MAGNETICALLY TARGETED DRUG DELIVERY SYSTEM BY IMAGING TECHNOLOGY

HAFSA INAM

NUST201463179MSMME62414

Supervisor

DR. UMAR ANSARI

DEPARTMENT OF BIOMEDICAL ENGINEERING AND SCIENCES

SCHOOL OF MECHANICAL & MANUFACTURING ENGINEERING

NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY

ISLAMABAD

APRIL 2017

MAGNETICALLY TARGETED DRUG DELIVERY SYSTEM BY IMAGING TECHNOLOGY

HAFSA INAM
NUST201463179MSMME62414

A thesis submitted in partial fulfillment of the requirements for the degree of
MS Biomedical Sciences

Thesis Supervisor:
UMAR ANSARI

Thesis Supervisor's Signature: _____

DEPARTMENT OF BIOMEDICAL ENGINEERING AND SCIENCES
SCHOOL OF MECHANICAL & MANUFACTURING ENGINEERING
NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY,
ISLAMABAD
APRIL, 2017

THESIS ACCEPTANCE CERTIFICATE

Certified that final copy of MS/MPhil Thesis written by **Ms. Hafsa Inam**, Registration No. **NUST201463179MSMME62414F** of **MS Biomedical Sciences and Engineering (SMME)** has been vetted by undersigned, found complete in all respects as per NUST Statutes /Regulations, is free of plagiarism, errors, and mistakes and is accepted as partial fulfillment for award of MS/MPhil Degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have also been incorporated in the said thesis.

Signature (Supervisor): _____

Dr. Umar Ansari

Date: 24-04-2017

Signature (HOD): _____

Date: 24/04/2017

Signature (Principal): _____

Date: 24-04-2017

FORM TH-4

National University of Sciences & Technology

MASTER THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by: **Ms. Hafsa Inam** regn no. NUST201463179MSMME62414F Titled: **Magnetically Targeted Drug Delivery System by Imaging Technology"** be accepted in partial fulfillment of the requirements for the award of **MS Biomedical Sciences** degree. (Grade A)

Examination Committee Members

1. Name: Dr. Murtaza Najabat Ali Signature:

2. Name: Dr. Nabeel Anwar Signature:

3. Name: Dr. Adeeb Shahzad Signature:

Supervisor's name: Dr. Umar Ansari Signature:

Head of Department

24/04/2017
Date

COUNTERSIGNED

Date: 24-04-2017

Principal

DECLARATION

I certify that this research work titled "*Magnetically Targeted Drug Delivery System by Imaging Technology*" is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources it has been properly acknowledged / referred.

Signature of Student

HAFSA INAM

NUST201463179MSMME62414

ii

PLAGIARISM CERTIFICATE (TURNITIN REPORT)

This thesis has been checked for Plagiarism. Turnitin report endorsed by Supervisor is attached.

Signature of Student

HAFSA INAM

NUST201463179MSMME62414

Signature of Supervisor

iii

COPYRIGHT STATEMENT

ACKNOWLEDGEMENTS

I am thankful to my Creator Allah Subhana-Watala to have guided me throughout this work at every step and for every new thought which You setup in my mind to improve it. Indeed I could have done nothing without Your priceless help and guidance. Whosoever helped me throughout the course of my thesis, whether my parents or any other individual was Your will, so indeed none be worthy of praise but You.

I am profusely thankful to my beloved parents who raised me when I was not capable of walking and continued to support me throughout in every department of my life.

I would also like to express special thanks to my supervisor Dr. Umar Ansari for his help throughout my thesis and also for MDDS and Selected topics of Biomedical Engineering which he has taught me. I can safely say that I haven't learned any other engineering subject in such depth than the ones which he has taught.

I would also like to pay special thanks to Faizan Saifullah for his tremendous support and cooperation. Each time I got stuck in something, he came up with the solution. Without his help I wouldn't have been able to complete my thesis. I appreciate his patience and guidance throughout the whole thesis.

I would also like to thank Dr. Murtaza Najabat Ali, Dr. Nabeel Anwar and Dr. Adeeb Shehzad for being on my thesis guidance and evaluation committee and express my special thanks to Mariam Mir for her help. I am also thankful to my lab members Aisha and Bakhtawar for their support and cooperation.

Finally, I would like to express my gratitude to all the individuals who have rendered valuable assistance to my study.

Dedicated to my exceptional parents

Mr. and Mrs. Muhammad Inam-ul-Haq

whose tremendous support and cooperation led me to this wonderful accomplishment.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

DDS	Drug Delivery System.
MNP	Magnetic nanoparticle.
PWM	Pulse-Width Modulation.
PID	Proportional-Integration-Derivative.
Kp	Proportional Coefficient
Kd	Derivative Coefficient
Ki	Integration Coefficient.
mm	Millimeter
V	Volts
A	Amperes

ABSTRACT

Conventional dosage methods such as capsules, gels etc. which are used traditionally have severe side effects including rising of blood sugar level by dissolution of drug in blood which can be overcome by replacing traditional drug delivery with specifically targeted and regulated drug delivery system. The main concept of using magnetic levitation for drug delivery purposes are when delivering the drug to a specific point via magnetic actuation, magnetic material encoated in drug can rupture the artery by attracting towards externally applied strong magnetic field. By taking magnetically levitated drug to the targeted area, it will minimize the risk of rupturing of the artery. Another problem that will be minimized is the dispersion of drug in the blood stream because the drug-coated core will be under the influence of strongly applied electromagnetic field and drug can be released by the alteration of electromagnetic fields In this study, the system uses one-dimensional force system. Two forces counter each other i.e. the electromagnetic force and the gravitational force. Addition of Ki to Kp and Kd though speeds up the motion when reaching to the targeted set point, blob stays in levitated condition around the set point thus stability is increased by the addition of Ki but oscillation are still present that hinders the stability of the system. Addition of exponential function to smooth out the power. Power was too much, to decrease the power of Kp, we added an exponential function, in result, it supplies the power when the error is large, power gets zero when error is reduced to zero. In this stable system, Kp and Kd gain are applied to minimize the oscillations and keep the blob levitated at targeted set point.

Key Words: *Electromagnet, Drug Delivery System, Magnetic Levitation, Liquid Medium, Arduino Uno, Real-Time Image processing*

CHAPTER 1

INTRODUCTION

Conventional dosage methods such as capsules, gels etc. which are used traditionally have severe side effects including rising of blood sugar level by dissolution of drug in blood (Sheweta P et al.,), which can be overcome by replacing traditional drug delivery with specifically targeted and regulated drug delivery system by using magnetic nanoparticles. Another difficulty is the hindrance for the drug to reach the diseased site, as in case of cancer, where the hypoxic environment and avascular nature of the tumor does not allow the drug to be delivered properly. Studies and researches are being conducted to overcome these problems through nanotechnology. Nanoparticles are being used for diagnosis, treatment and therapy of various diseases. (Gupta & Gupta, 2005, Xie et al., 2006).

The main concept was originated in late 1970's in which use of magnetic nanoparticles propose that drug delivery can be done directly to the targeted area by using magnets, for therapeutic purposes (Widder et al., 1978; Senyei et al., 1978; Mosbach and Schroder, 1979).

Among various Nano systems, magnetic nanoparticles (MNPs) are of special interest. Besides clinical diagnosis and therapeutic techniques, application of MNPs which are widely studied are: magnetically enhanced transfection, magnetically induced hyperthermia, magnetically assisted gene therapy and magnetic force based tissue engineering (Corchero & Villaverde, 2009).As an enhanced therapeutic approach, Nanotechnology has been used for the diagnosis of diseases. The main advantage of using nanoparticles over conventional medication is their small size, which covers larger surface area of diseased tissue, allows for maximum interaction with the surroundings and more efficient release of the drug whereas the small size makes it easy for the particle to reach areas that are otherwise inaccessible (Tari A et al., 1979, Perez JM et al., 2003).

This technique can be used to provide targeted therapeutic effects by regulating externally controlled magnets (such as in deep tissues and tumors), this is considered as a magnetically responsive procedure thus named as magnetic drug targeting (Lubbe AS et al., 1996). Magnetic nanoparticles and micro-particles were advanced by Widders et al to which fusion of cytotoxic drugs could be done (Widder et al., 1978; Senyei et al., 1978; Mosbach and Schroder, 1979).

1.1 Devices:

There are number of implantable devices that are being used for targeted drugdelivery and are in the stages of production and clinical assessment. For alternate and continuous deleverage of drug, drugs are being delivered at several quantities by these devices. As per patients need, these are designed to release drug for both shorter (days) and longer (~1 year) period of time. On the basis of designs, some of these can be refilled and some cannot. Number of factors comes under consideration when working on these drug delivery approaches such as long-term use and expenses. An intelligent feedback control system should be added as the devices are usually designed by micromachining. The need of oral drug delivery can be replaced by the ingestible devices that can be taken to the targeted tissue for the delivery of drug. There are multiple combinations of polymers that are being made for the targeted drug delivery to the specific tissue such as intestinal wall (Woodley, J. 2003).

1.2 Principle of Magnetic Targeting:

Drug Delivery System (DDS) is considered as a favorable technique for the delivery of drug, by

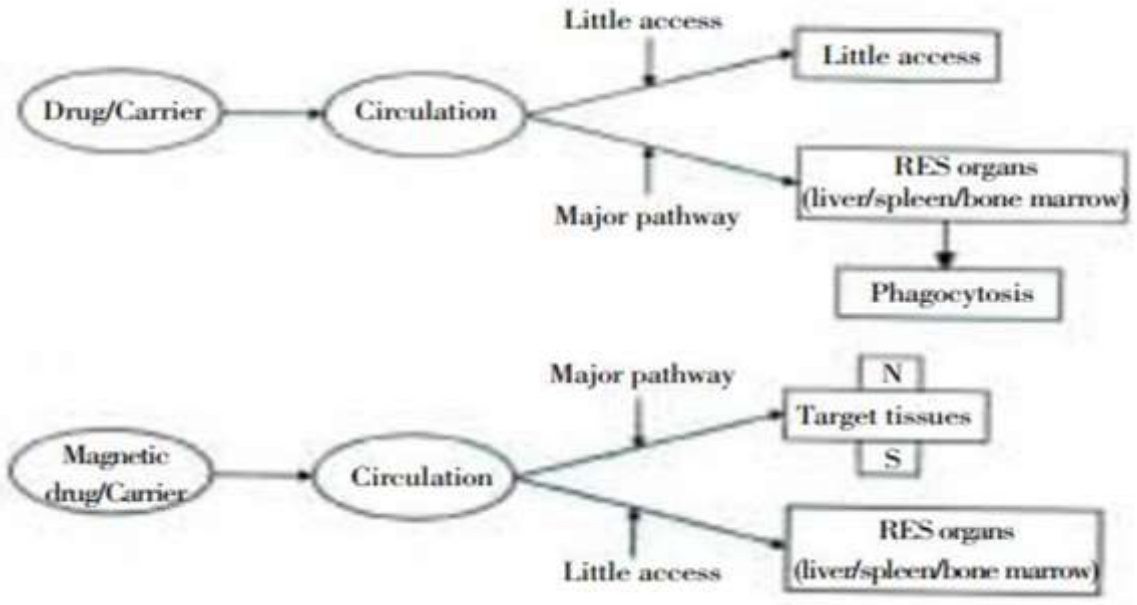


Figure 1: Principle of Magnetic Drug Targeting (Amit Chandna et al., 2013)

using magnetic nanoparticles, it allows the targeted drug delivery and releases them to local

targeted site. The capability of concentration of MNPs will be dependent on the blood flow rate and the strength of magnetic field (Widder KJ et al., 1983). There is higher chance of accumulation of efficient drug in blood vessels having smaller area and low rate of blood flow than in central vessels such as aorta which have high rate of blood flow. The drug, which is required to be given to the diseased area, and magnetically active nano-components are developed in pharmacologically stable environment. A huge range of polymeric carriers including poly (lactic acid) and poly (glycolic acid) has been formulated for the controlled release of drug (Brannon and Blanchette 2004). The main focused areas are polymer's biodegradability and the mutual compatibility of the polymer and drug.

This setup is designed to control and store real time data of movement of the drug. For this setup, external camera is attached to get real time data and Arduino Uno is connected to electromagnet and power supply to control the electromagnetic field of electromagnet.

The main concept of using magnetic levitation for drug delivery purposes are when delivering the drug to a specific point via magnetic actuation, magnetic material encoated in drug can rupture the artery by attracting towards externally applied strong magnetic field. By taking magnetically levitated drug to the targeted area, it will minimize the risk of rupturing of the artery.

Another problem that will be minimized is the dispersion of drug in the blood stream because the drug-coated core will be under the influence of strongly applied electromagnetic field and drug can be released by the alteration of electromagnetic fields.

CHAPTER 2

LITERATURE REVIEW

Many techniques have been developed by which natural and synthetic particles are incorporated with magnetic particles. The polymeric decomposed-able nanoparticles grasped the significant attention as they are capable of evading the reticuloendothelial system (RES) though remain engaged, which delays the circulation time in the blood and reduce the ability to effect the target site by releasing the drug and decrease side effects (Lubbe AS et al., 2001). The injection of drug/carrier complex was done by subjecting them intravenously into the body. Permanent rare earth magnets were used for creating the high gradient electric field, which was provided externally, which was used to concentrate the drug at the specific tumorigenic sites (Figure 1). Therapeutic agent will be released once the magnetic carrier reached at the specific diseased/tumorigenic site from the magnetic carrier via enzymatically induced activity or by changing the physiological conditions such as pH, osmolality or temperature, such changes with lead to the improved uptake of drugs by diseased area of the targeted site (Alexiou et al., 2000). These principles have also been used in vivo for the delivery of gene for therapeutic purposes to the specific targeted area (Mah et al., 2002)

2.1 History of Magnetic Drug Delivery System

In early 1940s, magnetic carriers were first introduced for the treatment of waste water as a novel approach (Arias JL et al., 2001). The key focus on the drug targeting is dependent on the magnetically targeted drug carriers which were prepared for delivery of drug by using Fe_2O_3 or Fe_3O_4 as core of the magnetic particles which were coated then with biocompatible polymer (Hu FX et al., 2006). In 1963, the evolution of drug delivery through magnetic carriers was explained by Meyers et al., they managed to collect small iron particles which were injected into the dog's leg intravenously by using a large horseshoe magnet whose magnetic field was externally applied (Meyers et al., 1963). In 1974, small magnets were engineered at the end of the catheters. The main idea of having magnetic ends of catheters was to use them supposedly for the selective embolism of arterio-venous irregularities (Hilal*et al.,* 1974*).* In late 1970s, Albumin microspheres

were designed by coating Adriamycin, a chemotherapeutic agent, on magnetically susceptible magnetite (Widder*et al* 1979). The therapeutic benefits of magnetic carriers, containing drug adsorbed on their surface, was directed to the capillaries for deposition of drug, and was first manifested in animals by Widder and his associates. This research was not carried forward because of the limitation of magnetically targeted drug delivery to the surface tumors only. In the era of 1980s magnetic microspheres and microcapsules were industrialized for the delivery of different drugs (Kato et al., 1984, Gupta et al., 1989). In 1994, for targeted radiotherapy, by combining magnetite and b⁻emitter ^{90}Y, biodegradable microspheres of poly(lactic acid) were manufactured (Häfeli*et al*., 1994) which were effectively applied to subcutaneous tumors (Häfeli et al., 1995).

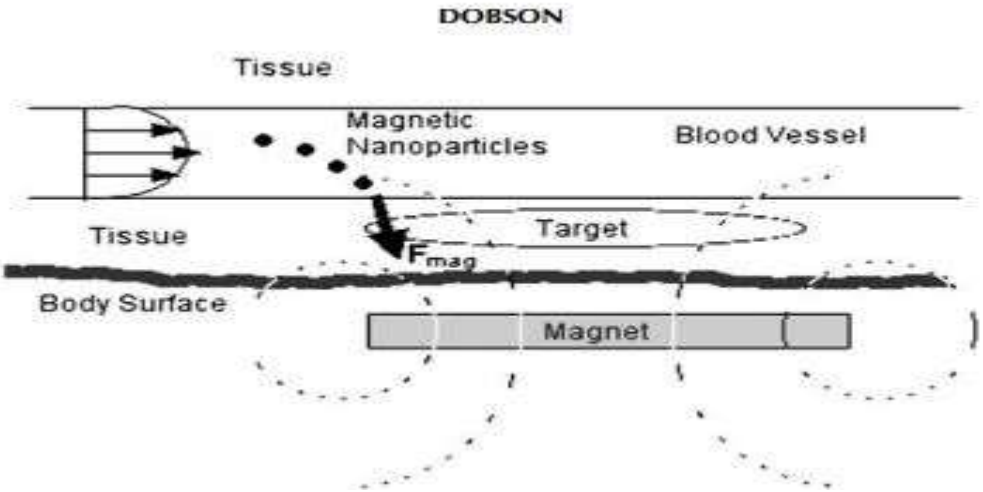


Figure 2: Schematic representation of a magnetic nanoparticle-based drug delivery system. Figure redrawn after Pankhurst et al. [2003].

These methodologies which were initially used were microsized. In animal models, magnetic nanoparticles were first introduced by Lübbe*et al* in 1996.The first clinical tried was carried out in 1996 where unsuccessfully pretreated cancer was injected with magnetic nanoparticles coated with epirubicin (Lübbe*et al* 1996). More than 50% of the nanoparticles accumulated in liver after the first phase of clinical testing.

2.2 Medical Applications of Functionalized Magnetic Nanoparticles

There is huge range of biomedical applications where magnetic nanoparticles can be used for therapeutic purposes as shown in figure 6 (Pankhurst et al., 2003) that ranges from usage of magnetic nanoparticles as contrast agent for magnetic resonance imaging (MRI) to the destruction

of tumor cells through hyperthermia treatment. The required therapeutic effect will be obtained by précised and controlled interactions of magnetic nanoparticles and living tissues.

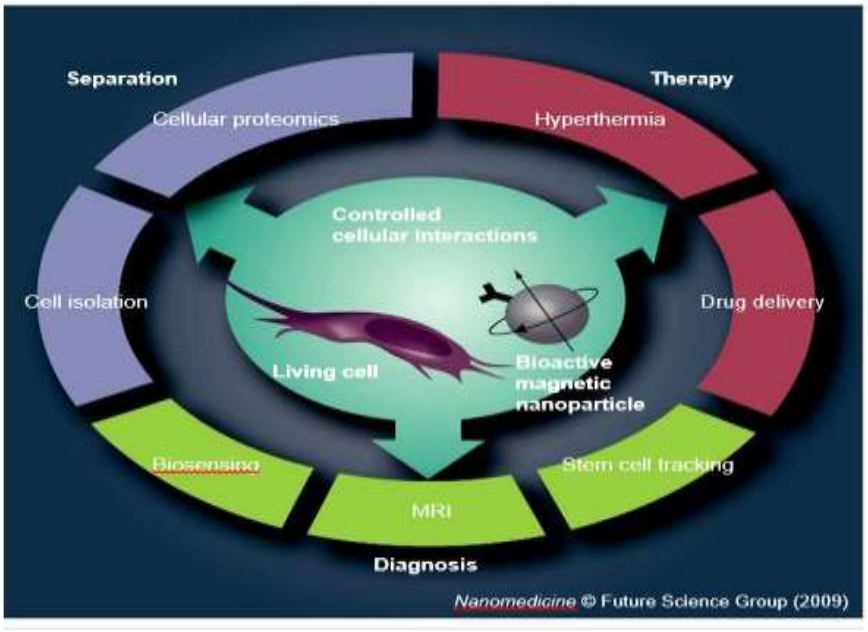


Figure 3: Biomedical applications of magnetic nanoparticles.

2.3 Site Specific Drug Delivery System

2.3.1 Magnetic delivery of cancer treatment:

Chemotherapy balanced between efficiency and toxicity of the cancerous cells and healthy tissues. There are number of strategies that have been used to counter the toxicity of healthy tissues. In chemotherapy, active agent is directed to the tumor, through regional artery, in concentrated form while restricting its concentration systemically. This intravenous administration of chemotherapeutic agent tries to reduce the side effects. Magnetic nanoparticles carrying drugs may attain wide dispersal throughout the cancerous tissue when magnetic force is applied externally on the magnetic carriers. Though this regional therapy via drug delivery will not be effective in the treatment of distant tumor regions (Rudge et al., 2001).

2.3.2 Magnetic delivery to the lungs:

Delivery of drugs to lungs via magnetically targeted drug delivery system has already been studied in literature (Gonda 2000). Various immunotoxins have been used for the localized treatment of malignant tumors of lungs. By designing a targeted drug delivery system, the drug particles would be directed towards the localized area of tumorous tissue of lungs by using the required magnetic field. To cross the mucociliary barrier, magnetic field would have to be strong. To determine the

pattern of aerosol particles, aerosol particles motion model in a magnetic field have also been developed on numerical basis (Ally et al., 2005). This model showed promising results in the delivery of chemotherapeutic agent which were magnetically aerosolized.

2.3.3 Topical magnetic delivery:

Magnetic nanoparticles have been used in delivering the drugs to the topical region including stratum corneum, epidermis and dermis. The increased concentration of drug was observed in stratum corneum, dermis and epidermis when given through magnetic nanoparticles. Since 1990s, Photodynamic therapy has been used for the treatment of cancer, this therapy is dependent on preservation of photosensitizers in tumor cells following the treatment of tumor cells with visible light. Keen interest has been shown in using biocompatible magnetic fluid for biomedical applications (Lacava et al., 2002). Improvement in an active nano-emulsion played an important role in biomedical applications and they have been used in controlled targeted drug release with efficient targeting. By entrapping photosensitized drugs in magnetic nanocarriers leads to a magnetically modulated nano-drug delivery system that will act synergically by photodynamic therapy (Primo et al., 2007).

2.3.4 Magnetic Fluid Hyperthermia:

One of the important approaches in treatment of cancer is hyperthermia. There are number of factors that induce hyperthermia includes, hot water usage, inductive and capacitive heating etc (Ikeda et al., 1994). Submicron magnetic particles have been developed to induce intracellular hyperthermia (Wada et al., 2001). One of the unavoidable problem that has been faced in hyperthermia procedure is the issue of heating the targeted tissue up to a temperature required for a treatment without damaging the healthy tissues surrounding the tumors. This hindrance has been overcome by the utilizing magnetic nanoparticles instead of providing heat treatment (Jordan et al., 1993). During magnetic fluid hyperthermia, super-paramagnetic particles are introduced into the tumor region, where magnetic nanoparticles are exposed to alternating magnetic field and heat is generated by hysteresis loss. **(**Figure 7**).** Different physical mechanisms are involved in generation of heat by transforming energy of alternating magnetic field. This transformation of energy into heat is strongly dependent on frequency of the externally applied magnetic field and the nature of particle in which surface modification and magnetism are included (Ma et al., 2004,

Hergta 2004). The application of Fe_3O_4 has gained attention in biomedical and biotechnology field due to its strong magnetic properties and low toxicity.

2.3.5 Magnetic drug delivery to the musculoskeletal system:

In future, one of the important application of magnetic targeted drug delivery system will be focused on musculoskeletal system in human and animals (Neuberger T, 2003). During disease or infection, local inflammatory response usually occur in musculoskeletal system and generally treated with non-steroidal anti-inflammatory drugs (NSAIDs) (Zavisova et al., 2007). These drugs usually cause systemic side effects including gastric ulcers or bleeding and maintaining drug concentration systemically may also be a problem. Magnetic nanoparticles can be used to serve the purpose of delivering drug in appropriate concentration to the inflamed tissues that will result in the supply of appropriate dosage and undesirable side effects will be reduced. Super-paramagnetic iron oxide (SPIO) nanoparticles are used in combination with magnets to serve the purpose of drug delivery carrier, this combination would allow the switching of magnetic field from off to on thus magnetic field and strength would be controlled that would manage the time, dosage and elimination of the drug.

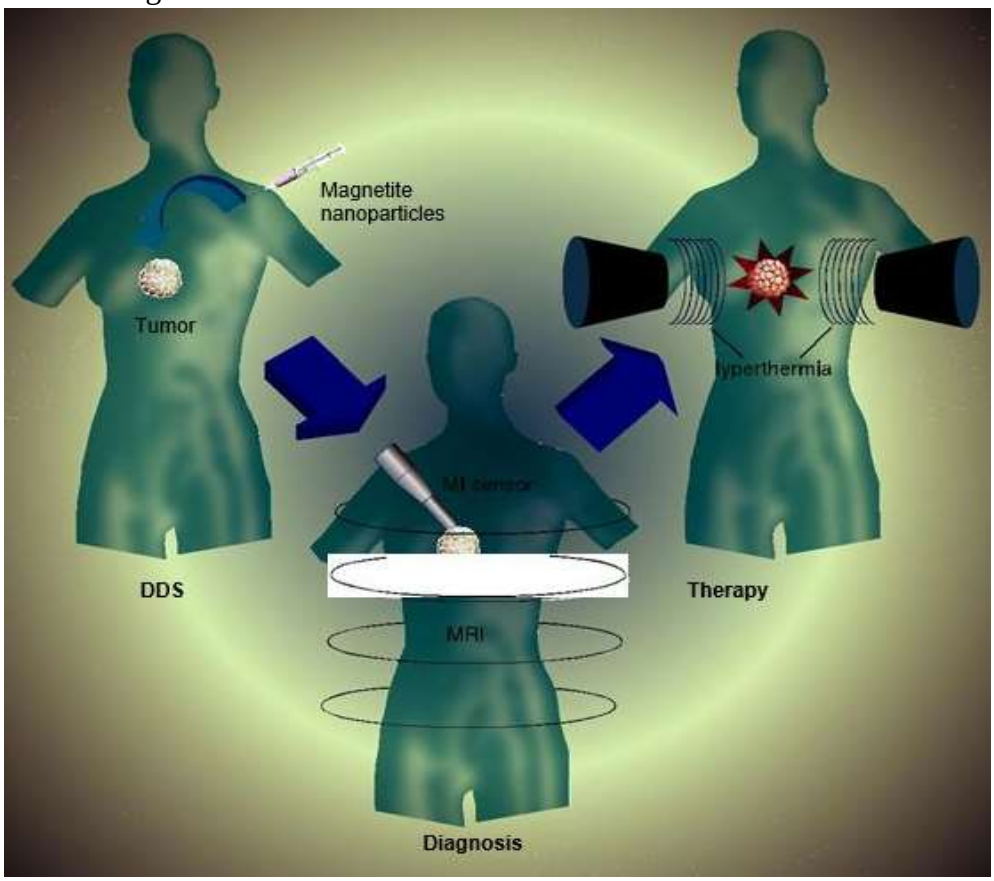


Figure 4. Therapeutic strategy using magnetic particles. Functionalized magnetic nanoparticles accumulate in the tumor tissues via the DDS. Magnetic nanoparticles can be used as a tool for cancer diagnosis by MRI. Hyperthermia can then be induced by alternating magnetic field exposure, thus, magnetic nanoparticles can be used for cancer therapy at the same time

as diagnosis. DDS: Drug-delivery system; MI: Magneto-impedance. Adapted from (Ito et al., 2005).

2.4 Limitations

There are number of disadvantages that are associated with magnetic drug delivery system.

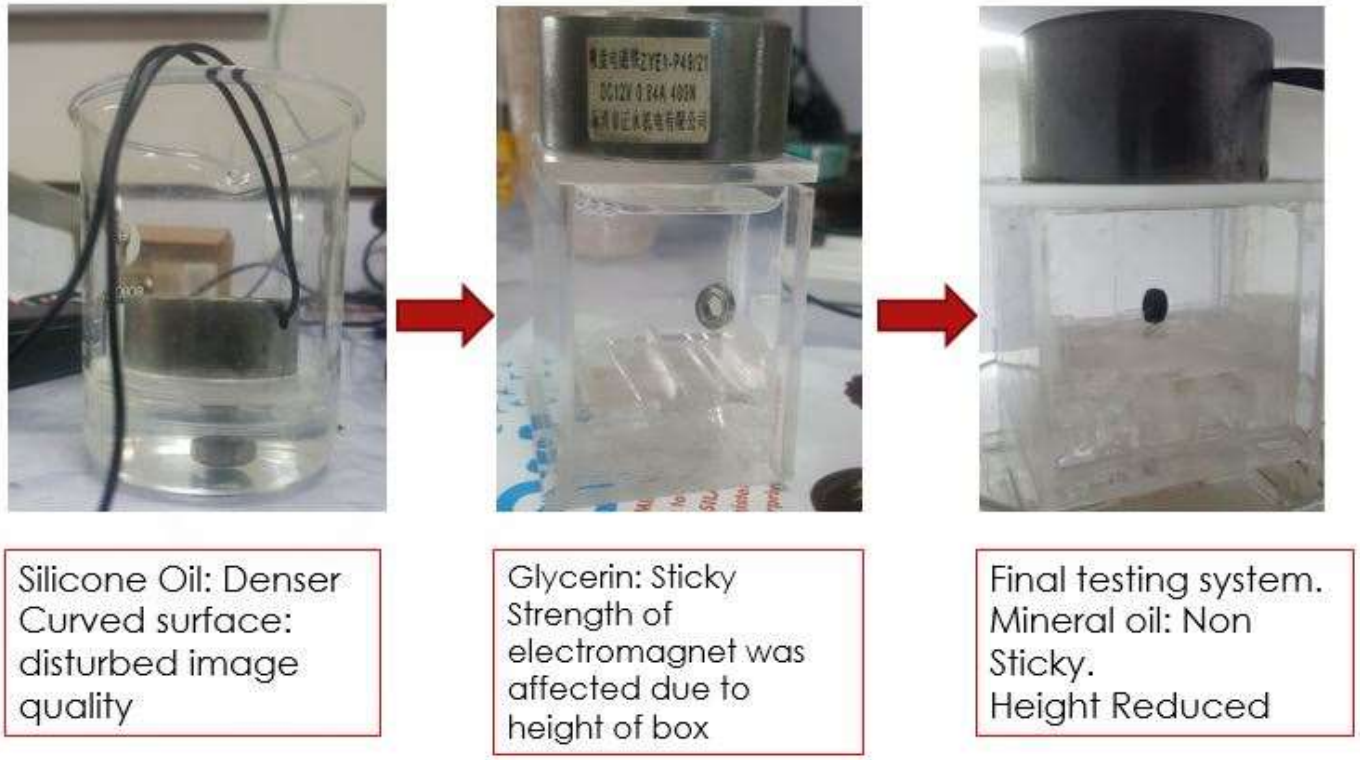
- The problem of toxicity may arise due to the smaller size of nanoparticles, nanoparticles gets accumulated at the site of targeted area that leads to their irrepressible activity.
- These nanoparticles can only be inserted in the body only after being characterized with specific cell receptor when using as regenerative medicine.
- Therapy effectiveness decreases with the continuous use of magnetic nanoparticles for delivery due to degradation of drug transporters.
- The accumulation of nanoparticles produces toxicity that disturbs the metabolic system thus reduces the effectiveness.
- Immunological responses arise due to toxicity and accumulation of nanoparticles, may lead to dysfunctionality of organs and detrimental consequences. (H. Markides et al., 2012).

CHAPTER 3

METHODOLOGY

3.1 Proof of Concept

In given images, following tests were performed in the lab to prove the concept of movement of material in a liquid medium under the influence of electromagnetic field.



In figure 5: a beaker, silicone oil, electromagnet and a nut was used to test the attractive forces of electromagnet. In this setup, curved beaker was used but when acquiring the data, curved surface distorted the images hence flawed data. Silicone oil was denser leading to difficulty in the movement of nut.

In Figure 6: Acrylic box was used to that clear images were taken without distortion and glycerin was used which was less dense as compared to silicone oil, but due to stickiness of glycerin to acrylic sheets, movement of blob was hindered as it used to stick to the acrylic base.

In Figure 7: The area for the movement of blob was decreased by adding piles of acrylic sheets. This helped in catering the electromagnetic range of electromagnet. Glycerin is replaced by mineral oil as it was non-sticky towards acrylic box and the density was closer to blood.

3.2 Selection of Materials

i. Selection of blob
ii. Selection of electromagnet
iii. Selection of the medium

3.2.1: Selection of Blob:

Blob is the driven circular material made up of ferritic stainless steel, which was responsive towards electromagnetic waves. As seen in figure 8(i) and 8(ii), multiple sizes, dimensions and materials were tested and their response was recorded towards electromagnet. Ferritic stainless steel response was strong towards electromagnet. Right most material and shape is selected.

Figure 8(i), 8(ii): Multiple sizes and dimensions of different materials.

3.2.2: Selection of Electromagnet:

A round solenoid electromagnet is used for providing external magnetic field named- zye1-p49/21. Its diameter is of 1.9 inches and height 0.8 inches. The mechanism is voltage driven, the optimal operating requirement was found to be 12 Volts and 0.84 Amperes for the electromagnetic field generation. This electromagnet provides the driving force to the blob.

3.2.3: Selection of Medium:

Mineral oil is used as a medium for the movement of blob. This is done to mimic the movement of blob in blood, as the ultimate goal is to deliver the drug in liquid medium. Mineral oil is used as its density is closer to blood. Density of mineral oil is 0.85g/mL and blood 1.08g/mL.

Acrylic box is used for the storage of medium and to control the movement of blob in the mineral oil. Acrylic box was fabricated using CO2 laser, Laser cutting equipment Jinan Jinqiang Laser CNC JQ9060-was used. Laser cutting was carried out at a Power 40W and Speed 2 mms-1.

Figure 9: Main setup used for navigation, electromagnet provides driving force to drive the blob in mineral oil.

3.3 Limitations

There are certain limitations in this setup which are as follows:

1. Electromagnet-heats up:

   Heating of electromagnet by continuous flow of current due to which heat dissipation occurs.

2. Image Threshold:

   Difference of image threshold occurs due to movement of material under the influence externally provided magnetic field.

3. Range of electromagnet:

   Due to presence of medium, electromagnetic range was compromised to 2cm.

4. Camera adjustment:

   Camera was needed to be adjusted at a specific height to get a leveled image so that accurate data acquisition can be done.

3.4 Main Setup

1. **Arduino Uno:**

   Electromagnetic field of electromagnet is controlled by PWM on an Arduino™ UNO® Board, with the core Atmel™ Atmega328P microcontroller.

2. **External Fan:**

   A basic direct current operated external fan is used to control the heating of electromagnet.

3. **Lamp:**

   A lamp is used to fix the threshold of image and data acquisition.

4. **Range of electromagnet:**

   To cater this problem, acrylic box which was of 4cm, blob movement area is restricted to 2cm by piling up acrylic layers in acrylic box.

5. **Camera Adjustment:**

   To get accurate movement of blob and clear data acquisition, camera and medium-filled acrylic box are adjusted at same level.

6. **MATLAB Setup:**

   MATLB 2013b setup is used for data acquisition and real-time data processing acquired from images.

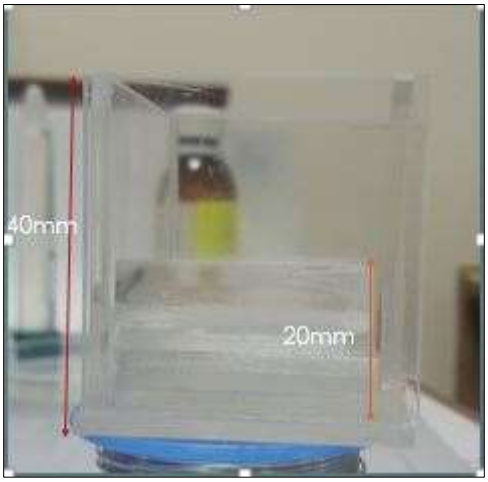
Figure 10: Final assembled setup of magnetically controlled drug delivery setup.

Main setup as shown in figure 10 consists of an external camera, Arduino™ UNO® Board, with the core Atmel™ Atmega328P microcontroller, external fan, a lamp, Laptop core i5 containing MATLAB and Arduino software and a box containing acrylic box-medium-electromagnet setup.

3.5 Parameters

Parameters of materials used for system setup are given in 3.5.1:

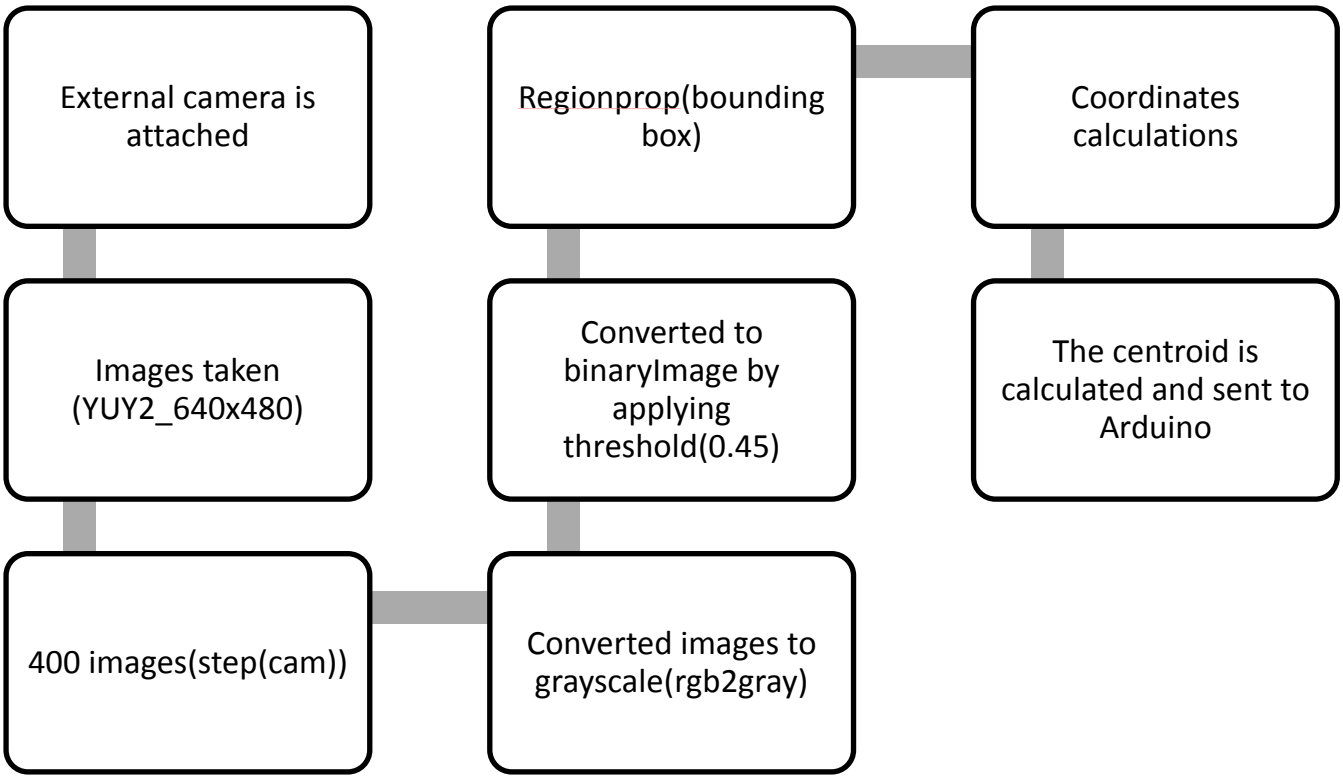
Parameters	***Values***
Original Box Height	50mm
Original Box Width	40mm
Height for levitation	22mm
Blob Height	5mm
Blob Weight	0.174g
Material	Ferritic Stainless Steel
Camera	10MP, Manual focus



3.6 System Setup Flow Diagram:

A flow diagram in figure 11, is mentioned, that explains the whole procedure of controlled and real time position tracking of the blob in liquid medium. Real time data acquisition is performed in MATLAB on the images captured by external camera, according to the position specific Pulse-width modulation (PWM) is applied to control the electromagnetic strength applied on to levitated blob and then Proportional-integrational-derivative is applied to control the movement of blob and to levitate it at set-point.

3.7 Image Acquisition & Processing – MATLAB



Data acquisition, image processing and data extraction is performed in MATLAB. External camera, attached to laptop via usb cable, is placed in front of the acrylic box containing mineral oil medium and blob. Images are taken when MATLB algorithm is run, these images are taken at the resolution of YUY2_640x480. Number of images taken are 400 by using the command of (step(cam)) which was fastest to capture real time images, images taken are then converted to grayscale by using command grayscale(rgb2gray), these images are then converted to binary images by applying a fixed threshold of 0.45. To extract the properties of region of moving blob

under the influence of magnetic field, Region prop (bounding box) command is used, this command helps in calculating the coordinates of specific region from an image. Form extracted coordinates, centroid value of the bounding box region is calculated which is then sent to Arduino to control electromagnetic field according to the set point of the blob where it is supposed to be levitated. MATLAB code is given below in figure 12 (i, ii, iii).

Figure 12 (i, ii, iii): MATLAB code for Image acquisition and data extraction

```
clear all
close all
arduino=serial('COM7','BaudRate',115200);
  fopen(arduino);

cam = imaq.VideoDevice('winvideo', 2, 'YUY2_640x480');
prevx=0;
sp=128;
Kp=1.0828;
Kd=0.0028;
Ki=0.01;
lasterror=0;
error=0;
sumerror=0;
a=ones(2,400);
for i=1:400

pics{i} = step(cam);
g=rgb2gray(pics{i});

%THRESHOLD
thresholdValue=0.46;
binaryImage=g<thresholdValue;
binaryImage = bwareaopen(binaryImage, 30);

[Ilabel, num]=bwlabel(binaryImage, 8);
```

```
% // Calculate bottom right corner
bottomRightCoords = [topLeftCoords(:,1) + bboxCoords(:,3) ...
    topLeftCoords(:,2) + bboxCoords(:,4)];

% // Calculating the minimum and maximum X and Y values
finalCoords = [topLeftCoords; topRightCoords; bottomLeftCoords; bottomRightCoords];
minX = min(finalCoords(:,1));
maxX = max(finalCoords(:,1));
minY = min(finalCoords(:,2));
maxY = max(finalCoords(:,2));
 minY=minY+20

if isempty(minY)==0
  error(i) = minY-sp;
%   x = Kp*error(i)  + Kd*(lasterror - error(i)) + Ki*sumerror;
  x = Kp*error(i)+ 0.01*exp(0.1*error(i));
```

Figure 12 (ii)

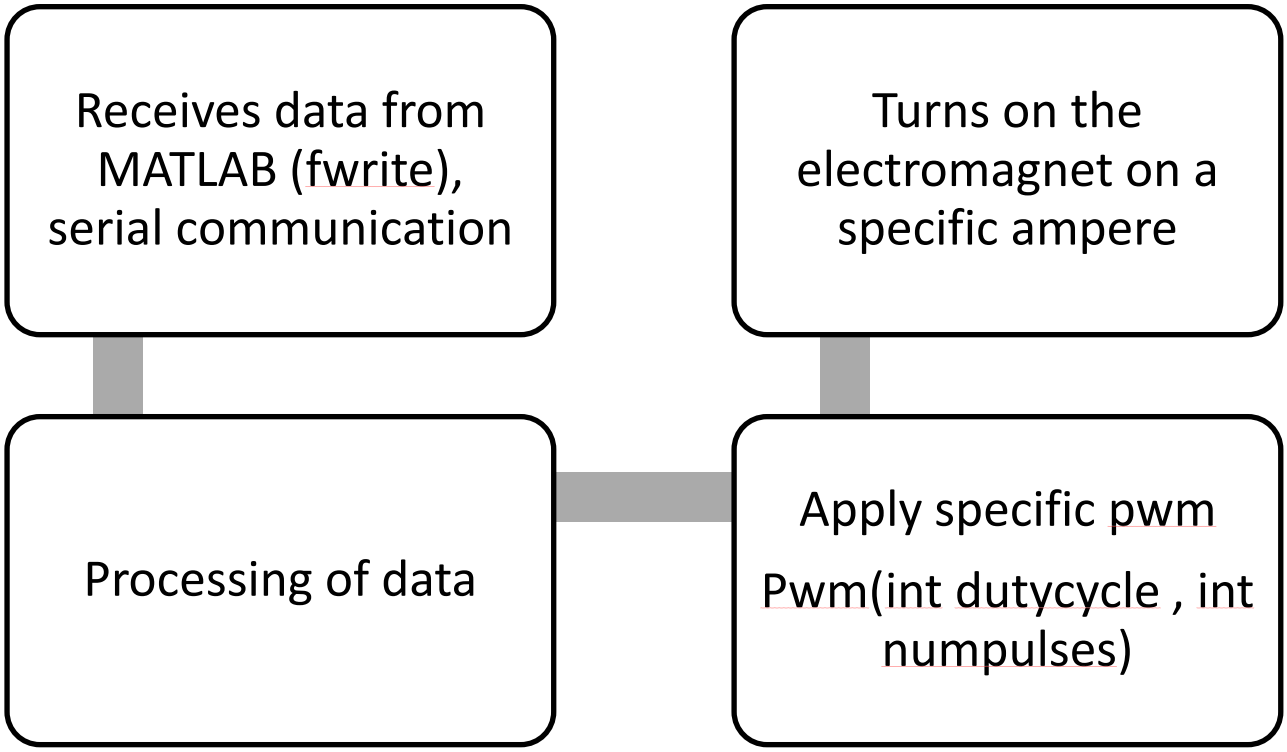
```
if isempty(minY)==0
  error = minY-sp;
  x = Kp*error + Kd*(lasterror - error) + Ki*sumerror;

  if (x >100)
  x = 100;
  end
  if (x <0)
  x = 0;
  end
  a(1,i)=minY;
 a(2,i)=error;
  lasterror = error;
  sumerror = sumerror + error;
 fwrite(arduino,x ,'char')

pause(0.0471)

else
    break;
end
end
fclose(arduino)
delete (arduino)
imaqreset
plot(a(1,:))
```

Figure 12 (iii)

3.8 Arduino UNO Micro-controller

Arduino Uno AT328 connected to electromagnet, this mechanism is ampere driven, the optimal operating requirement was found to be 0.84A. The flowchart of processes going on in Arduino are given below in figure 8:
Figure 8: Flow chart of processes going on in Arduino-UNO.

Arduino-UNO microcontroller is powered by usb cable from Laptop and PWM pin 11 is connected



to electromagnet that provides power to electromagnet to turns on and off. Arduino receives the extracted data from MATLAB via serial communication by using command of fwrite, at the baud rate of 115200. The received data is processed in Arduino, required PWM is applied according to the data obtained from MATLAB image acquisition and data processing, For PWM, pwm (int dutycyle, int numpulses) command is used in Arduino to apply required power to electromagnet connected to it. The applied PWM turns on the electromagnet by providing required ampere to move blob at certain height.

Algorithm used in Arduino for controlling pwm applied to electromagnet is given below in figure 13.

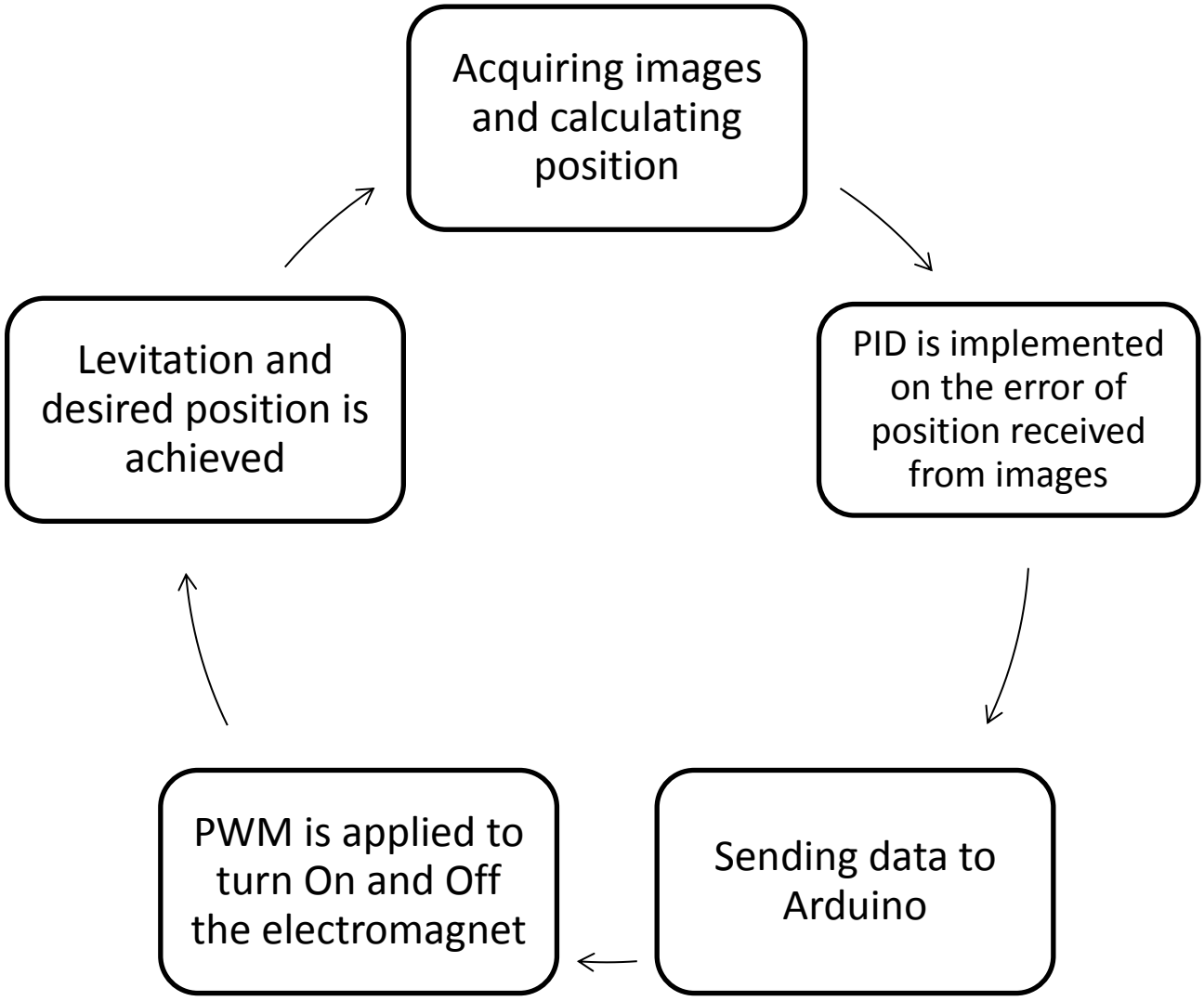
Figure 13: Algorithms used in Arduino.

```
int outPin = 11;
//int matlabData;
int temp;
int dutycycle;

void pwm(int dutycycle,int numpulse)
{

int i,a,b;

a=100*dutycycle; //on time for 5kHz
b=100*(100-dutycycle); //off time for 5kHz

for(i=1;i<=numpulse;i++)
{
 digitalWrite(outPin, LOW);
  delayMicroseconds(a);
  digitalWrite(outPin, HIGH);
  delayMicroseconds(b);
}
}
void setup()
{
 // matlabData = 0;
  temp = 0;
  Serial.begin(115200);
void setup()
{
 // matlabData = 0;
   temp = 0;
   Serial.begin(115200);

   pinMode(outPin, OUTPUT);
   digitalWrite(outPin, HIGH);
}
void loop()
{

   while(!Serial.available());
   temp = Serial.read();
   //Serial.println(temp);;
 pwm(temp, 3); //50 duty cycle for 100 pulses
```

3.9 Feedback control loop system

To control the position of the blob at the set point, a feedback system is required to take the blob at set-point. For that Proportional-integration-derivative – PID control system is used that will provide feedback from the images taken by camera and processed in MATLAB, that's when PID will be applied so that specific PWM is applied to keep blob levitated at required point. This is applied on real-time data acquisition, processing and on extracted centroid data to keep the blob levitated. In figure 14, flowsheet diagram is given based on control feedback loop system. In this flow sheet diagram, it is explained that images are taken and position is calculated in MATLAB, PID is implemented on the error of position received from images, data is sent to Arduino where PWM is applied to turn on and off the electromagnet to achieve the desired levitated position.

Figure 14: Flow diagram of control feedback loop system.



CHAPTER 4

RESULTS AND DISCUSSION

Testing of the system consists of two phases. In one phase PID – all 3 coefficients are applied to test the response of the system, and in testing phase II, combinations of P, I and D are applied to check the response of minimizing the error.

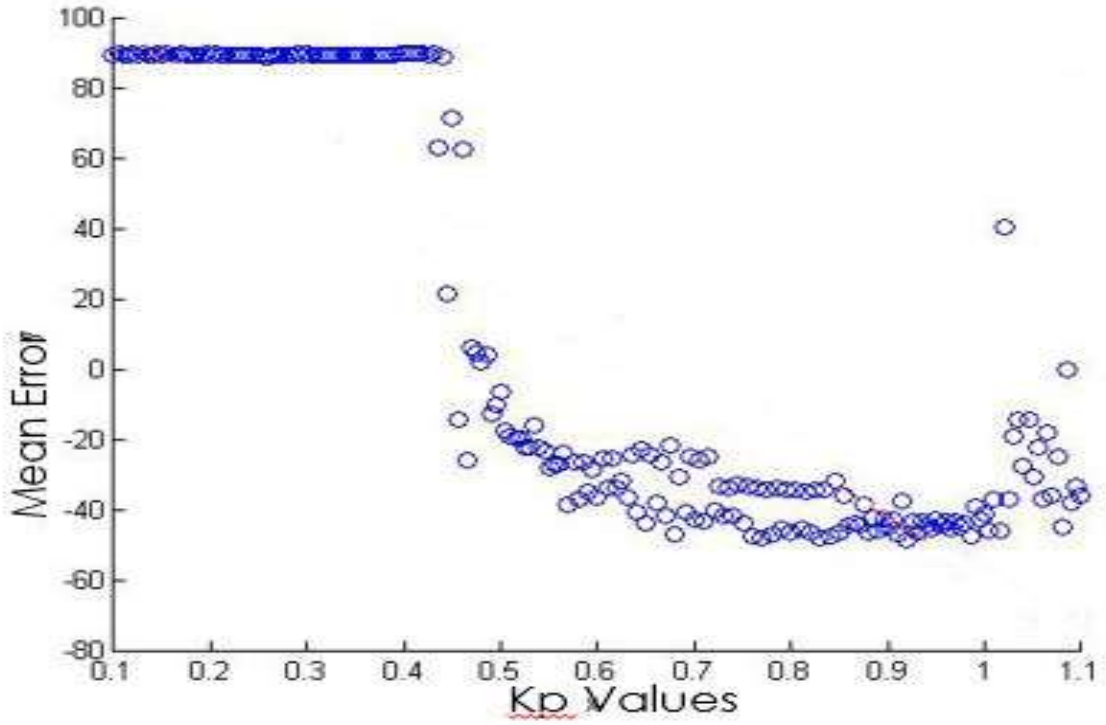
4.1 Testing Phase – I

In testing phase I, first thing to do was to specify the range of Proportional, Integration and Derivative independently to record the response towards minimizing the error in reaching to the set point or desired position.

In phase I, all three graphs of Kp, Kd and Ki are generated on MATLAB by running the algorithm for 100 images to test the range from 0.1 to 1.1 with the increment of 0.005.

4.1.1 Proportional Co-efficient Kp:

In figure 15, a graph is shown, generated on MATLAB by having serial communication with Arduino. This graph is calculated to identify the range of optimal value of proportional gain Kp



for minimizing the error of reaching to set point. On x-axis, Kp value range is given and on y-axis mean error is calculated. Total number of loops are 201 and in each loop, 100 images are taken to calculate the mean error at certain Kp value. In this graph, 1.085 is calculated as optimal value where calculated mean error is -0.29 which is closest to zero which depicts that at this value of Kp, error was very close to zero.

4.1.2 Integration Co-efficient Ki:

In figure 16, a graph is shown, generated on MATLAB by having serial communication with Arduino. This graph is calculated to identify the range of optimal value of integration gain Ki for minimizing the error of reaching to set point. On x-axis, Ki value range is given and on y-axis mean error is calculated. Total number of loops are 201 and in each loop, 100 images are taken to calculate the mean error at certain Ki value. In this graph, 0.96 is calculated as optimal value where calculated mean error is 0.01 which is closest to zero which depicts that at this value of Ki, error was very close to zero.

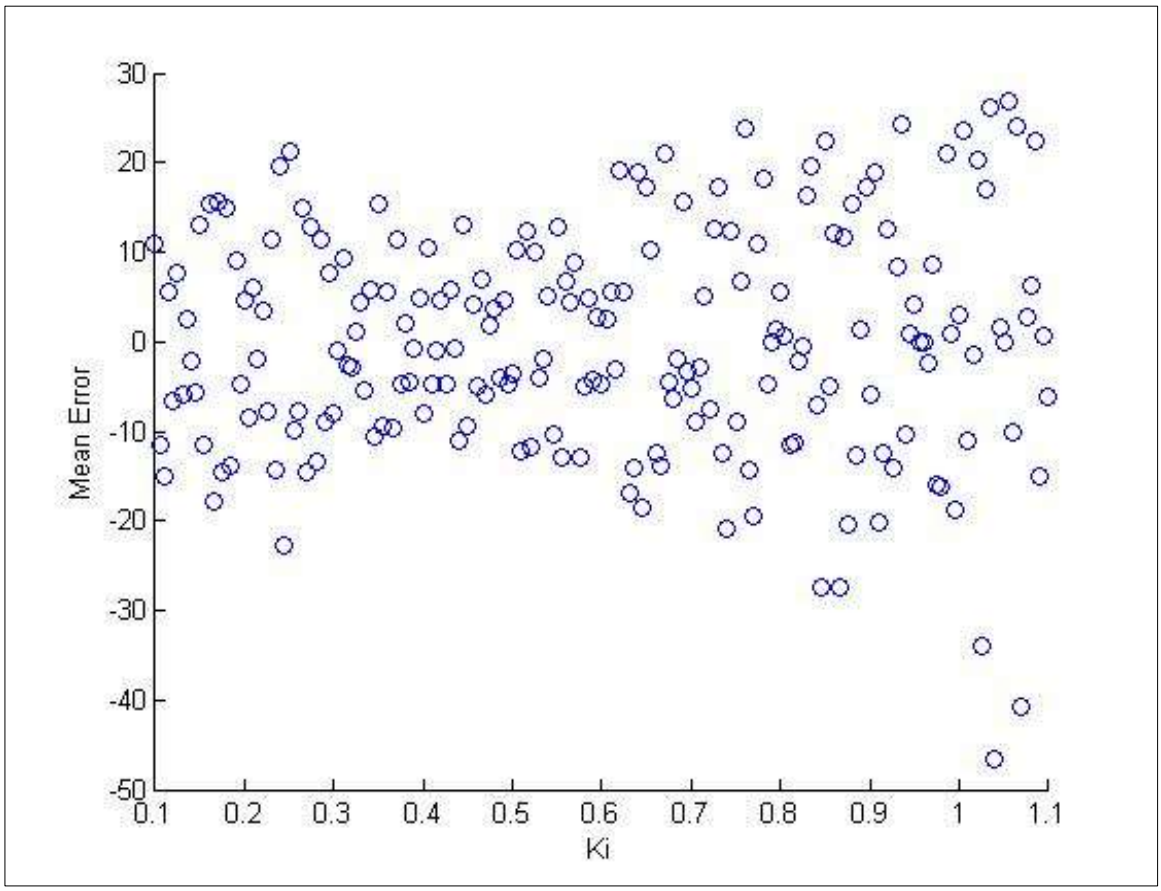


Figure 16: Optimal range of Ki value is calculated on x-axis.

4.1.3 Derivative Coefficient Kd:

In figure 17, a graph is shown, generated on MATLAB by having serial communication with Arduino. This graph is calculated to identify the range of optimal value of derivative gain Kd for minimizing the error of reaching to set point. On x-axis, Kd value range is given and on y-axis mean error is calculated. Total number of loops are 201 and in each loop, 100 images are taken to calculate the mean error at certain di value. In this graph, 0.43 is calculated as optimal value where calculated mean error is -6.225 which is closest to zero which depicts that at this value of Kd, error was very close to zero.

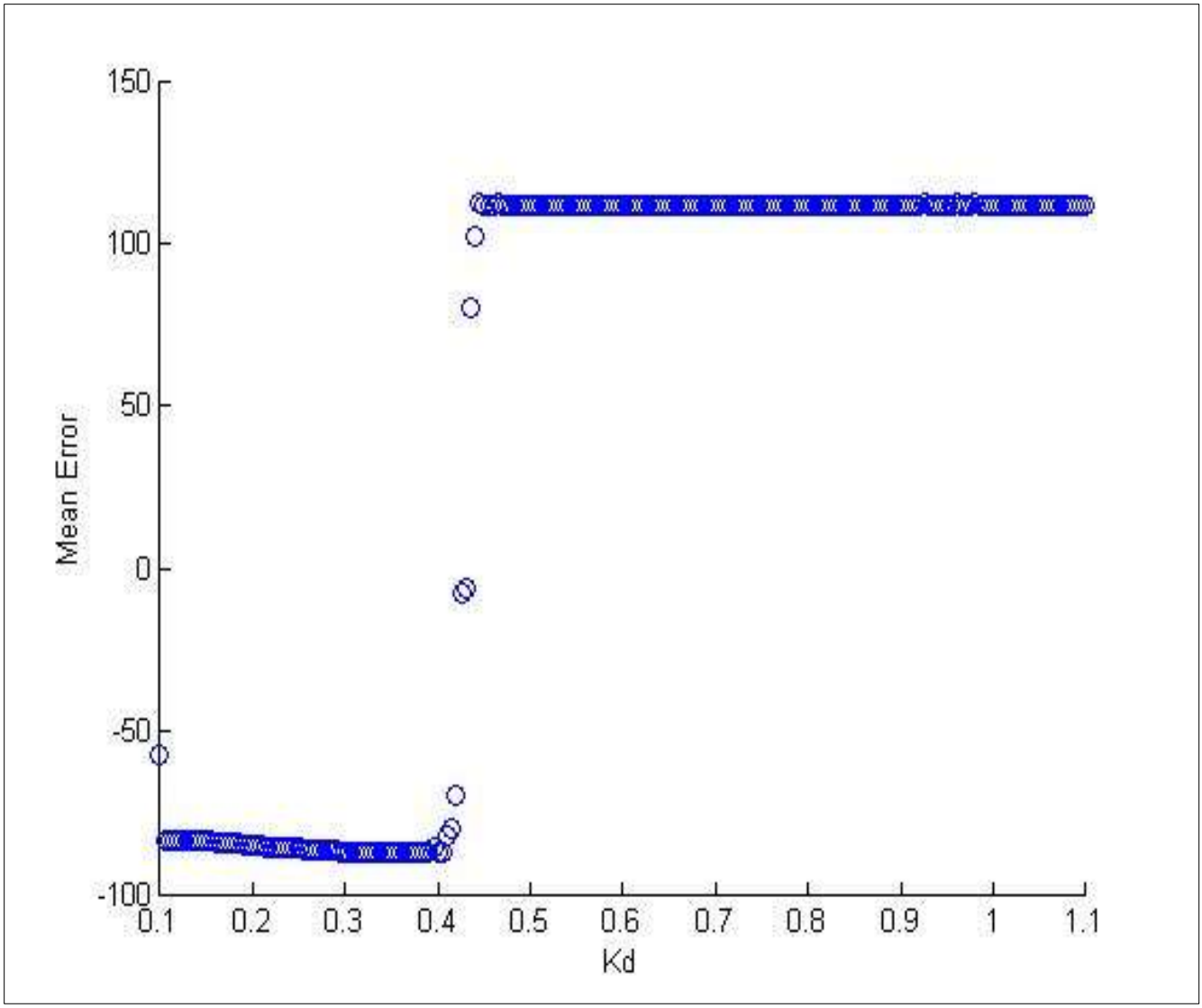


Figure 17: Optimal range of Kd value is calculated on x-axis.

4.2 Testing of the device

Testing of the device is done by applying Kp gain first, then Kp and Kd gain together and then all Kp, Kd and Ki gain are applied together to know its effect on the system and its ability to minimize the error and stability of the system by combined effect of Kp, Kd and Ki.

Test is performed on 400 images and executed in total time of 18.2secs.

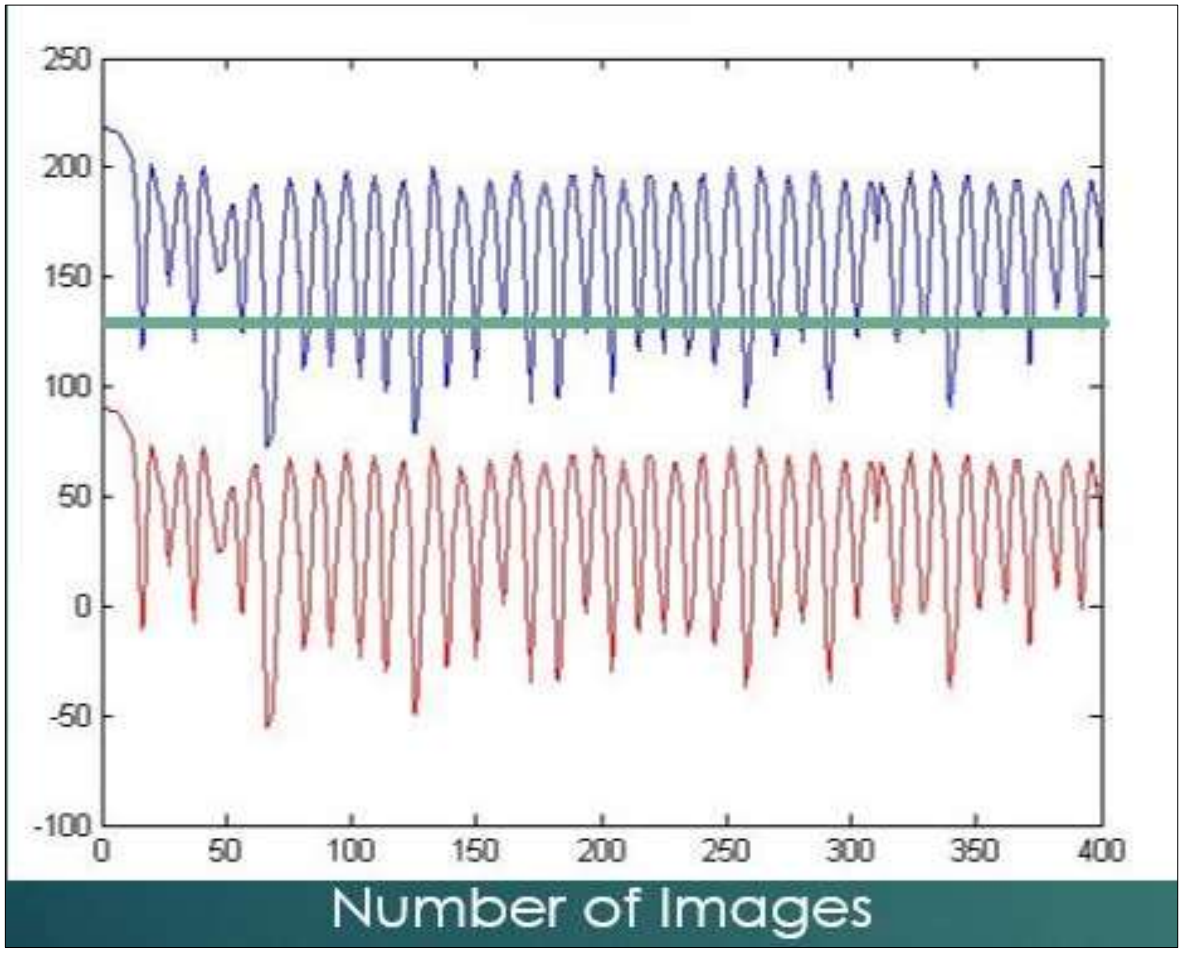


Figure 18: Kp value graph of minimizing the error.

In figure 18, a graph is presented, on x-axis number of images are given. 128 is the set point, shown by green line, blue waves exhibit the movement of the blob around the set point and red waves show the error in reaching to the set point. The blob remains levitated around the set point. Kp testing started from 1.005. This graph is calculated at Kp value of 1.0828 at which error in reaching to the set point 128 is closer to zero.

In figure 19, a graph is presented, on x-axis number of images are given. 128 is the set point, shown by green line, blue waves exhibit the movement of the blob around the set point and red waves show the error in reaching to the set point. The blob remains levitated around the set point. This graph calculated the combination of proportional gain Kp with addition of derivative gain Kd. Addition of Kd started from 0.0005 because addition of Kd damps the movement of blob in liquid medium in order to reach the set point. Addition of Kd to Kp though dampens the motion but when reaching to the targeted set point, power given to blob overshoots to keep it levitated but in overshooting, blob stayed in oscillated position around the set point thus not stabilized.

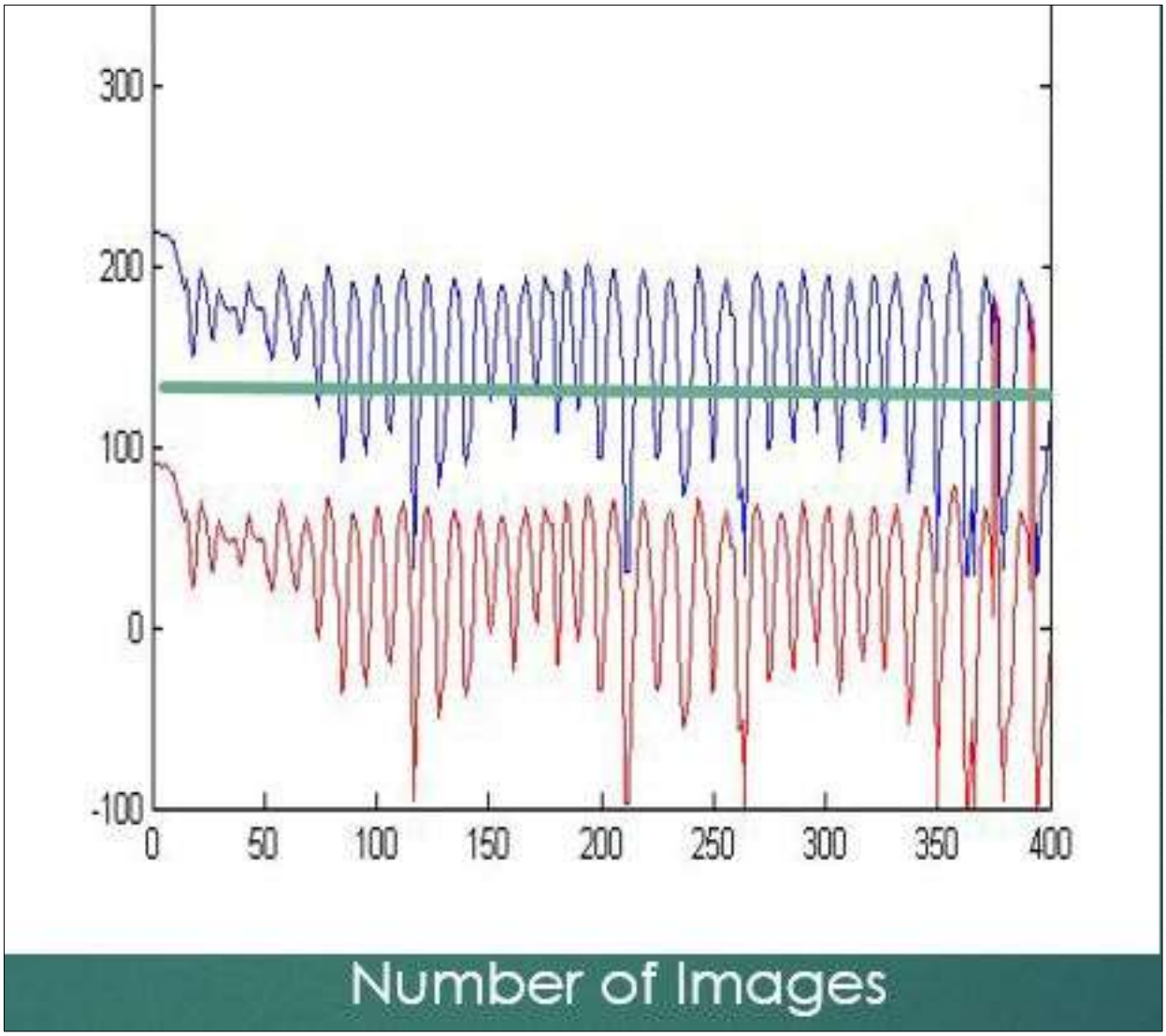


Figure 19: Kp and Kd values graph of minimizing the error.

In figure 20, a graph is presented, on x-axis number of images are given. 128 is the set point, shown by green line, blue waves exhibit the movement of the blob around the set point and red waves show the error in reaching to the set point. The blob remains levitated around the set point. This graph calculated the combination of proportional gain Kp with addition of derivative gain Kd and proportional gain Ki. Addition of Ki started from 0.0001 because addition of Ki calculates all the previous errors the movement of blob in liquid medium in reaching the set point. This graph is calculated at the Ki value of 0.0004. Addition of Ki to Kp and Kd though speeds up the motion when reaching to the targeted set point, blob stays in levitated condition around the set point thus stability is increased by the addition of Ki.

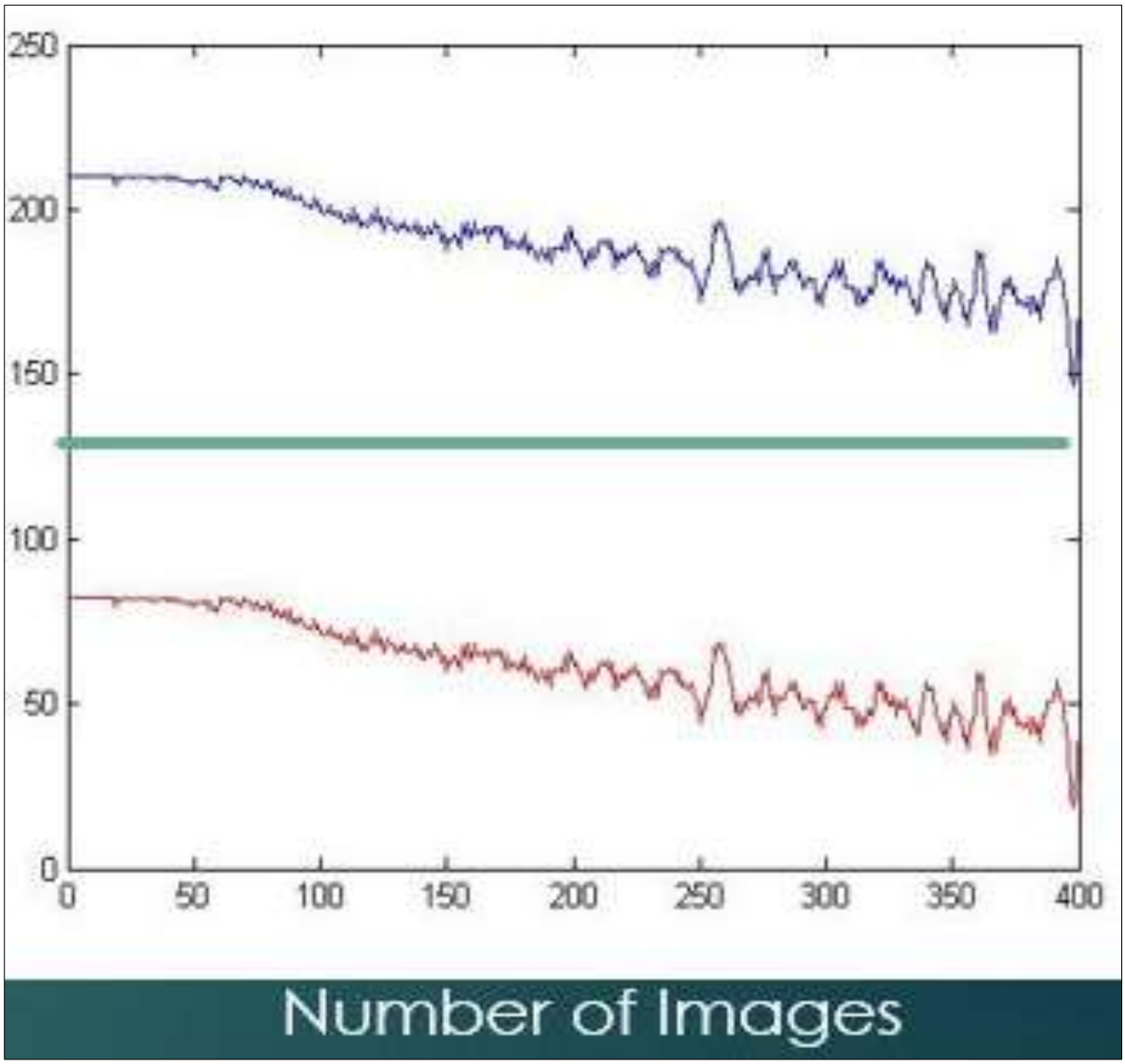


Figure 20: Kp , Kd and Ki values graph of minimizing the error.

4.3 Testing Phase – II

In testing phase II, first thing to do was to specify the range of Proportional gain independently to record the response towards minimizing the error in reaching to the set point or desired position.

4.3.1 Range Testing:

In phase II, A graph of Kp is generated on MATLAB by running the algorithm for 100 images to test the range from 1.1 to 1.3 with the increment of 0.005. This graph was generated to access the value of Kp where the error is minimized to zero and blob levitate at the targeted set point. In figure 21, at x-axis, minY, the position is mentioned and on y-axis Kp value is given. Blob reached the targeted set point 128 at Kp value of 1.15. Hence zero error achieved at Kp value of 1.15. As Kp value is increased, oscillations are increased, blob oscillates around the set point in levitated state.

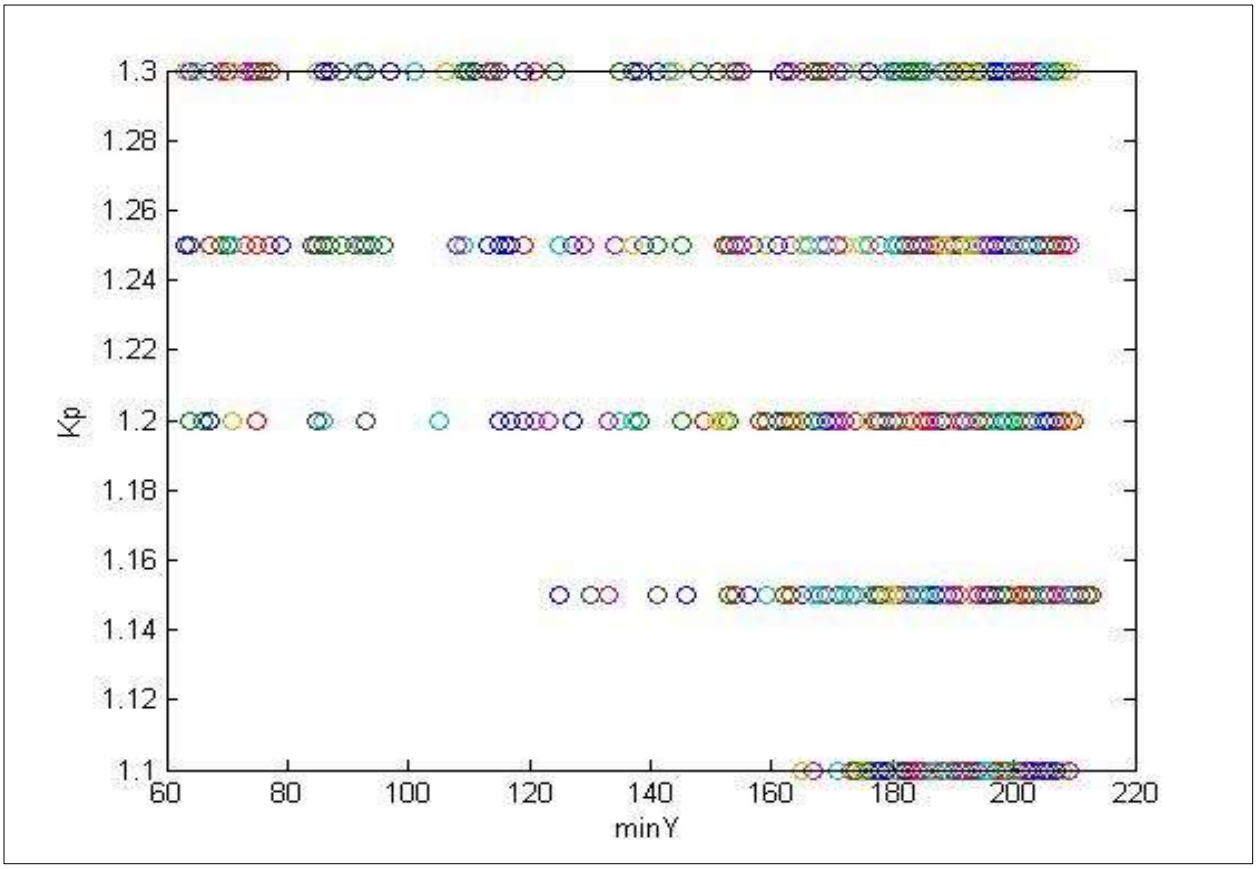


Figure 21: Range testing of Kp value to achieve the targeted set point and zero error.

4.3.2 Exponential Power:

Electromagnetic waves were too strong and pulling blob towards the electromagnet when reaching the targeted point because of increased Kp gain, to decrease the power of Kp gain, we added an exponential function. It supplies the power when the error is large, power gets zero when error is low.

In figure 22, on x-axis, error is given and on y-axis PWM is mentioned. Exponential value is added to add up more power to lift the blob from base. This graph shows that exponential value decreased when error reaches towards zero and exponential value increased with increase in error. Same is the case with PWM, with increased error, PWM is high to lift blob from base and PWM reduces to zero when blob reaches the targeted set point where error is zero.

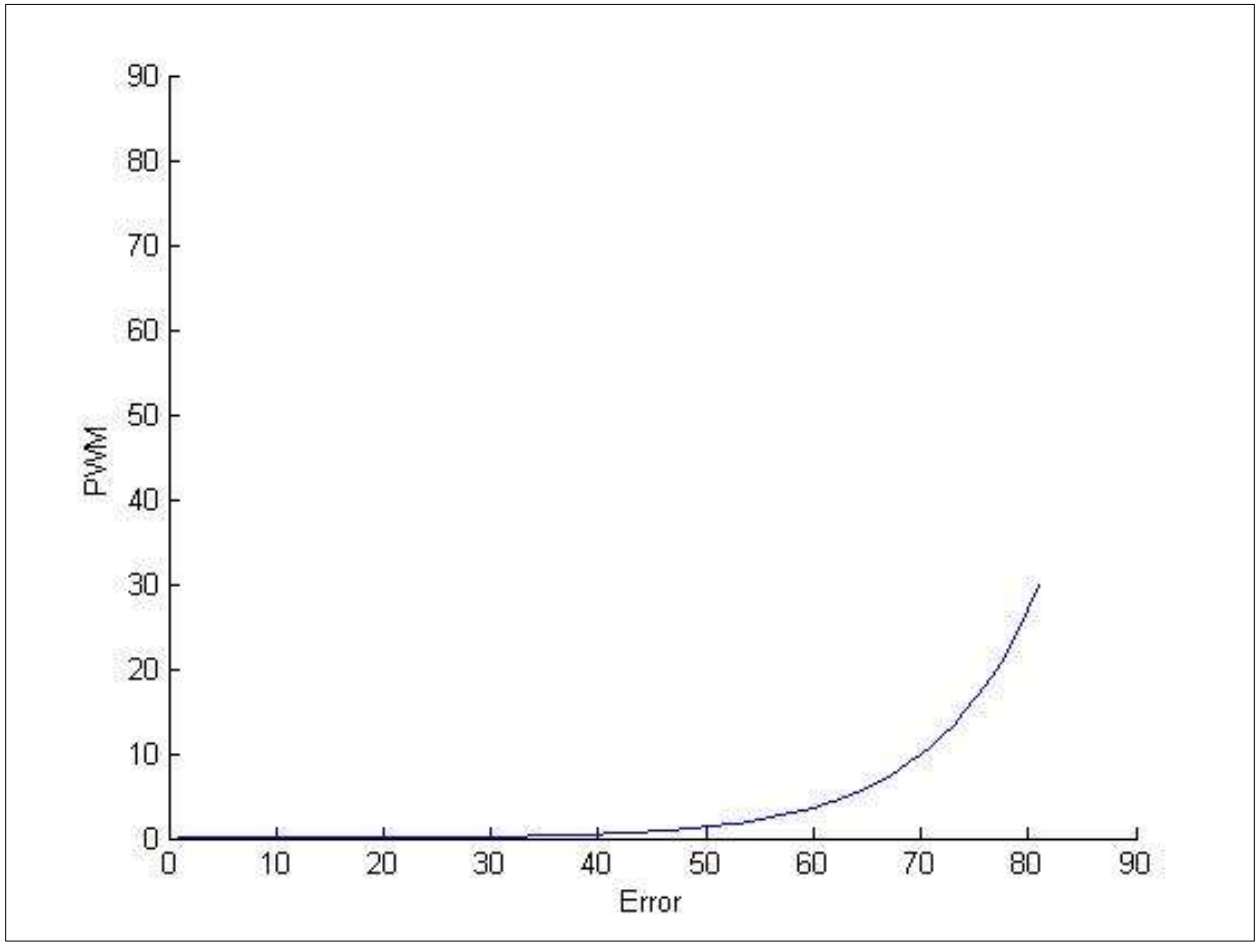


Figure 22: Addition of exponential power to decrease the error

4.3 3 Implementation of exponential function:

Exponential function is implemented on real time error data.

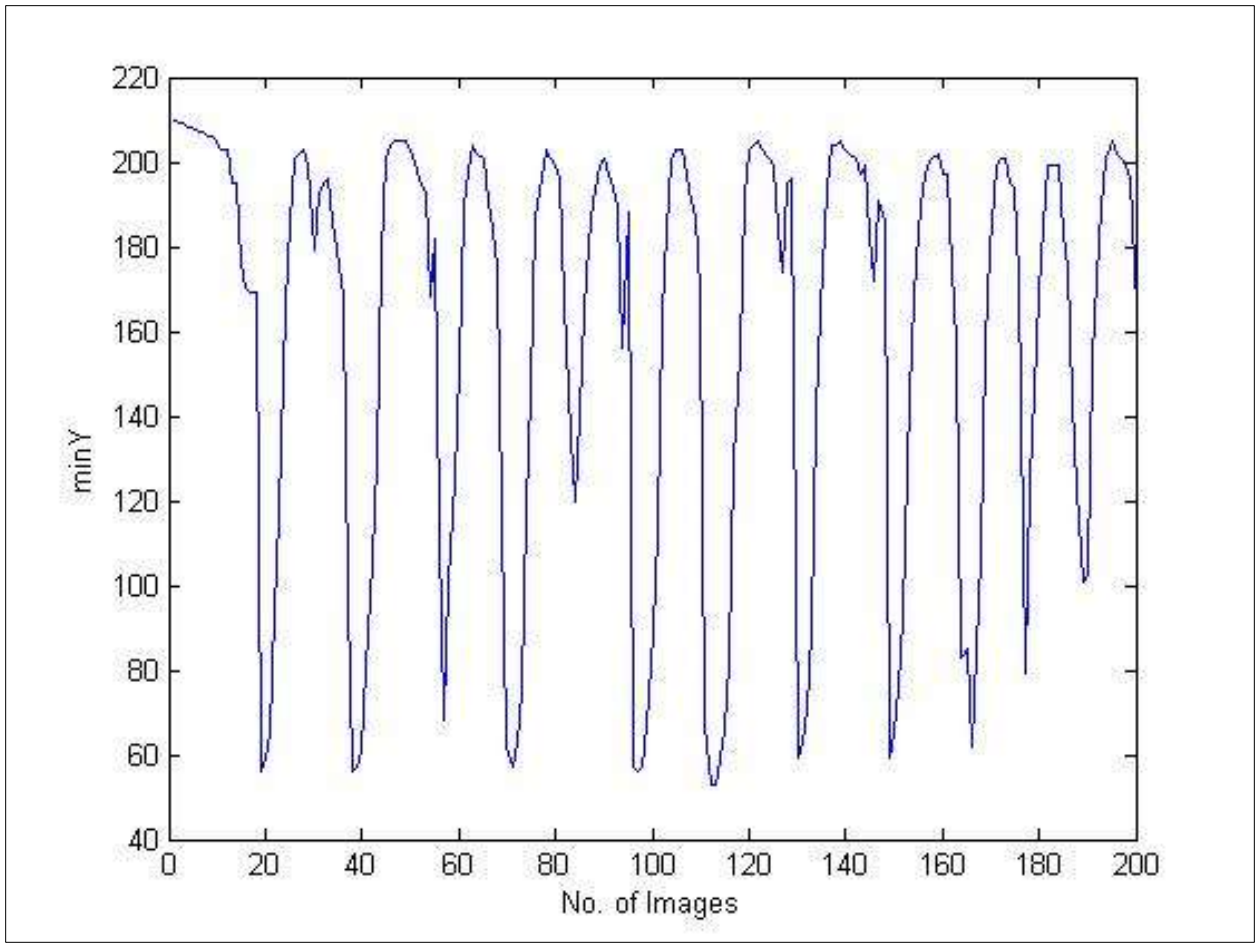


Figure 23: exponential function is applied to make the system stabilized.

In figure 23, a graph is presented in which position is mentioned on y-axis and total number of images taken are mentioned on x-axis. Blob was placed at base i:e 210. With Kp gain of 1.13, Kd gain of 0.01, exponential function is also applied to minimize to error when reaching the set point. The blob kept oscillating around the set point of 128 but oscillations are strong and system is not stabilized. When error is large, exponential function adds up more power to blob till it reaches near the required point and when error decreased, exponential function reduces its power to zero.

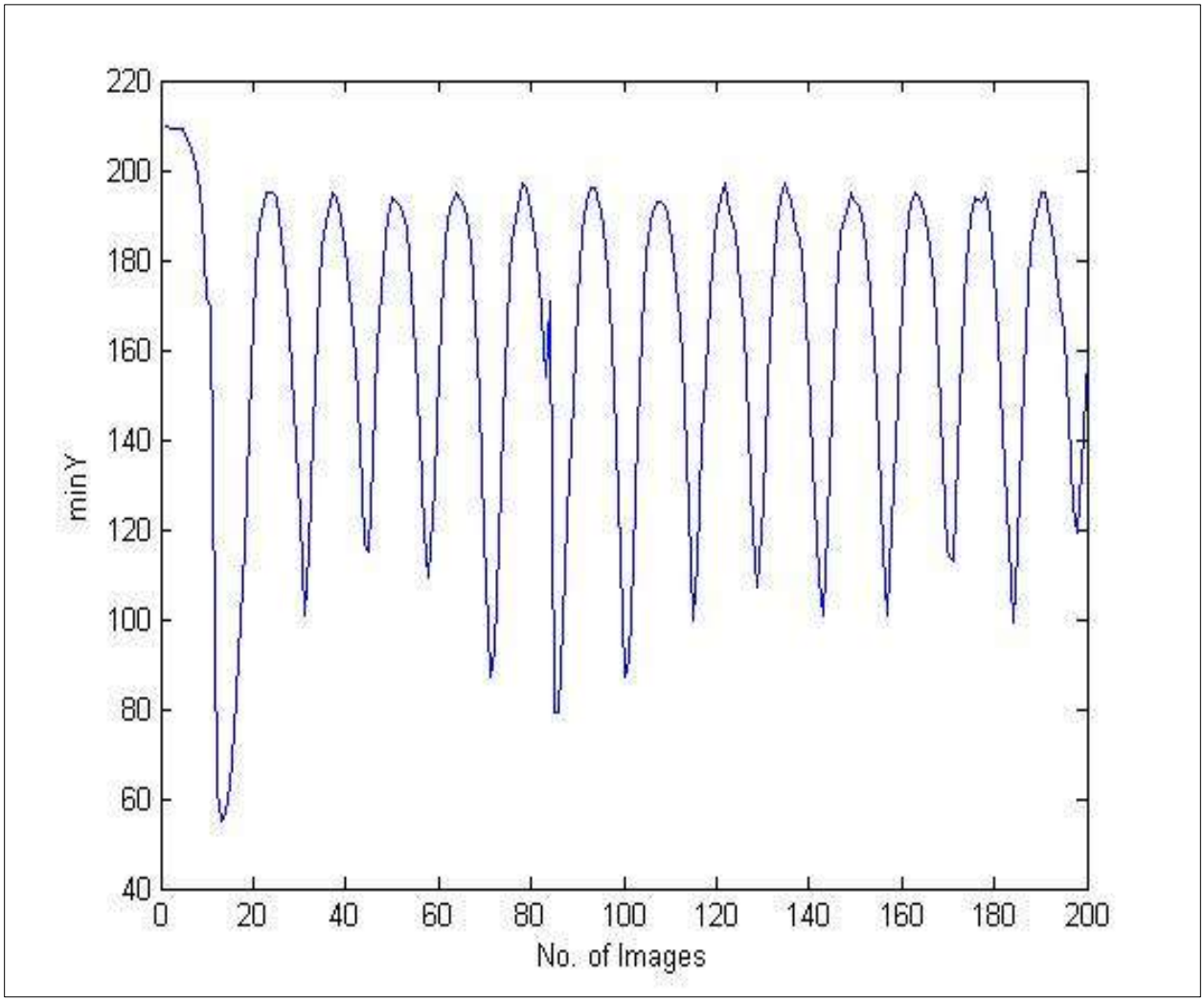


Figure 24: Kp tuning after the implementation of exponential function.

In figure 24, a graph is presented in which position is mentioned on y-axis and total number of images taken are mentioned on x-axis. Blob was placed at base i:e 210. With Kp gain is adjusted at 0.92 along with Kd gain of 0.01, exponential function is also applied to minimize to error when reaching the set point. The blob kept oscillating around the set point of 128, oscillations are damped and system is close to stabilizations. Kp tuning is done to get closer to the desired set point to take system towards stability

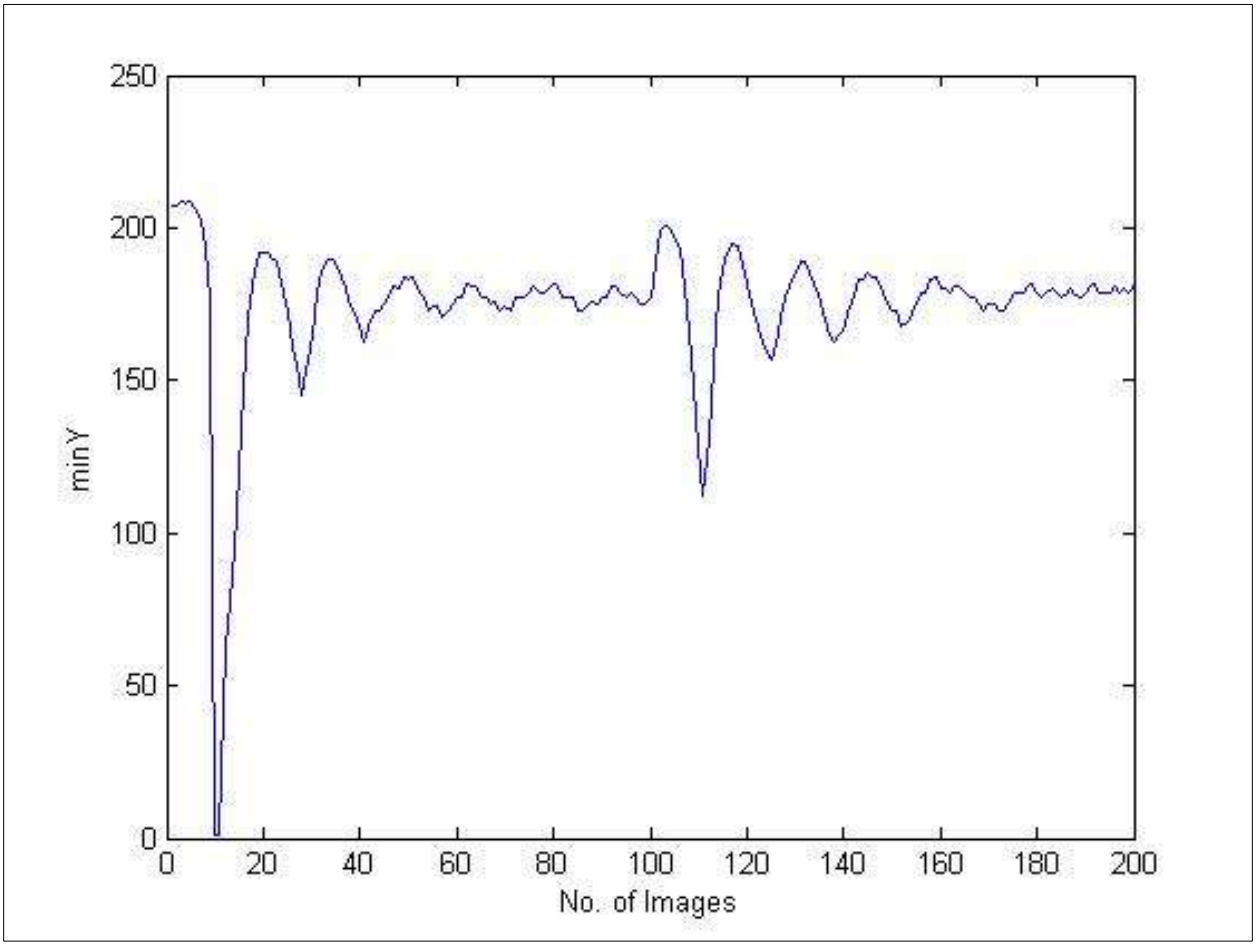


Figure 25: System stability after Kp tuning after the implementation of exponential function.

In figure 25, a graph is presented in which position is mentioned on y-axis and total number of images taken are mentioned on x-axis. Blob was placed at base i:e 210. With Kp gain is adjusted at 0.85 along with Kd gain of 0.01, exponential function is also applied to minimize to error when reaching the set point. The blob kept oscillating around the set point of 175, oscillations are damped and system is close to stabilizations. Kp tuning is done to get closer to the desired set point. This graph shows stability of the system, the strong wave in graph shows external disturbance was caused purposefully to examine the stability of the system.

In this stable system, Kp and Kd gain are applied to minimize the oscillations and keep the blob levitated at targeted set point.

4.4 Final Levitated-Stabilized State

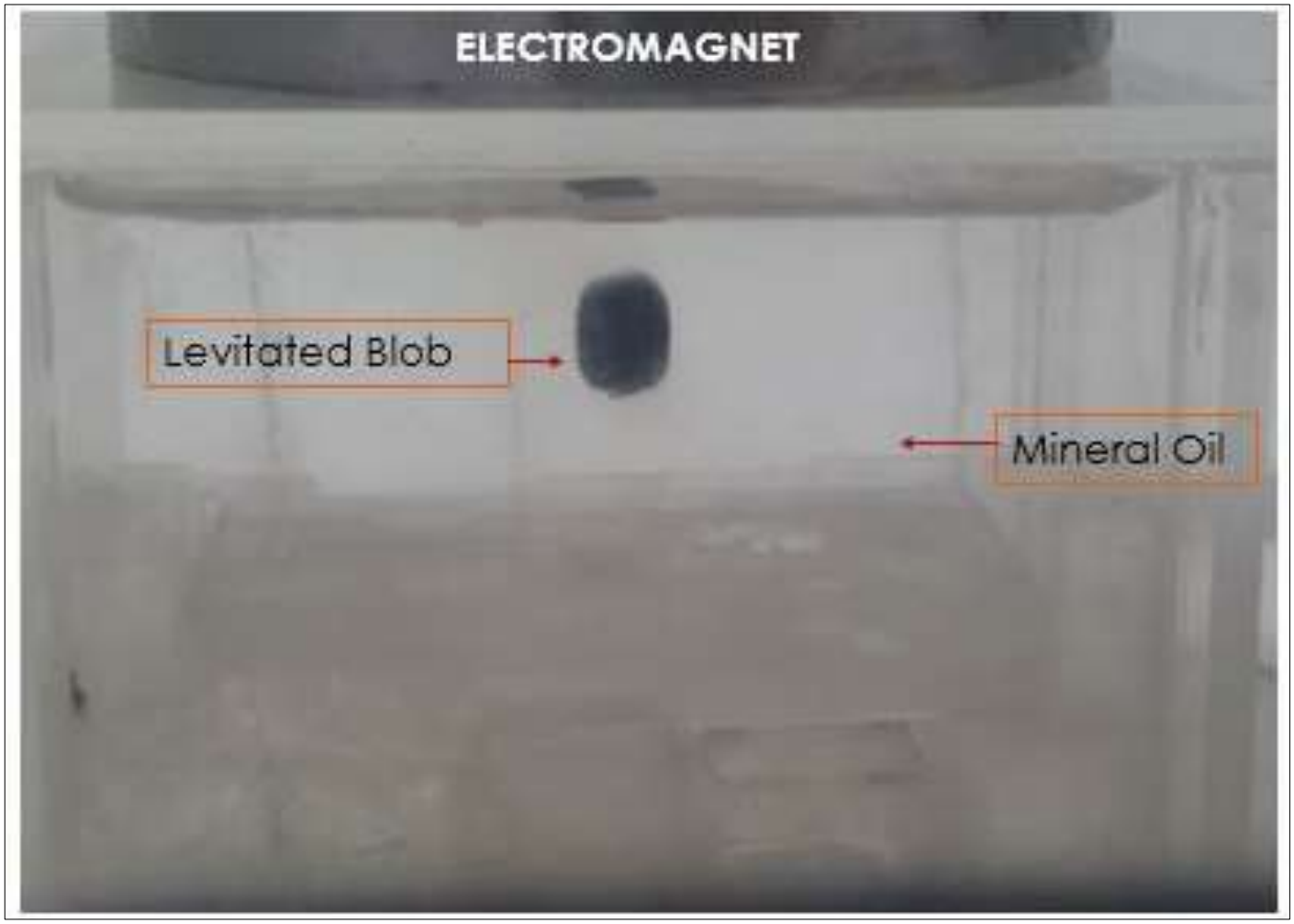


Figure 26: Levitated state of blob by balancing out gravitational and electromagnetic forces.

CHAPTER 5

CONCLUSION

The system uses one-dimensional force system. Two forces counter each other i.e. the electromagnetic force and the gravitational force. Addition of Ki to Kp and Kd though speeds up the motion when reaching to the targeted set point, blob stays in levitated condition around the set point thus stability is increased by the addition of Ki but oscillation are still present that hinders the stability of the system. The blob crosses the set point in momentum under proportional gain value and then falls back in free fall until it is again below the required height. Addition of exponential function to smooth out the power. Power was too much, to decrease the power of Kp, we added an exponential function, in result, it supplies the power when the error is large, power gets zero when error is reduced to zero. The exponential function adds additional power to the blob when it is at rest, when the error is reduced, this extra exponential power decreases.

In this stable system, Kp and Kd gain are applied to minimize the oscillations and keep the blob levitated at targeted set point.

The apparatus models the targeted drug delivery system. Levitation and position control has been successfully achieved by using real time data processing and imaging technology. The system fine tunes to a very minute error.

5.1 Future Perspective

This system can be controlled and more stabilized in 2D and 3D by using more magnets to design a more stable setup for drug delivery. The system can be more stable by using more magnets and by increasing the number of images and time to increase the stability of system.

REFERENCES

[1] Alexiou C, Arnold W, Klein RJ, Parak FG, Hulin P, Bergemann C, Erhardt W, Wagenpfeil S, Lubbe AS. 2000. Locoregional cancer treatment with magnetic drug targeting. Cancer Res 60:6641–6648.

[2] Ally J, Martin B, Khamesee MB, Roa W, Amirfazli A: Magnetic targeting of aerosol particles for cancer therapy. J. Magn. Magn. Mater. 293, 442–449 (2005).

[3] Amit Chandna, Deepa Batra, Satinder Kakar, Ramandeep Singh: A review on target drug delivery: magnetic microspheres. Journal of Acute Disease (2013)189-195. doi: 10.1016/S2221-6189(13)60125-0.

[4] Arias JL, Gallardo V, Gómez-Lopera SA, Plaza RC, Delgado AV: Synthesis and characterization of poly(ethyl-2-cyanoacrylate) nanoparticles with a magnetic core. J. Control. Rel. 77, 309–321 (2001).

[5] Brannon-Peppas L, Blanchette JO: Nanoparticle and targeted systems for cancer therapy. Adv. Drug Deliv. Rev. 56, 1649-1673 (2004)

[6] Gonda I: Preface. Major issues and future prospects in the delivery of therapeutic and diagnostic agents to the respiratory tract. Adv. Drug Deliv. Rev. 5, 1–9 (2000).

[7] Grief AD, Richardson G. 2005. Mathematical modelling of magnetically targeted drug delivery. J Magn Mater 293:455–463

[8] Gupta PK, Hung CT, Rao NS: Ultrastructural disposition of adriamycin-associated magnetic albumin microspheres in rats. J. Pharmacol. Sci. 78, 290–294 (1989).

[9] Häfeli UO, Sweeney SM, Beresford BA, Sim EH, Macklis RM: Magnetically directed poly(lactic acid) 90Y‐microspheres: novel agents for targeted intracavitary radiotherapy. J. Biomed. Mater. Res. 28, 901–908 (1994).

[10] Häfeli UO, Sweeney SM, Beresford BA, Humm JL, Macklis RM: Effective targeting of magnetic radioactive 90Y-microspheres to tumor cells by an externally applied magnetic field. Preliminary in vitro and in vivo results. Nucl. Med. Biol. 22, 147–155 (1995).

[11] Hergta R, Hiergeista R, Zeisberger M et al.: Enhancement of AC-losses of magnetic nanoparticles for heating applications. J. Magn. Magn. Mater. 280, 358–368 (2004).

[12] Hilal SK, Michelsen WJ, Driller J, Leonard E: Magnetically guided devices for vascular exploration and treatment. Radiology 113, 529–540 (1974).

[13] Hu FX, Neoh KG, Kang ET: Synthesis and in vitro anti-cancer evaluation of tamoxifen-loaded magnetite/PLLA composite nanoparticles. Biomaterials 27, 5725–5733 (2006).

[14] Ikeda N, Hayashida O, Kameda H, Ito H, Matsuda T: Experimental study on thermal damage to dog normal brain. Int. J. Hyperthermia 10, 553–561 (1994).

[15] Ito A, Hibino E, Kobayashi C et al.: Construction and delivery of tissue-engineering human retinal pigment epithelial cell sheets using magnetite nanoparticles and magnetic force. Tissue Eng. 11, 489–496 (2005).

[16] Jordan A, Wust P, Fahling H, John W, Hinz A, Felix R: Inductive heating of ferrimagnetic particles and magnetic fluids: physical evaluation of their potential for hyperthermia. Int. J. Hyperthemia 9, 51–68 (1993).

[17] Kato T, Nemoto R, Mori H: Magnetic microcapsules for targeted delivery of anticancer drugs. Appl. Biochem. Biotechnol. 10, 199–211 (1984).

[18] Lacava LM, Lacava ZGM, Azevedo RB et al.: Use of magnetic resonance to study biodistribution of dextran-coated magnetic fluid intravenously administered in mice. J. Magn. Magn. Mater. 252, 367–369 (2002).

[19] Lubbe AS, Alexiou C, Bergemann C: Clinical application of magnetic drug targeting. J. Surg. Res. 95, 200–206 (2001).

[20] Lubbe AS, Bergemann C, Riess H, Schriever F,Reichardt P, Possinger K, Matthias M, Dörken B, Herrmann F, Gürtler R. Clinical experiences with magnetic drug targeting: a phase i study with 4'-epidoxorubicin in 14 patients with advanced solid tumors. Cancer Res (1996), 56:4686–4693.

[21] Lübbe AS, Bergemann C, Huhnt W et al.: Preclinical experiences with magnetic drug targeting: tolerance and efficacy. Cancer Res. 56, 4694–4701 (1996).

[22] Lübbe AS, Bergemann C, Riess H et al.: Clinical experiences with magnetic drug targeting: a Phase I study with 4´-epidoxorubicin in 14 patients with advanced solid tumors. Cancer Res. 56, 4686–4693 (1996).

[23] Mah C, Fraites TJ, Zolotukhin I, Song SH, Flotte TR, Dobson J, Batich C, Byrne BJ. 2002. Improved method of recombinant AAV2 delivery for systemic targeted gene therapy. Mol Ther 6: 106–112

[24] Ma M, Wu Y, Zhou J, Sun Y, Zhang Y, Gu N: Size dependence of specific power absorption of Fe3O4 particles in AC magnetic field. J. Magn. Magn. Mater. 268, 33–39 (2004).

[25] Mosbach K, Schro¨der U. 1979. Preparation and application of magnetic polymers for targeting of drugs. FEBS Lett 102: 112–116.

[26] Meyers PH, Cronic F, Nice CM: Experimental approach in the use and magnetic control of metallic iron particles in the lymphatic and vascular system of dogs as a contast and isotopic agent. Am. J. Roentgenol. Radium Ther. Nucl. Med. 90, 1068–1077 (1963).

[27] Neuberger T: Doctoral thesis. University of Zurich, Switzerland (2002).

[28] Pankhurst QA, Connolly J, Jones SK, Dobson J: Applications of nanoparticles biomedicine. J. Phys. D Appl. Phys. 36, R167–R181 (2003).

[29] Perez JM, Simeone FJ, Saeki Y, Josephson L, Weissleder R. Viral-induced self-assembly of magnetic nanoparticles allows the detection of viral particles in biological media. J Am Chem Soc. 2003;125(34): 10192–10193.

[30] Primo FL, Michieleto L, Rodrigues MAM et al.: Magnetic nanoemulsions as drug delivery system for Foscan: skin permeation and retention in vitro assays for topical application in photodynamic therapy (PDT) of skin cancer. J. Magn. Magn. Mater. 311, 354–357 (2007).

[31] Ranney DF. Biochem Pharmacol.; 35: 1063-1069 (1986) .

[32] Rudge S, Peterson C, Vessely C et al.: Adsorption and desorption of chemotherapeutic drugs from a targeted carrier (MTC). J. Control. Rel. 74, 335–340 (2001).

[33] Tari A, Chantrell RW, Charles SW, Popplewell J. The magnetic properties and stability of a ferrofluid containing Fe3O4 particles. Physica B and C. 1979;97(1):57–64.

[34] T.K. Indira and P.K. Lakshmi, Magnetic Nanoparticles A Review, International Journal of Pharmaceutical Sciences and Nanotechnolgy, Vol. 3, Issue 3 (2010), 1035-1042.

[35] Wada S, Yue L, Tazawa K et al.: New local hyperthermia using dextran magnetite complex (DM) for oral cavity: experimental study in normal hamster tongue. Oral Dis. 7, 192-195 (2001).

[36] Widder KJ, Marino PA, Morris RM, Senyei AE: In: Targeted Drugs. Goldberg E(Ed.). John Wiley and Sons, NY, USA, 201 (1983).

[37] Widder KJ, Morris RM, Poore GA, Howard DP, Senyei AE. 1983. Selective targeting of magnetic albumin microspheres containing low-dose doxorubicin - total remission in Yoshida sarcoma-bearing rats. Eur J Cancer Clin Oncol 19:135–139

[38] Widder KJ, Senyei AE, Scarpelli DG. Magnetic microspheres: a model system for site specific drug delivery in vivo. Proc Soc Exp Biol Med 58:141–146.(1978)

[39] Widder KJ, Senyei AE, Ranney DF: Magnetically responsive microspheres and other carriers for the biophysical targeting of antitumour agents. Avd. Pharmacol. Chemother. 16, 213–217 (1979).

[40] Zavisova V, Koneracka M, Strbak O et al.: Encapsulation of indomethacin in magnetic biodegradable polymer nanoparticles. J.Magn. Magn. Mater. 311, 379–382 (2007).

TURNITIN REPORT

thesis 2

ORIGINALITY REPORT

5%	1%	5%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Nahla S Barakat. "Magnetically modulated nanosystems: a unique drug-delivery platform", Nanomedicine, 10/2009 Publication	3%
2	Jon Dobson. "Magnetic nanoparticles for drug delivery", Drug Development Research, 01/2006 Publication	1%
3	Sašo Gyergyek. "Influence of synthesis method on structural and magnetic properties of cobalt ferrite nanoparticles", Journal of Nanoparticle Research, 12/30/2009 Publication	<1%
4	Chomoucka, J.. "Magnetic nanoparticles and targeted drug delivering", Pharmacological Research, 201008 Publication	<1%
5	Islam, M. S., J. Kurawaki, Y. Kusumoto, M. Abdulla-Al-Mamun, and M. Z. Bin Mukhlish. "Hydrothermal Novel Synthesis of Neck-	<1%

structured Hyperthermia-suitable Magnetic (Fe_3O_4, γ-Fe_2O_3 and α-Fe_2O_3) Nanoparticles", Journal of Scientific Research, 2011.
Publication

6	www.bupa.co.uk Internet Source	<1%
7	Lignos, D, D Moreno, and S Billington. "Hybrid simulation of a 2-story steel MRF retrofitted with HPFRC infill panels", Behaviour of Steel Structures in Seismic Areas STESSA 2012, 2011. Publication	<1%
8	trialx.com Internet Source	<1%

EXCLUDE QUOTES ON
EXCLUDE BIBLIOGRAPHY ON
EXCLUDE MATCHES OFF