixed time of one minute, the adsorption kinetics were obtained. Adsorption kinetic results were unstable and noisy, leading to conclusions that ineffective coating has taken place. Due to the nature of IONP to agglomerate and sedimentate, clusters were formed even before experimental work began, hence leading to kinetics representing the diffusion motion, rather than the adsorption kinetics that is intended. It was concluded that rapid stopped-flow method was not feasible to form multicore iron oxide nanoparticle.

CHAPTER ONE

INTRODUCTION

1.1 Research Background

The application of magnetic nanoparticles (MNPs) in a biomedical context is a fast developing field. For example, the MNP suspended in aqueous liquids can be introduced into blood streams or tissue of a human body, allowing for the utilization of magnetic effects, such as magnetic losses, magnetic forces and localized sources of magnetic fields.

Small sized iron oxide nanoparticles depicts a superparamagnetic behavior with zero remanent magnetization due to thermal relaxation processes within the crystal lattice. With increasing particle volume, the particles become ferrimagnetic and show hysteresis during magnetic reversal. Medical applications do utilize larger particles with ferrimagnetic behavior because they have higher specific heating losses and higher magnetization than small superparamagnetic cores. However, since ferrimagnetic particles have remanent particle magnetization, it leads to agglomeration of MNP, even without an external magnetic field or use of coating. This can pose a serious hazard to health if say such particles agglomerate in the body.

To prevent such agglomeration, multicore nanoparticles (MCNP) (Suzdalev, 2001) are key. MCNP have very weak remanent magnetization, way smaller compared to single core particles of the same size of cluster, and this translates to lower tendency to form agglomerates, which is an excellent basis for medical applications.

MCNPs can be prepared by different routes. Generally, for preparation of biocompatible iron oxide MCNP, alkaline co-precipitation is used. Besides that, there is the inverse microemulsion, sol-gel synthesis, flow injection, electrospray synthesis, sonochemical method and thermal decomposition.

With the lines of literature, each structure of agglomerated particles, which is due to dipole-dipole interaction of single cores with a remnant magnetization, is termed as multicore. In magnetic bioseparation, MCNP show a higher separation efficiency due to their larger particle volume, which translates to higher magnetic attraction force. This is certainly more favourable thansmall MNPs with high surface to volume ratio, which results in inefficient separation. Moreover, due to their smaller hydrodynamic diameter, MCNPs show higher diffusion coeffection which leads to higher binding kinetics to targeted cells in biomedical cases. (Dutz, 2009)

The rapid stopped flow is a rapid kinetics technique where small volumes of solutions are rapidly driven from syringes into a high efficiency mixer to initiate a fast reaction. The resultant reaction volume then displaces the contents of an observation cell, hence filling it with freshly mixed reagents. The volume injected is limited by the stop syringe which explains the “stopped-flow”. Just prior to stopping, a steady state flow is attained. The solution entering the flow cell is only milliseconds old. The age of this reaction volume is known as dead time of the stopped-flow system. As solution fills the stopping syringe, the plunger hits a block, causing the flow to stop instantaneously. Using right techniques, kinetics of the reaction can be measured in the cell.

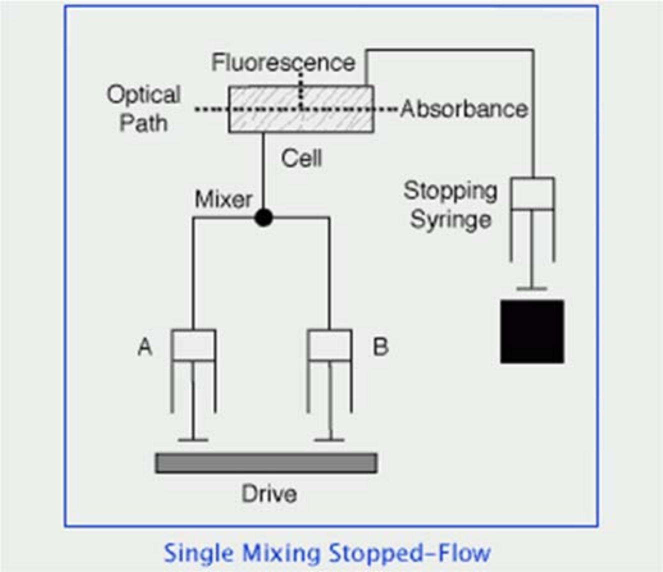


Figure 1.1: Single Mixing Stopped Flow unit

1.2 Problem Statement

The synthesis of iron oxide nanoparticles (IONP) is a complex process because of their colloidal nature. The preparation of colloidal dispersions of the IONP can be achieved in two ways, firstly, dispersion (size reduction to nanometer range and dispersing solid phase) of iron or iron oxides in aqueous medium and second, condensation of precursors from liquid or gaseous phase, well known procedures for ferrofluid preparation, called as top down (mechanical attrition) and bottom up (chemical synthesis) methods in fabrication of magnetic nanoparticles.

Focusing on bottom-up synthesis procedures, there are many methods such as chemical coprecipitation, hydrothermal reactions, sol-gel reactions, polyol methods, flow injection syntheses, electrochemical, biomineralization and various more.

A relatively interesting method to work on would be the flow injection method. Flow injection method has many advantages, and this method was first introduced by Ružicka and Hansen (1982). There are many advantages of the flow injection method, such as :

- Injection of reactant is direct into the carrier stream, achieving laminar flow conditions consistently (Re < 2000) which provides high mixing homogeneity
- There is a chance of segmenting the flow with inert fluids, hence providing system with pool zones confined to volumes of few microliters
- Gives control over the dispersion of the injected reactants by manipulation over the geometric and hydrodynamic condition of the system
- High degree of automation by integrating a computerized detector in the manifold array
- High reproducibility in injection volumes and residence times

With all these taken into account, our problem is, how do we synthesize multicore iron oxide nanoparticles? Is the rapid stopped-flow injection method suitable to be used for the synthesis of multicore iron oxide nanoparticle?

Hence, the aim of this project will be to use this flow injection method to successfully assemble multicore iron oxide nanoparticles. To point out the fact that the assembled IONP will be multicore, the advantages are of improved magnetic response of particles, while keeping the superparamagnetic behavior and satisfactory colloidal stability in spite of greater hydrodynamic size of dispersed particles, actually iron oxide nanoparticle clusters (Tombacz, 2015). In addition, the multi-core IONP increases magnetic moment.

1.3 Research Objectives

This research aims:

i) To assemble multicore iron oxide nanoparticle by rapid stopped-flow method

ii) To characterize the multi-core iron oxide nanoparticle prepared in terms of colloidal stability and magnetophoretic response

CHAPTER TWO

LITERATURE REVIEW

2.1 Single Core and Multi-core Nanoparticle

It is safe to use the term “core” to describe an individual nanoparticle and “multi-core” to describe a collection of cores held by a matrix to form a fixed structure. Basic definitions of single and multi-core particles would say these materials as discrete identifiable entities that possible agglomerate further. However, it is important to note that agglomeration would always be a result of weak physical interaction in a reversible process. To differentiate this reversible agglomeration, the term “aggregate” is used to define the stronger assemblage that occurs in multi-core iron oxide nanoparticles. For our case, the magnetic properties of the nanoparticles present magnetic forces between them due to magnetic dipole-dipole interactions or exchange interactions if the particles are in close contact. These dipolar interactions are relatively long range and their strength depend on other factors such as particle size, shape, inter-particle distance and many other. Hence, when magnetic nanoparticles aggregate, the dynamic and DC magnetic properties of the magnetic nanoparticle systems are changed due to magnetic interactions (L. Gutierrez, 2014) (X. Batlle, 2002), and most importantly changing the properties of the material compared to a non-interacting system. Despite knowing that synthesis of nanoparticles naturally lead to materials with different aggregation degree, little focus has been given to find out the functionality of those for engineering and industrial applications. For example, multi-core IONP seem to have superior performance for magnetic hyperthermia.

Magnetic multi-core nanoparticles are composed of several cores per particle as shown in Figure 2.1

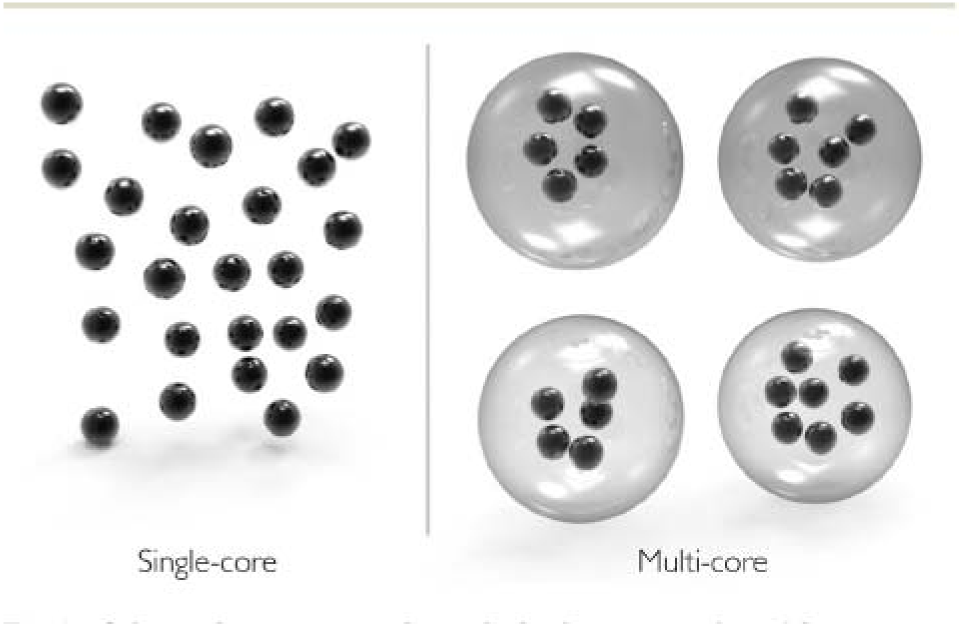


Figure 2.1: Schematic representation of single and multicore particles

In contrast with agglomerates of single-core particles, multi-core particles are assembled within a matrix that prevents further alterations to the number of cores per particle with time. These systems could present strong magnetic interaction between cores as a direct results of the cores being of close proximity to one another (Gustafsson, 2010). In this case, the number of magnetic cores per particle, their sizes, distance between them and the spatial distribution in general will strongly affect the magnetic properties of the material. The complexity of understanding multi-core nanoparticle lies in the reliable methods used to reproduce nanoparticle size, shape and structural homogeneity. Stable and reproducible analysis methods are vital to characterize the different magnetic particle system.

2.2 Application of Magnetic Nanoparticle

In applications of nanomedicine and biology, applications like magnetic resonance imaging, magnetic drug delivery system, magnetic fluid hyperthermia and magnetic labelling and separation of cells are utilizing magnetic nanoparticles, especially superparamagnetic iron oxides (Tombacz, 2015). Complex nanosystems of coated and multiple functionalized superparamagnetic iron oxide nanoparticle (IONPs) have become important as they give the best compromise between good magnetic properties and minimum toxicity.

In wastewater treatment, IONP can be utilized as adsorbents which gives an external magnetic field for separation and removal of contaminants in polluted water (Carlos, 2013). These IONP can quickly remove elements of chlorine and immobilize heavy metals contained in contaminated groundwater. In addition, as stated by (Hong, 2009), IONP can remove arsenic from water.

2.3 Polymer coating

It is certainly a challenge to control size, shape, stability and dispersibility of nanoparticles in desired solvents. Magnetic IONP have a large surface-to-volume ratio hence have high surface energies. This leads to aggregation as to naturally minimize the surface energies. Plus, the naked IONP have high chemical activity, and are easily oxidized in air (especially magnetite), which results in loss of magnetism and dispersibility. Thus, it is vital to provide proper surface coating and develop some effective protection strategies to keep the stability of magnetic IONP. Strategies include small organic molecules or surfactants, polymers and biomolecules, inorganic coating such as metal or silica, or even nonmetal elementary substance like metal oxide or metal sulfide. This will also help further functionalization.

Nano-scale magnetic structures has great potential for development in optio-electronics, electronics, biomedical applications, magnetic storage and wastewater treatment. Over recent times, there is a huge interest to prepare well-defined, discrete magnetic nanoparticles for global benefits. These nano-sized magnetic material display properties that are different from their respective bulk material counterparts. Size and surface effects play vital role in the magnetic behavior of nanomagnetic particles.

Researches around the world are focusing on applications in scientific technology. In the electronics field, research are underway to investigate the design of superstructures comprised of magnetic ordered arrays in order to boost sensitivity in magnetic sensors. The idea to produce thickness of magnetic nanoparticle just adequately to control the interparticle interactions is being studied to attempt to halt defects in layered magnetic films and enable packing efficiencies leading to better magnetization and high susceptibilities. Moreover, there is large interest in creating a well-defined superstructure which would surpass current electronic technology in magnetic storage and overcome the present superparamagnetic limits.

2.4 Colloidal Stability

Colloidal stability is referred to as the ability of colloid dispersion to resist aggregation for a certain time. With sufficient repulsion force, the particles will disperse enough to resist aggregation and the colloidal stability of the system is maintained. However, if there are no repulsion, then particles will experience aggregation and sedimentation as shown in Figure 2.2. An energy barrier in the interparticle interaction potential is required for colloidal stability. (Phenrat.T, 2010)

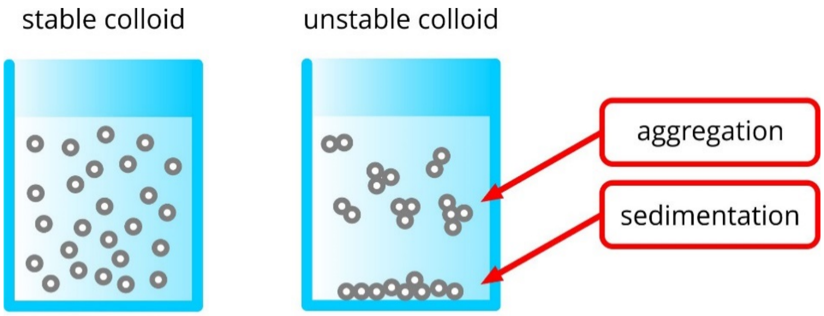


Figure 2.2: Colloidal stability illustration

Derjaguin-Landau-Verwey-Overbeak (DLVO) theory explains that the stability of the system comprises of charged particles (Ohshima, 2012). The theory is developed due to nanoparticle coatings.

2.5 DLVO Theory of Colloid Stability

The DLVO theory can be used to describe the role of polyelectrolyte on the surface of MIOPs. The theory comprises of both magnetic attraction and steric repulsions in addition to van der Waals force and electrostatic double layer forces where the magnetic properties play a vital role in ensuring stability and transport of magnetic nanoparticles. (Phenrat.T, 2010), (Hong, 2009). Generally, there are three kinds of stabilization mechanism of particles. Those are steric stabilization, electrosteric stabilization and electrostatic stabilization. Steric stabilization involves a polymer that is added to the system to adsorb onto a particle surface which prevents the particle surface from sticking to each other or be attracted to one another. Say adequate volumes of polymer are adsorbed, the layer thickness would be sufficient to abstain particles by steric repulsion between polymer layers as stated by (Ohshima, 2012).

Electrostatic stabilization on the other hand happens when particles interact due to distribution of charge species in system where it forms an electrical double layer around the particle itself. The strong repulsion is then experienced when the two double layers meet and start to overlap. This will eventually surpasses strength of van der Waals forces.

In this Figure 2.3, the two types of stabilization are shown.

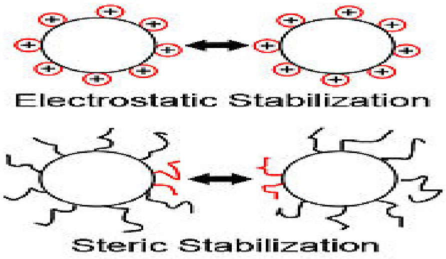


Figure 2.3: Electrostatic and Steric Stabilization

2.6 Magnetophoretic Response

Separation of a specific entity for diagnostic purpose is viable through the application of magnetophoretic force. The concept is to magnetically isolate the target entity, either ones with or without magnetic responsive characteristic (target entity without magnetic property needs to be magnetically labelled with magnetic particles (Gijs MAM, 2010), from a complex mixture by applying an external magnetic field. Under the presence of a magnetic field with gradient, magnetic materials will be magnetically aligned and undergo motion to the region of highest magnetic field strength by magnetophoretic force (Lim J.K. 2014). This process is known as magnetophoresis which basically is the motion of magnetic particles relative to their non-magnetic surrounding medium under a non-homogenous magnetic field (Andreu Js, 2011). The magnetically captured and concentrated samples then can be utilized for analysis of target identification. This technique is generally known as “magnetic separation” MS. Advantages of this method are the omission of physical contact between the magnetic source and biological samples, which translates to the method of MS being biologically non-invasive and do not exert a detrimental effect on biological components (Pamme N, 2007). Moreover, MS has high throughput, economically (Probst CE, 2011) and less energy intensive when a permanent magnet is made to be the magnetic source(Yavuz CT, 2009).

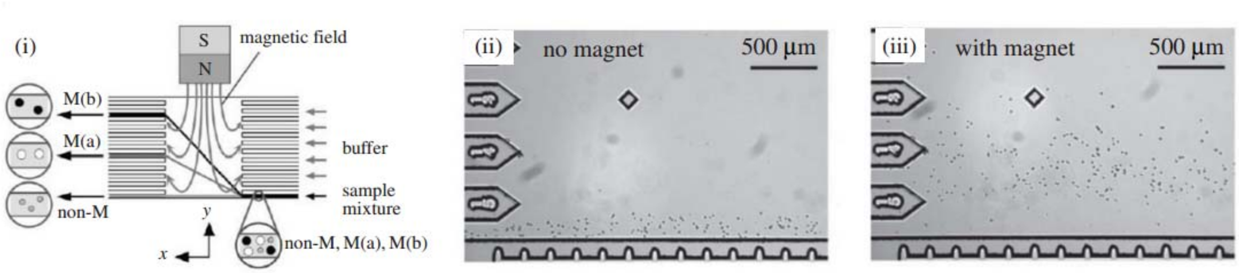


Figure 2.4: Magnetophoresis

(i) Schematic diagram illustrating a free-flow MS technique at which laminar flow was applied in x-direction (ii) flowing straight through the channel without applied external magnetic field (iii) deflected in flow of direction upon application of external magnetic field (Pamme N and Wilhelm C, 2006).

CHAPTER THREE

MATERIALS AND METHOD

3.1 Materials

Table 3.1: List of material

Materials	Supplier	Usage
Magnetic Iron Oxide	Nanostructured & Amorphous	Main Sample
Poly(sodium(4)styrenesulfonate) (PSS)	Sigma Aldrich	Polyelectrolyte for coating MIOP
Hydrochloric Acid (HCl)	Fisher Scientific	As a reagent
Nanoparticles(MIOPs)	Materials (NanoArmor)	General use

Table 3.2: Properties of MIONPs

Properties	Magnetic Iron Oxide Nanoparticles
Other name	Magnetite and Maghemite
Molecular formula	Fe_3O_4, Fe_2O_3,
Molecular weight (g/mol)	231.53
Density, g/cm3	4.8-5.1
Average size	20-30 nm
Melting point	Not Available

Table 3.3: Properties of PSS

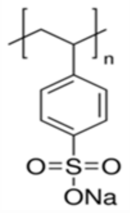
Properties	**Poly(sodium(4)styrenesulfonate)**
Other name	**Poly(4-styrenesulfonic acid) sodium salt** **(PSS 70k)**
Molecular formula	$(C_8H_7NaO_3S)_n$
Molecular weight (g/mol)	70 000
Density, g/cm3	0.801
Boiling point	Not Available
Melting point	Not Available
Chemical structure	

Table 3.4: Properties of HCL

Properties	**Hydrochloric Acid**
Molecular formula	HCl
Molecular weight (g/mol)	36.45
Density, g/cm3	1.18
Boiling point	Not Available
Melting point	Not Available

3.2 Equipment

Table 3.5: List of equipment

Equipment	Model/Brand	Usage
pH meter	Eutech pH 5+	To measure the pH values and adjust for IONP and PSS solutions
RX2000 Rapid Mixing Stopped-Flow Accessory	RX2000	To rapidly form multicore IONP with PSS coating
Uv-Vis Spectrometer	Agilent Cary 60 UV-Vis	To study adsorption kinetics and formation of multicore IONP
Zetasizer	Malvern	To measure hydrodynamic size of particles

3.3 Flow of research

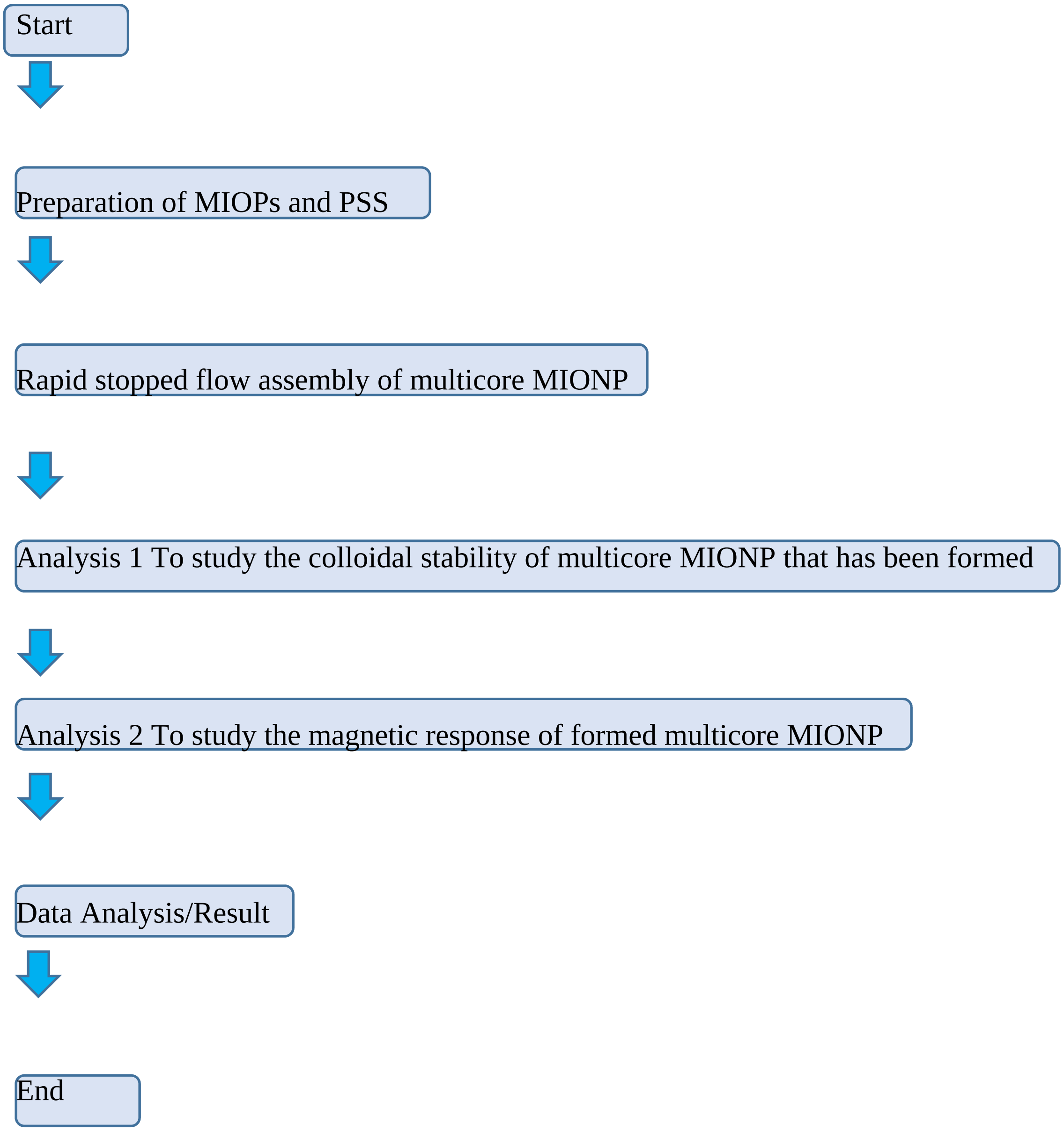


Figure 3.1: Process Flow of Experiment

3.4 Method of preparation of multicore MIONPs

3.4.1 Preparation of MIONPs

A suspension of 5000mg/L of MIONPs((Fe_3O_4) in deionized water was prepared using the glass scintillation vial. This was then dispersed by ultrasonification for at least one hour using sonicator (Elmasonic S10H) to induce well dispersed condition.

3.4.2 Preparation of PSS coating

5000 mg/L of PSS 70k suspension in deionized water was prepared using the glass scintillation vial. This was then sonicated for one hour to induce well dispersed conditions.

3.4.3 pH adjustments

The pH levels are important to enable the charged MIONPs and PSS to be of opposite poles in order of physisorption to occur via electrostatic attraction. To adjust, HCL was used accordingly and pH of 3.5 was targeted. The pH adjustment was done once the different concentration of iron oxide NP solution was prepared. In other words, pH was adjusted prior to sonication and reaction in the rapid mixing unit.

3.4.4 Fabrication of multicore IONPs

The MIOPs and PSS solutions, both well dispersed are now used in the syringes of the rapid mixing unit RX2000. Adequate samples are taken and injected simultaneously using the piston and reaction, which is the formation of polymer-coated multicore iron oxide nanoparticle is allowed to occur.

Figure 3.2: RX2000 rapid mixing unit

3.4.5 Identifying multicore IONPs

To identify if the formed particles are multicore, the reaction kinetics is analyzed and the wavelength of formed particles is checked to see if it falls in the range of standard multicore IONPs.

3.4.6 Colloidal stability analysis

Concentrations of MIONP was chosen to be a process parameter. Therefore, different concentrations such as 20ppm, 40ppm, 60ppm, 80ppm and 100ppm were used in the injection for rapid mixing. Once that was done, the formed multicore IONPs were allowed to disperse in a measuring cylinder of 20ml. and further dispersion was induced using a spatula. Once done, photos of the samples were taken at intervals of 1 minute for the next half an hour. This was to observe the sedimentation of multicore MIONPs at different concentrations.

3.4.7 Magnetophoresis analysis

An external magnetic field was induced around the cuvette the rapid mixing unit and the reaction kinetics were observed till the kinetics became zero. The duration taken for the formed multicore IONP to completely stick onto the side of the cuvette and allowing UV light to completely pass through would signal the complete attraction of the magnetic IONPs to the magnetic field. The shorter the duration, the faster the magnetic response.

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter represents the experimental path and discussion consisting of two main sections. The first section illustrates the experimental design applied for the formation of multicore IONP using rapid stopped-flow method and the results obtained whereas the second section describes the nature of the results and products formed.

4.1 Experimental Design

4.1.1 Wavelength analysis

Table 4.1: Wavelength results

Reading	Absorbance
Zero	0.1221
1	3.2736
2	3.2421
3	3.2542
4	3.2250
5	3.2267

To set the range of wavelength for the kinetic reaction analysis and calibration curve, first, the wavelength of the iron oxide NP solution had to be discovered. Over a range of selected wavelength, using the Simple Reads software, the absorbance was measured. Table 4.1 showed maximum absorbance for the wavelength size of 505 nm. Hence, a wavelength of 505nm was chosen for the rest of the experimental work.

4.1.2 Calibration curve

Once the wavelength was identified, the calibration curve was plotted. The calibration curve is a standard curve, and is used to determine the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration. It is also useful for extrapolation (Prof. Tom O'Haver, Professor Emeritus, 2008). Figure 4.2 and Table 4.1 shows calibration curve at 505nm.

Figure 4.1: Calibration Curve

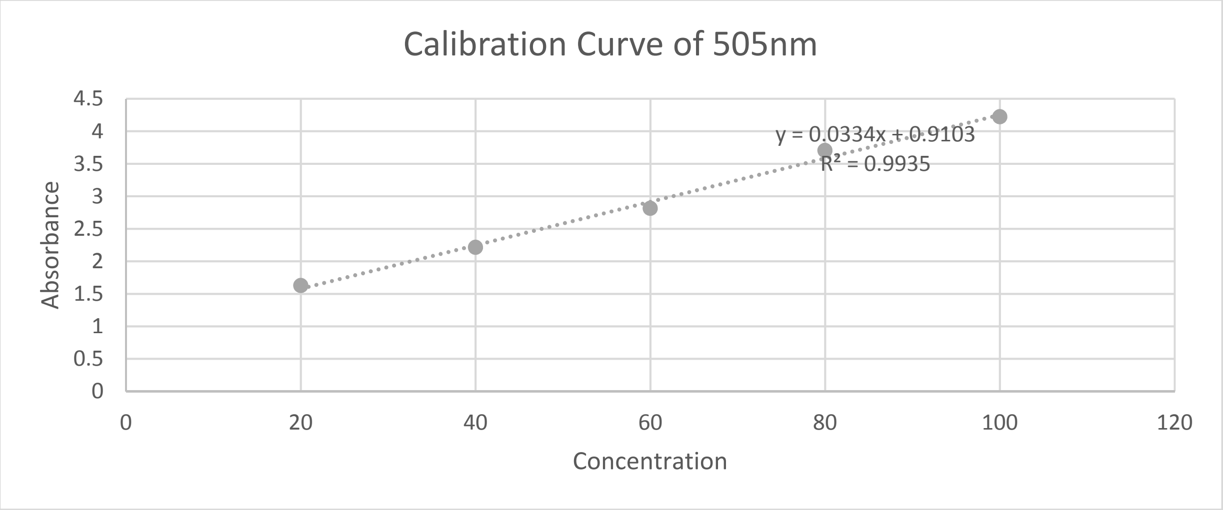


Table 4.2: Calibration data

Concentration (Ppm)	Absorbance			
20	1.6227	1.6234	1.6282	1.624767
40	2.2265	2.2097	2.2049	2.2137
60	2.8122	2.8104	2.8142	2.812267
80	3.7036	3.7021	3.7042	3.7033
100	4.321	4.231	4.112	4.221333

The R-squared value is at 0.9935, which indicates how close the data is to the fitted regression line.

Over here, we are analyzing the change in absorbance with varying concentration of iron oxide nanoparticle. Absorbance is an important detail throughout this experiment, and can be explained further using the Beer-Lambert Law (Douglas A. Skoog). The law is a linear relationship between absorbance and concentration of an absorbing species. The general law is written as:

$A = e\ b\ C$

Where : A = Absorbance. e = molar absorptivity, b = path length, C = concentration

However, it is important to note that this law has several limitations in both chemical and instrumental ways where deviations in absorptivity coefficients at high concentration due to electrostatic interactions between molecules in close proximity, scattering of light due to iron oxide particles’ nature and various other reasons. As for a general understanding, we can deduce that an increase in absorbance would indicate an increase in concentration due to its linearity.

4.1.3 Hydrodynamic size Test

Test A: Effect of coating on hydrodynamic size

Since the idea to form multicore iron oxide nanoparticle with polymer coating using a rapid stopped mixing unit is a novel research idea, it was first important to find out of coating and formation of multicore IONP actually happened. For this, a verification test was done by comparing hydrodynamic size of before and after of a low concentration IONP solution. Concentration used was 10ppm and the result is shown in Figure 4.3 and Figure 4.4.

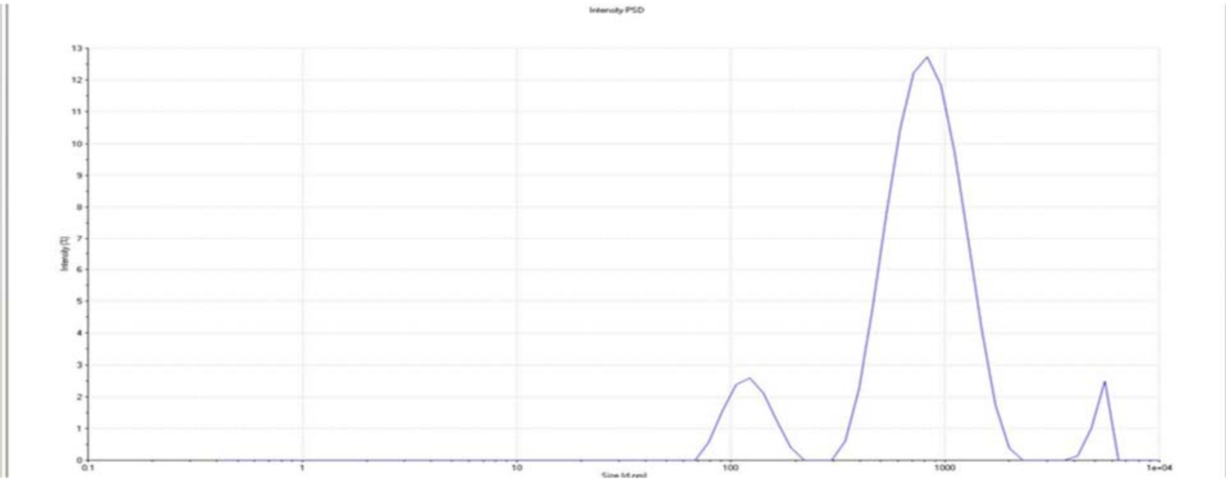


Figure 4.2: Bare 10ppm IONP

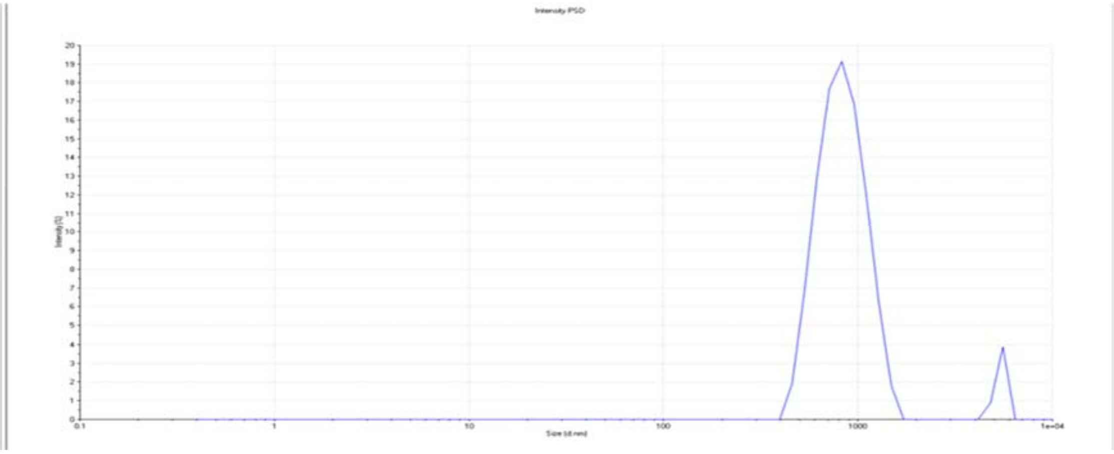


Figure 4.3: Coated 10ppm IONP