A

DISSERTATION REPORT

ON

"SURFACE ADHESIVES FOR REMOVAL OF KIDNEY STONE FRAGMENTS"

Submitted for partial fulfillment of the requirement for award of the degree of

INTEGRATED MASTERS OF TECHNOLOGY

IN

DISCIPLINE (Polymer Science & Technology)

Submitted By:

HARSHINI TAMMAREDDY

09120018

Under the Guidance of :

Dr. N.C. Mishra
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NUT, Singapore

DEPARTMENT OF POLYMER & PROCESS ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY ROORKEE
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5|6|2014
Date:

T. Harshini
Name of student
HARSHINI TAMMAREDDY

CANDIDATE'S DECLARATION

I hereby declared that the work which is being presented in this Dissertation Report entitled ***"Surface Adhesives for Removal of Kidney Stone Fragments"*** in partial fulfillment of the requirement for the award of the degree of Integrated Master of Technology in Polymer Science & Technology, IIT Roorkee is a record of my own work carried out, under the supervision of ***Dr.Terry Steele, NTU, Singapore and Dr.N.C.Mishra, Department of Polymer & Process Engineering, IIT Roorkee.***

The matter embodied in this project report has not been submitted by me for the award of any other degree.

Date: 5/6/2014

Place: Singapore

S. Harshini

Harshini Tammareddy

This is to certify that the above statement made by the candidate is correct to the best of our knowledge.

Dr.N.C.Mishra
Associate Professor
IIT Roorkee

Dr.Terry WJ Steele
Assistant Professor
NTU,Singapore

CONTENTS

Abstract

Current technologies implemented for removing kidney stones are complex and doesnot achieve 100% removal of kidney stones. The objective of this project is to prepare formulations of bio adhesives for removing kidney stone fragments that were left in the patient's body after Percutaneous Nephrolithotomy(PCNL) and ureteroscopy that pose significant morbidity to the patient with urinary stones with upto 50 % requiring intervention within 5 years.

Two kinds of nanoparticles were chosen for this study i.e., citrate coated gold nanoparticles and PVA coated iron oxide nanoparticles .Their surface properties were modified by coating them with various concentrations of Polydopamine(P-DOPA).

Dopamine undergoes self-polymerization under mild basic conditions forming P-DOPA onto various organic and inorganic materials. Nanoparticles were immersed into dopamine solution at mild basic conditions in Tris buffer which leads to uniform coating of P-DOPA onto nanoparticles. These prepared samples were analysed using Dynamic Light Scattering and UV Spectroscopy.

Polymerization reaction of P-DOPAonto nanoparticles was controlled by varying the ph of the buffer. On reducing the ph of solution P-DOPA coated nanoparticles were found to be aggregating which might be proven useful for improved iron loading efficiencies onto kidney stone fragments.

Stability studies of P-DOPA coated nanoparticles for one week has been conducted.

Adhesion of P-DOPA with kidney stone has been established by preparing Human Kidney stone pellet. Kidney stones, collected from Tan Tock Seng Hospital,Singapore, were crushed with pestle motar and pellet was prepared using Hydraulic press of FTIR .P-DOPA was coated onto these pellets and P-DOPA adhesion with kidney stone has been proved.

In conclusion P-DOPA has been proved to be an ideal polymer for extracting kidney stone fragments.

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Chapter 1

INTRODUCTION

1.1 Background

1.1.1 Motivation

A kidney stone is a solid piece of material that forms in a kidney when there are high levels of calcium, oxalate, and phosphorus in the urine. Calcium oxalate is the most common type of kidney stone. 70 %-93%of kidney stones that form in the human body is made of calcium oxalate[1]. Other than Calcium oxalate kidney stones can also contain uric acid, struvite, xanthine,brushite, quartz, whitlockite, dahlite and cystine [1]. Presence of kidney stones leads tocompromise in quality of life, extreme pain and also permanent damage to kidney in some extreme cases.

1.1.2 Current technologies

Earlier kidney stones were removed through complex and time consuming open surgeries.In the recent times three major techniques are relied upon for removing stones from Human kidneys.

Current treatment methods for kidney stones include

1. Shock wave lithotripsy,
2. Ureteroscope and
3. Percutaneous nephrolithotomy.

Shock wave lithotripsy

Shock Wave Lithotripsy (SWL) is the most common treatment method for kidney stones. Shock waves from outside the body are targeted at a kidney stone causing the stone to fragment. Stones are broken into "stone dust" or fragments that are small enough to pass through urine. Stonesthat are smaller than 2 cm in diameter can be effectively removed by SWL technique. Thistreatment is not effective for extraction of very large stones (> 2 cm diameter).

Ureteroscope

In this techniqueureteroscope is inserted through urethra and bladder into the ureter, to get towhere the kidney stone is located.Laser is used to break kidney stones into smaller pieces. Ureteroscopic techniques are generally more effective than SWL for treating stones located in the lower ureter. However, ureteroscopy treatment method of upper ureter is much more complex and challenging task. Generally, SWL act as a firstlinetreatment for stones of less than 10 mm, andureteroscopy for those greater than 10 mm in diameter.

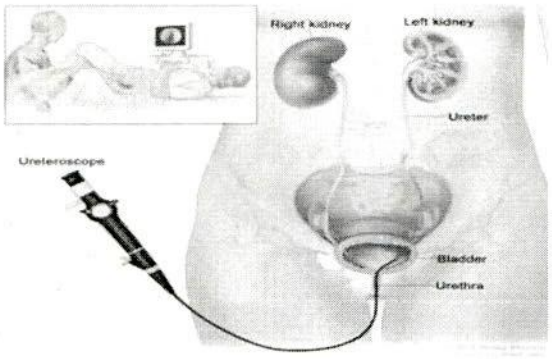


Figure 1 illustration of ureteroscope(Figure adopted from [8])

Percutaneous nephrolithotomy

Percutaneous nephrolithotomy (PCNL) is a surgical procedure to remove stones from the kidneyby a small puncture wound through the skin. It is most suitable to remove stones of more than 2cm in size and which are present near the pelvic region. It is difficult to remove to remove the fragmented kidney stones with this technique.

Figure 2 illustration of PCNL (Figure adopted from [15])

1.1.3 Prevailing challenge in current treatment techniques

Even with all the above mentioned treatment methods, we can only achieve 50% to 80% clearance of kidney stones in human body. Subsequent interventions are needed to remove the small stone fragments (*submicron particles*) which reside in the kidney post PCNL and ureteroscopy which will pose significant morbidity to the patients with urinary stones [2].

1.2 Objective

The objectives of this project are to

- Optimize P-DOPA coating on nanoparticles
- Prove that P-DOPA is the ideal bio-adhesive that will bind to nanoparticles as well as kidney stone allowing the safe removal of kidney stone fragments

1.3 Project Description

Previous work was reported by Raul Fernandez et.al. about a novel technique to remove kidneystone fragments. The work focused on designing a numeric model for binding a peptide coatediron oxide superparamagneticmicroparticles to kidney stone fragments to induce magnetization of the kidney stones and subsequent extraction using a magnetic tool [2]. In order to explore thisapproach for removing microfragmentsof kidney stones (< 2mm), previous research was donein our group under the direction of Dr. Terry Steele (NTU) to design a magnetic catheter toextract the sub-micron magnetized kidney stones [3].
To complement the previous work done on magnetized urethral catheter, my work will focus onremoving the kidney stones using a bio-adhesive linker which when coated on magnetic nanoparticles can selectively cross link with kidney stones. The coated magnetic nanoparticles cross linked with kidney stones can then be easily removed using the previously designed magnetic catheter in Dr.Terry's lab.

Reports have been published earlier demonstrating the superparamagnetism effects exhibited by nanoparticles [7]. Using nanoparticles which exhibit superparamagnetism will be a useful model for this study as superparamagnetic particles are known to possess higher magnetic susceptibility in the presence of external magnetic field. Hence we selected magnetic nanoparticles forcoating the bio adhesive linker in this study which will be further used for extraction of kidneystones. We will specifically target designing a method for extracting kidney stones in the rangefrom 0.5 mm to 2mm in this study.

Figure 2 is an illustration of the strategy that will be employed in this work. Specifically we plan touse Polydopamine (P-DOPA)as our kidney stone crosslinker. P-DOPAis a synthetic eumelanin polymer. It is derived from dopamine through oxidative self-polymerization under mild basic conditions. Figure 3 describes the polymerization of dopamine to P-DOPA.
Dopamine is awidespread catechol compound and is known to virtually bind to all the surfaces through self-polymerization due to the presence of highly reactive catechol groups. A fundamentalunderstanding regarding the mechanism of formation of P-DOPAis still lacking but it waspropose̲d that the reaction takes place through the oxidation of catechol groups into quinone [6].Also the pDOPAcoating is resistant to harsh environment and biocompatible making it idealcrosslinker for use in this study.

Reported mechanism of coating

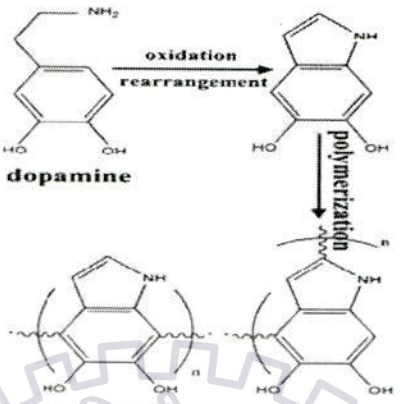


Figure 3 Mechanism of P-DOPA formation (Fig adopted from [4])

Project Proposal Plan

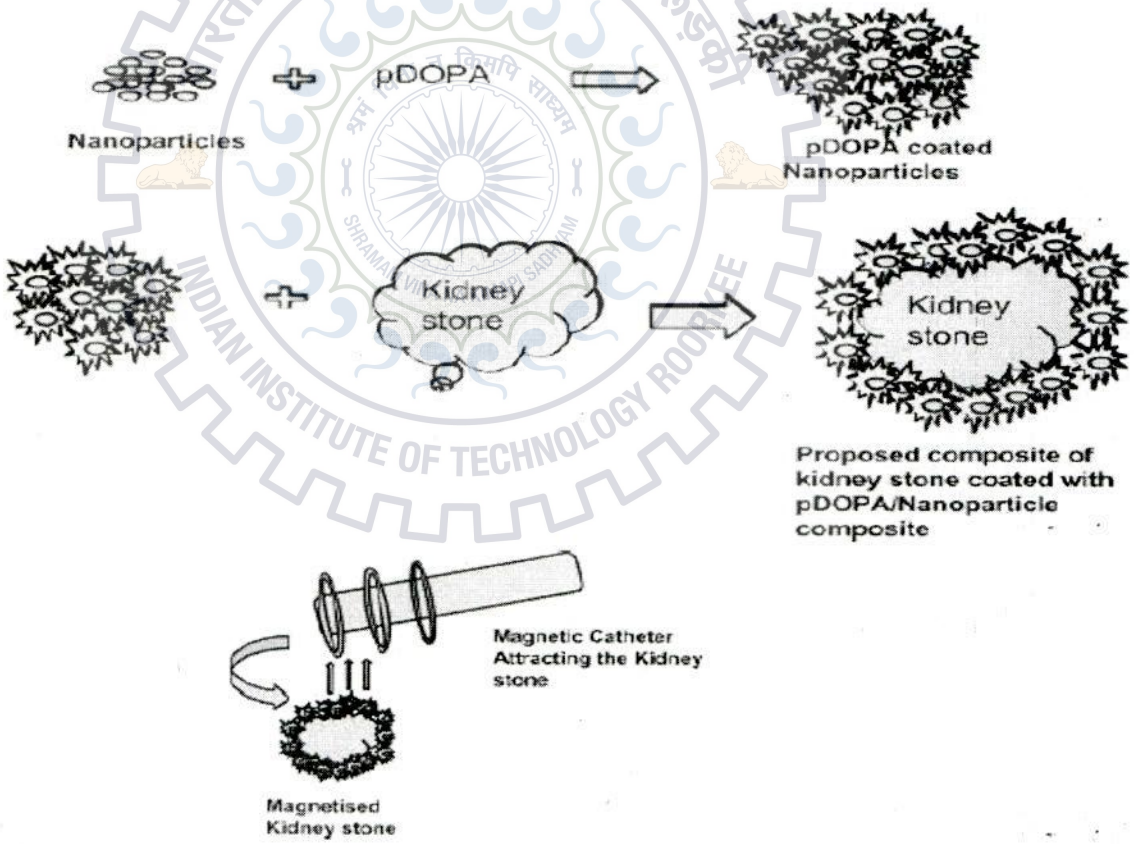


Figure 4 illustration of project proposal plan

1.4 Design parameters

- Kidney stone fragments have size less than 2mm
- Kidney stones are fragmented prior to the insertion of the urethral catheter

1.5 Terminologies associated within this report

Nanoparticles

Nanoparticles are super paramagnetic in nature and their size is less than 100nm.They generally possesses magnetic moment only in the presence of external magnetic field.

Kidney stone

Dietary minerals present in excessive quantities in urine leads to crystal aggregation which are called as kidney stones. They are mainly composed of calcium oxalate. 70 %-93% of kidney stones that form in the human body is made of calcium oxalate [1].

Chapter 2
LITERATURE REVIEW

2.1Polydopamine (P-DOPA)

Polydopamine is a proven bio-adhesive that strongly binds to a broad spectrum of organic and inorganic materials. P-DOPA can undergoself-polymerizationand deposit on any shape and type of material.P-DOPA is formed by oxidation of dopamine under mild basic conditions on various surfaces. The structure of P-DOPA is mainly composed of covalent bonds. The polymerization reaction of dopamine to P-DOPA is simple and the polymer layer formed is quiet strong drawing huge interest in this material's properties [9].

2.1.1Polymerization of Dopamine

Though it is a polymer that is widely used as bio adhesive the exact mechanism of oxidative self-polymerization that dopamine undergoes is yet to be found[9].But it is believed that polymerization of dopamine into P-DOPAis similar to melanin formation. The catechol groups present in dopamine undergoes oxidation into quinone under mild basic conditions and these quinone groups will further form into an adherent polymer layer. [9]

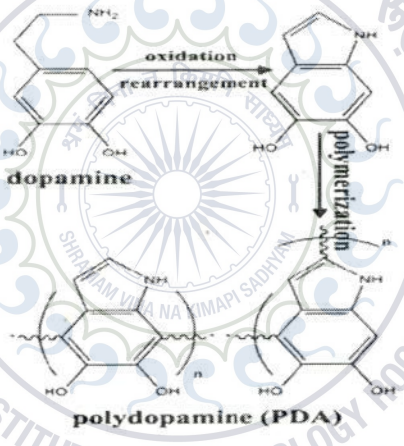


Figure 5 Polymerization of P-DOPA (Fig adopted from [4])

2.1.2 P-DOPA as Bioadhesive

P-DOPA is being researched extensively in the recent times considering its ability as a strong bio adhesive. P-DOPA adheres to the substrate through covalent bonding network proving its potential as a bio adhesive. Also the preparation of P-DOPA is quick and simple. [9]

P-DOPA has the ability to withstand harsh environment and is quiet stable proving it as a polymer suitable for adhesive applications. P-DOPA is hydrophilic in nature and is biocompatible making it suitable for biological applications. [10]

2.1.2 Structure of P-DOPA

The exact structure of P-DOPA is still under debate and has to be proven yet. The following are the various proposed structures of P-DOPA available in the literature. [9]

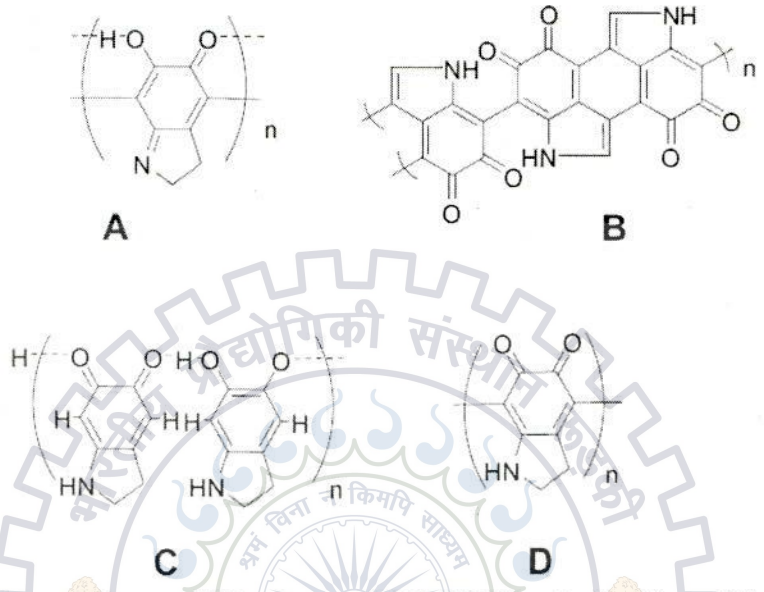


Figure 6 Proposed Structures of P-DOPA (Fig adopted from [9])

2.1.3 Effect of pH on P-DOPA

After adherent P-DOPA film is formed its surface charge varies with pH. P-DOPA is zwitterionic in nature. At low pH it has positive surface charge and high pHit exhibits negative charge on its surface. [11]

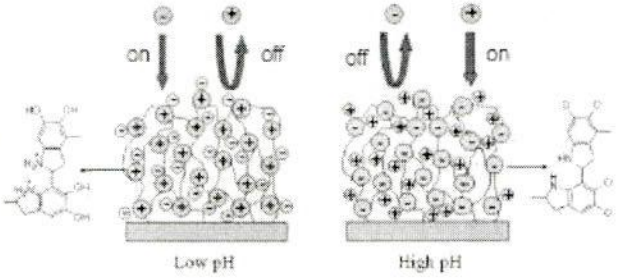


Figure 7 pH dependence of P-DOPA (Fig adopted from [11])

2.1.4 COATING P-DOPA ONTO NANOPARTICLES

Previous literature is available on coating nanoparticles with P-DOPA under mild basic conditions [5],[10]. This is a simple and easy process where nanoparticles are immersed in DOPA solution at pH 8.5[5] .Dopamine will react with atmospheric oxygen and undergo oxidation to form P-DOPA layer on nanoparticles. Polymerization reaction and coating thickness are dependent upon the pH, the amount of oxygen available and time of reaction. [5]

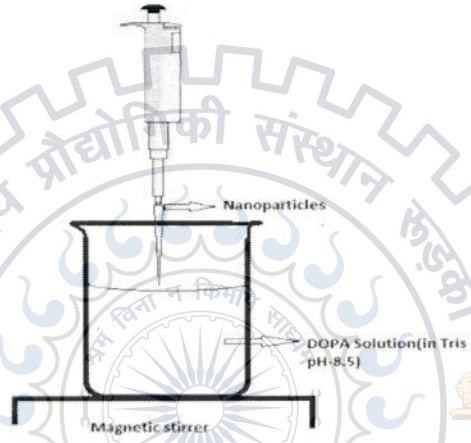


Figure 8 illustration of P-DOPA coating method

2.2 Nanoparticles

Nanoparticles general size range fall below 100nm. Magnetic nanoparticles are in general super paramagnetic in nature .They exhibit magnetic properties only in the presence of external magnetic field. [7].

For this project we have chosen two kinds of nanoparticles i.e., gold nanoparticles (Citrate coated) and PVA coated iron oxide nanoparticles. Gold nanoparticles were chosen to validate that P-DOPA can be coated onto nano sized particles.

Iron oxide nanoparticles are chosen for magnetizing the kidney stones. Iron oxide nanoparticles are biocompatible and possess good magnetic properties.

Biocompatibility and toxicology of Iron Oxide Nanoparticles [14]

- Iron oxide nanoparticles are biocompatible and can safely be incorporated into human body.
- Iron present in these particles enters body's metabolism and will be leaded by human body into the haemoglobin of red blood cells thus causing no harm to the human body.

2.3Magnetic Catheter [3]

A magnetic catheter is designed in Dr. Terry's lab by combining the design of urethral catheter and magnetic tip for removing magnetized kidney stone fragments. The catheter will be inserted into the kidney where kidney stones are located and then the magnetic tip will be pressed out. This magnetic tip will generate magnetic field that will attract the magnetized kidney stones.

Design specifications

Outer diameter of the rings 2 mm,
Inner diameter of the rings 1 mm,
Thickness of the ring 1 mm and
Maximum magnetic field 104 mT.

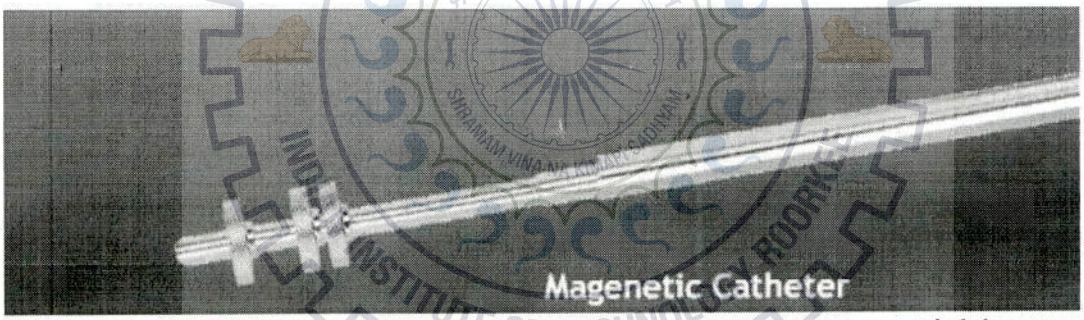


Figure 9 illustration of Designed Magnetic Catheter in Dr.Terry's lab

Chapter 3
MATERIALS AND METHODS

3.1 Materials

Dopamine (Molecular weight 189.64) used in this project was purchased from Sigma Aldrich, Singapore. Human kidney stones used in this project were collected from Tan Tock Seng Hospital,Singapore. PVA coated iron oxide nanoparticles were prepared in Dr.Raju Ramanujan's lab,NTU, Singapore through co-precipitation technique with concentration of 5mg/ml in Di-water.Citrate coated gold nanoparticles (5nm-30nm) were commercially purchased from nanocomposix and has a concentration of 0.5gm/gml in aqueous buffer. Tris buffer used in this project was prepared from Trizma base that has been purchased from Sigma. Tris buffer used for coating experiments has a concentration of 10 millM and pH8.5.

3.2 Methods

3.2.1 Coating P-DOPA onto Au nanoparticles

Coating method was adapted from [5]

- Coating of P-DOPA on to gold nanoparticles was obtained using an immersion method.

Citrate coated Gold Nanoparticles solution were immersed into predetermined amount of freshlyprepared dopamine solution in Tris buffer (10 mM, pH 8.5) under mild stirring (250 rpm).

- After stirring the solution at room temperature for sufficient time, gold nanoparticles were separated by centrifugation at 16000g.

Process of coating was optimized by controlling two variables
- *Coating time* (Coating thickness has been studied by controlling the time of immersion of gold nanoparticles into P-DOPA solution)
-*Concentration of P-DOPA solution* (Coating thickness was optimized by changing the concentration of P-DOPA coating solution).

P-DOPA coating was characterized with Malverin Zeta sizer and UV-Visible spectrometer.

3.2.2Particle Size Characterization

Malverin Zetasizer,UK has been used for measuring the Z-Average size of nanoparticles. All the measurements were taken at 25 ºC. Zeta sizer relies on Dynamic Light Scattering technique,to measure the size of nanoaprticles.Upon illuminating laser onto the particles the particles undergo Brownian motion and with the help of Stokes-Einstein relationship the instrument measures the Z-Average size of the particles.[12]

3.2.3 UV-Visible Spectrophotometer[13]

Ultraviolet-visible (UV-Vis) spectrophotometer measure the intensity of light passing through the sample curvette (I) with respect to the reference curvette (I_o).Transmittance value(I/ I_o)

isdeduced and with the help of Transmittance value Absorbance A is derived using Beer-Lambert Law.

$$A = - \log (I/I_o) \text{ or } A = \varepsilon bc$$

ε = extinction coefficient, b= path length, and c = concentration

On exposure to UV-VIS light depending on the material properties molecules absorb radiation of different in the electromagnetic spectrum. Light shone on the sample has photons carrying energy E= hc/λ(where E = energy, h = Planck's constant, c = speed of light, and λ = wavelength) and this will absorbed if the energy this photon carrying is sufficient enough for the occurance of electronic transition.

Solution placed in the cuvette absorbs light of a particular wavelength and the light intensity observed in converted into electrical signal and thus displays absorbance. For this study, samples are measured at 282nm.

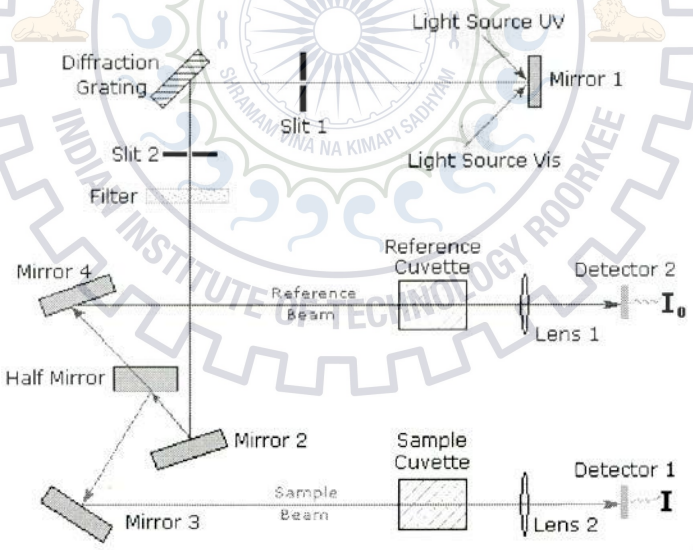


Figure 10: illustration of working principle of UV-VIS Spectrometre (Fig adopted from [13])

3.2.4 Shear adhesion Test

Shear adhesion test was designed to identify the adhesion strength between the P-DOPA coated nanoparticles and kidney stones.

Kidney stones were crushed into fine powder and a pellet was prepared using hydraulic press of FTIR.P-DOPA was added to different pairs of calcium hydroxide stones and kidney stones. To measure the adhesion strength chatillon tester was used. But due to P-DOPA cohesive failure could not identify the adhesion bond strength.

Pellet preparation

Calcium hydroxide pellets and kidney stone pellets were prepared using the hydraulic press of FTIR by applying a pressure of 0.5 tons for 60 seconds uniformly on all the samples. Kidney stone pellets were prepared using the hydraulic press of FTIR by applying a pressure of 1 tons for 60 seconds.

- Dimensions of pellet were measured using Vernier Callipers.

Figure 11: Hydraulic press used for pellet preparation

Chapter 4
RESULTS AND DISCUSSION

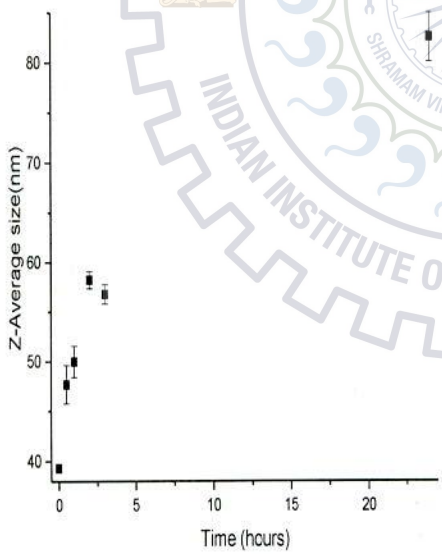
4.1 DLS Results of Au nanoparticles

15ml DOPA solution were prepared in Tris buffer (pH-8.5) and 50µl of citrate coated gold nanoparticles (conc-0.52mg/ml) were added. Samples were taken out at specified time intervals to perform DLS.

a) Initial concentration of DOPA - 80µg/ml
Mass ratio of nanoparticles to DOPA taken – 1:46.1

Time	Average size(in nm)
0.5	47.68
1	49.97333
2	58.24333
3	56.79667
24	82.53333

Table 1: Z-Avg size of DOPA (80 µg/ml) coated Au nps

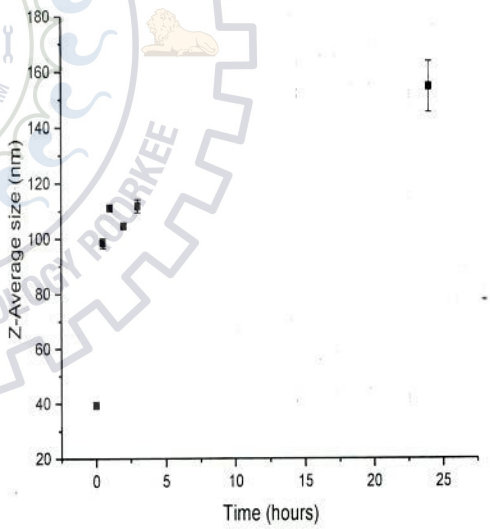


Graph 1: Z-Avg size of DOPA (80 µg/ml) coated Au nps

b) Initial concentration of DOPA - 40µg/ml
Mass ratio of nanoparticles to DOPA taken – 1:23.05

Time	Average size(in nm)
0.5	98.38333
1	111.1333
2	104.6667
3	111.8
24	151.2

Table 2: Z-Avg size of DOPA (40 µg/ml) coated Au nps



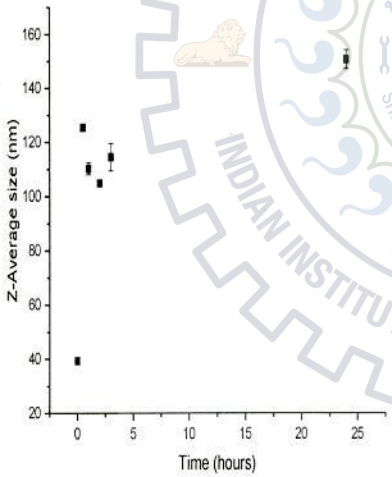
Graph 2: Z-Avg size of DOPA (40 µg/ml) coated Au nps

c) Initial concentration of DOPA - 20µg/ml

Mass ratio of nanoparticles to DOPA taken – 1:11.5

Time	Average size(in nm)
0.5	125.4667
1	110.2
2	104.8333
3	114.5
24	145

Table 3: Z-Average size of DOPA (20 µg/ml) coated Au nps



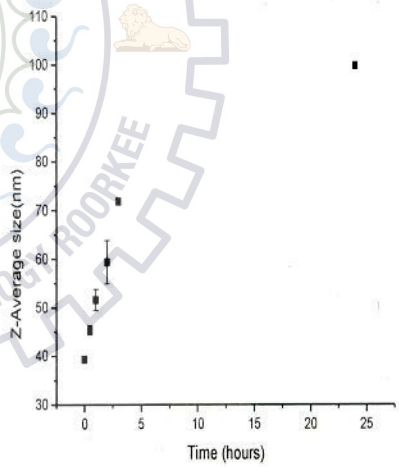
Graph 3: Z-Avg size of DOPA (20 µg/ml) coated Au nps

d) Initial concentration of DOPA - 10µg/ml

Mass ratio of nanoparticles to DOPA taken – 1:5.75

Time	Average size(in nm)
0	39.31667
0.5	45.402
1	51.616
2	59.372
3	71.65
24	99.81667

Table 4: Z-Average size of DOPA (10 µg/ml) coated Au nps

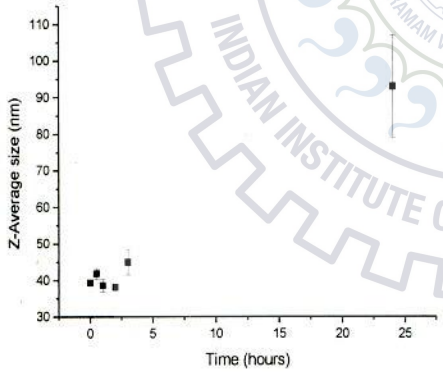


Graph 4: Z-Avg size of DOPA (10 µg/ml) coated Au nps

e) Initial concentration of DOPA - 5µg/ml
Mass ratio of nanoparticles to DOPA taken – 1:2.875

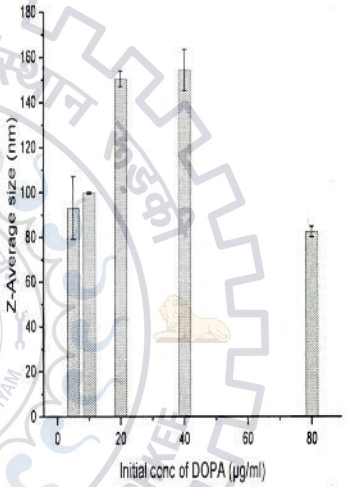
Time	Average size(in nm)
0	39.31667
0.5	41.86167
1	38.60833
2	38.09333
3	45.00833
24	93.06667

Table 5: Z-Avg size of DOPA (5 µg/ml) coated Au nps



Graph 5: Z-Avg size of DOPA (5 µg/ml) coated Au nps

Summary of coating of all the conc of DOPA for 24 hours



Graph 6: Size of nanoparticles after coating 24hrs with different conc of P-DOPA

- Gold nanoparticles were coated steadily with 10µg/ml DOPA concentration(initial)
- Not much coating was observed within 24 hours for 5 µg/ml DOPA concentration (initial).
- DOPA coating on Gold nanoparticles followed a similar trend from 5 µg/ml to 40 µg/ml which can be observed from Graph 6.

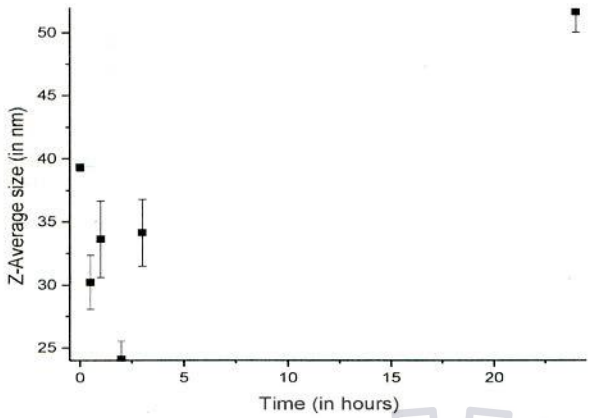
4.2 **Control Experiment of Au nanoaprticles**

Control experiment of Au nanoparticles in Tris has been conducted to prove that the increasing trend in Z-Average size observed in the experiments in section 4.1 are not because of aggregation of Gold nanoparticles.15ml Tris buffer (pH-8.5) was taken and 50 µlof citrate coated gold nanoparticles (conc-0.52mg/ml) were added. Samples were taken out at specified time intervals to perform DLS.

Results

Time	Average size(in nm)
0	39.31667
0.5	30.2075
1	33.6275
2	24.1
3	34.14
24	51.7

Table 6: Z-Average size of Au nps in Tris buffer



Graph 7: Z-Average size of Au nps in Tris buffer

T-Test was performed and the variation in Z-Average size of Gold nanoparticles in Tris buffer within 24hrs is found to be insignificant indicating that the Citrate coated Gold nanoparticles were quiet stable in Tris buffer

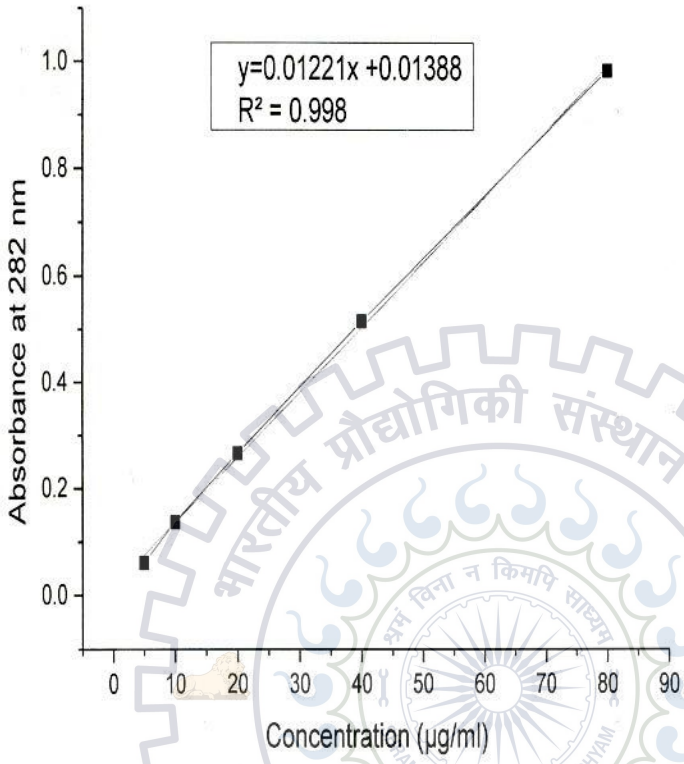
4.3 UV RESULTS

4.3.1 STANDARD CURVES OF DOPAMINE

Calibration curves of dopamine in Tris were prepared to identify the concentration of DOPA in the solution during coating DOPA onto nanoparticles from concentrations 80µg/ml to 0.5 µg/ml.

Concentration(in microgram/ml)	Absorbance			Net average absorbance
5	0.061	0.061	0.061	0.061
10	0.134	0.14	0.136	0.137
20	0.267	0.266	0.265	0.266
40	0.52	0.507	0.519	0.515333
80	0.991	0.986	0.972	0.983

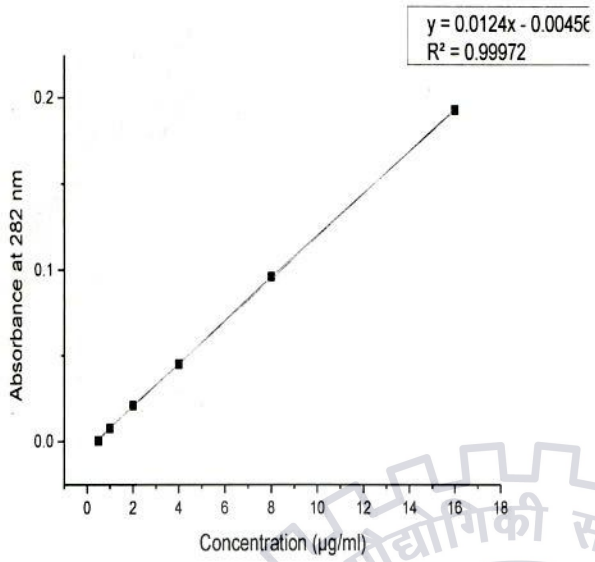
Table 7: UV Absorbance of Dopamine (5µg/ml-80µg/ml) in Tris buffer



GRAPH 8: Standard curve of DOPA (Conc range 5µg/ml-80µg/ml)in Tris

Concentration(in microgram/ml)	Absorbance			Net average absorbance
0.5	0	0.001	0	0.000333
1	0.008	0.007	0.008	0.007667
2	0.022	0.02	0.021	0.021
4	0.045	0.045	0.045	0.045
8	0.097	0.096	0.096	0.09633
16	0.192	0.193	0.194	0.193

Table 8: UV Absorbance of Dopamine (0.5µg/ml-16µg/ml) in Tris buffer



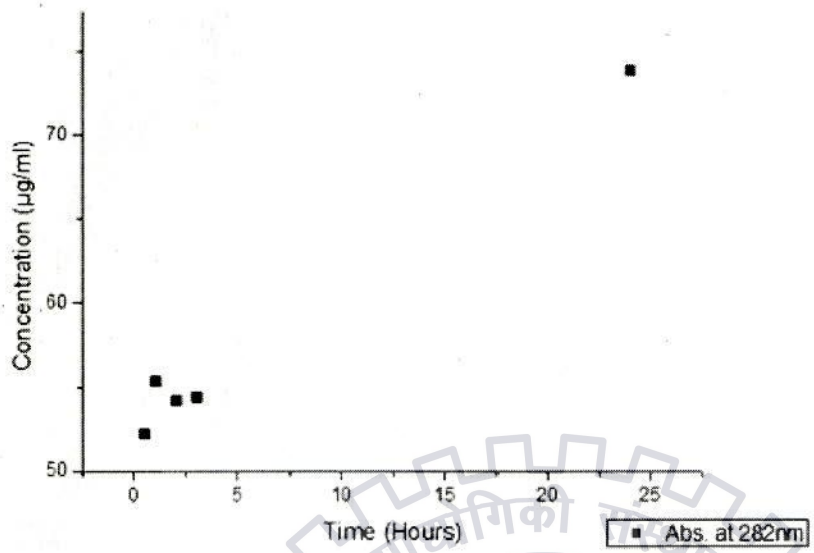
GRAPH 9: Standard curve of DOPA (Conc range 0.5µg/ml-16µg/ml)in Tris

4.3.2 UV Results of Au Nanoparticles coated with P-DOPA

15ml DOPA solution were prepared in Tris buffer (pH-8.5) and 50 µl of citrate coated gold nanoparticles (conc-0.52mg/ml) were added. Samples were taken out at specified time intervals centrifuged at 16000g for 15 minutes uniformly to conduct UV spectroscopy studies.

Mass ratio of nanoparticles to DOPA taken – 1:23.05

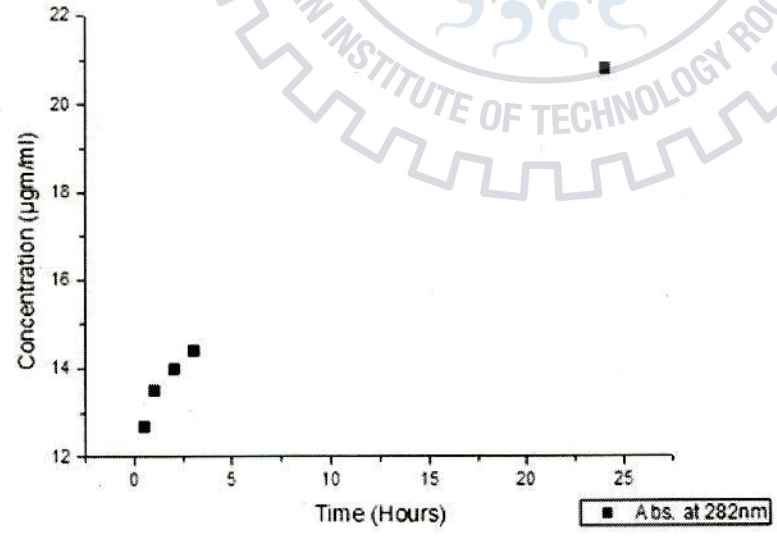
Initial conc. of DOPA 40 µg/ml



GRAPH 10: UV result of DOPA (conc 40 µg/ml) coating onto Au nanoparticles

Initial conc. of DOPA 10 µg/ml

Mass ratio of nanoparticles to DOPA taken – 1:5.525



GRAPH 11: UV result of DOPA (conc 10 µg/ml) coating onto Au nanoparticles

With time the concentration of DOPA in supernatant increased which is contrary to DLS results which has proved that P-DOPA is being coated onto Au nanoparticles in which case there should be a decrease in the concentration of DOPA with time. The reason for this is P-DOPA formation in the solution. To establish this fact P-DOPA solutions of 80μg/ml and 40μg/ml were prepared in Tris buffer at pH 8.5 and a significant color change has been observed with time .Due to the complex kinetics involved and DOPA and P-DOPA having highest absorbance at 282 nm UV method has been proved unsuccessful for identifying the DOPA concentration in the solution. Hence UV study has not been conducted further for Iron oxide nanoparticles.

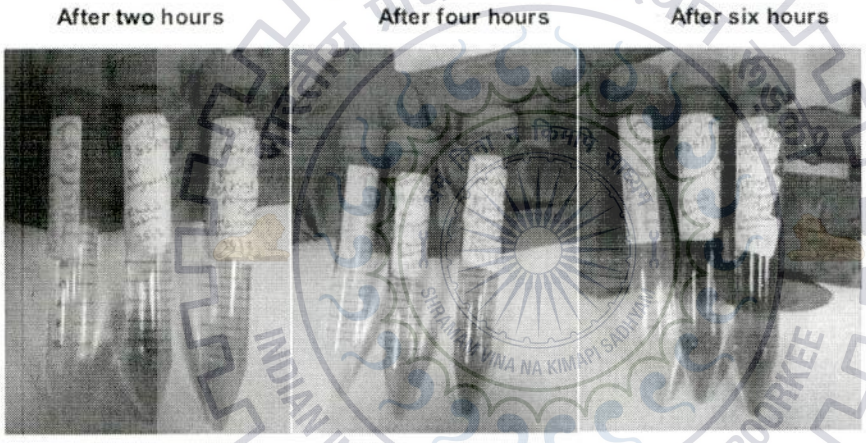


Figure 12: Polymerization of DOPA in solution

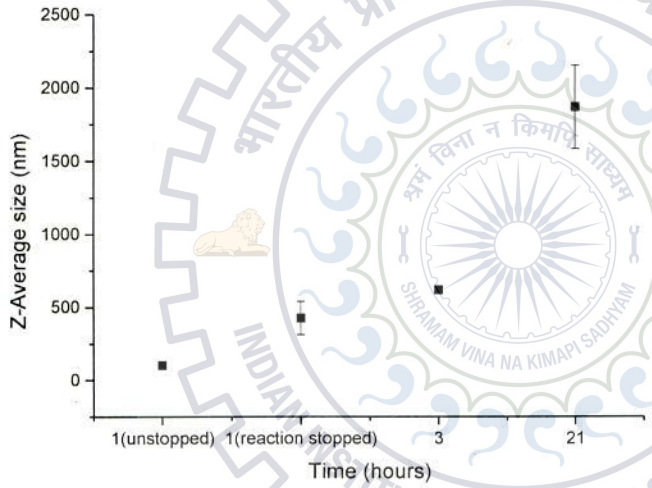
4.4 pH dependence of Au nanoparticles with p-DOPA

15ml DOPA solution (Initial conc 10μg/ml) were prepared in Tris buffer (pH-8.5, conc 10mM) and 50 μl of citrate coated gold nanoparticles (conc-0.52mg/ml) were added. Reaction was stopped after specified time by adding a few drops of Hydrochloric acid and bringing down the pH<5.5. Samples were taken out at specified time intervals to perform DLS.

Reaction stopped after 1hr

Time(in hours)	Z-Average size(in nm)
1 (reaction unstopped)	105
1 (reaction stopped)	427.38
3	618.63
21	1870.83

Table 9: Z-Average size of DOPA coated Au nanoparticles (pH<5.5)-Reaction time 1hour

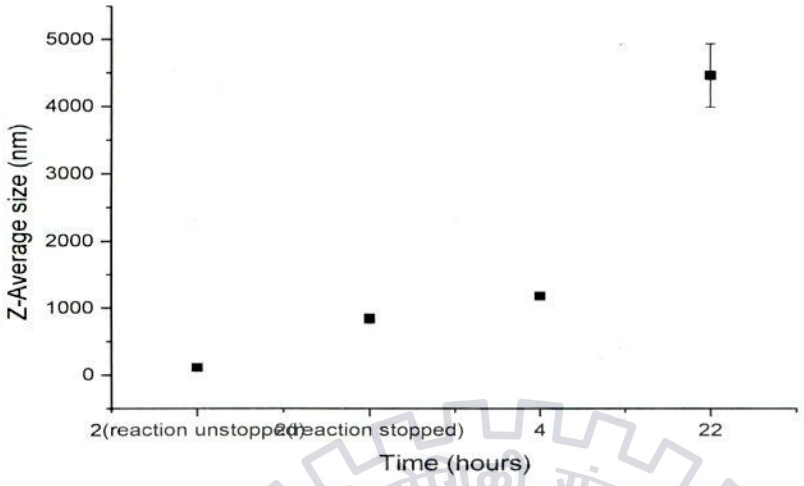


GRAPH 12: Z-Average size of DOPA coated Au nanoparticles (pH<5.5)-Reaction time 1hour

Reaction stopped after 2 hours

Time(in hours)	Z-Average size(in nm)
2 (reaction unstopped)	116.9666667
2 (reaction stopped)	845.3666667
4	1188
22	4491.167

Table 10: Z-Average size of DOPA coated Au nanoparticles (pH<5.5)-Reaction time 2hours



GRAPH 13: Z-Avg size of DOPA coated Au nanoparticles (pH<5.5)-Reaction time 2hours

Instantaneous aggregation is observed immediately after reducing the pH . This feature of P-DOPA can be useful for improving iron loading efficiency onto Kidney stones.

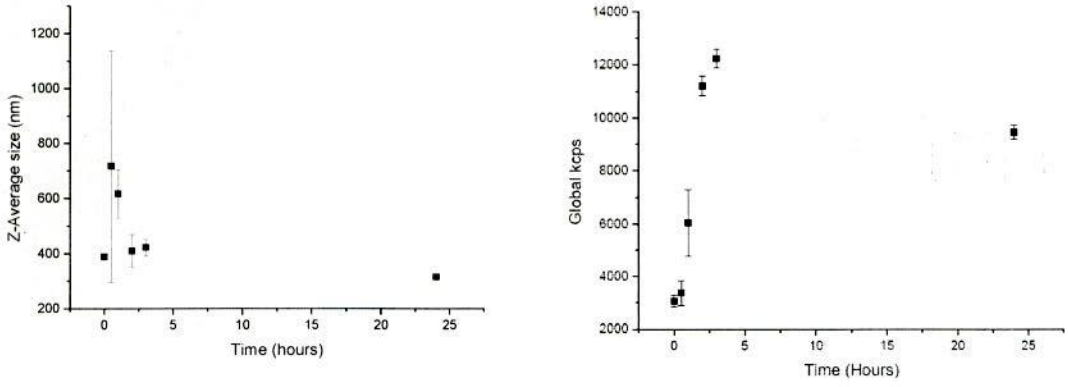
4.5 DLS RESULTS OF IRON OXIDE NANOPARTICLES

To analyze the trend of p-DOPA coating on Iron oxide nanoparticles15ml DOPA solution were prepared in Tris buffer (pH-8.5,conc 10mMolar) and 5 µl of PVA coated iron oxide nanoparticles (conc-5mg/ml) were added. Samples were taken out at specified time intervals to perform DLS

a) **Initial concentration of DOPA 80 µg/ml**

Time	Average size(in nm)	Global kcps
0	389.3	340.91667
0.5	717.33	373.4
1	617.4167	426.366666666667
2	410.25	141.416666666667
3	423.1167	154.366666666667
24	316.75	119.683333333333

Table 11: Z-Average size & Global kcps of DOPA(conc- 80 µg/ml)coated Iron oxide nanoparticles

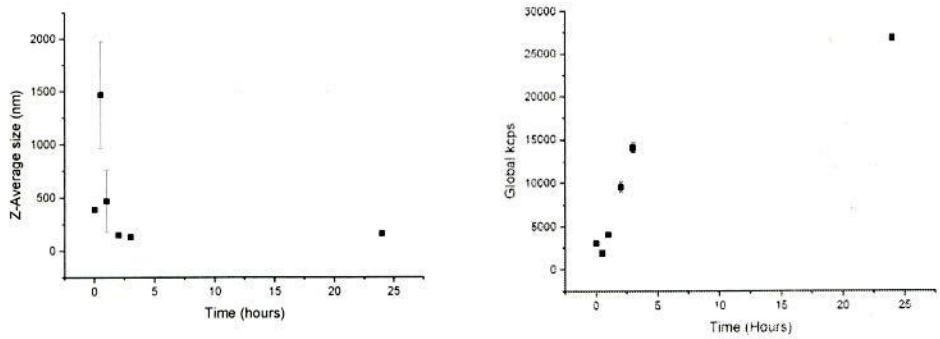


GRAPH 14:Z-Avg size & Global kcps of DOPA(80µg/ml) coated Fe3O4 nanoparticles

b) Initial concentration of DOPA 40 µg/ml

Time	Average size(in nm)	Global kcps
0	389.3	340.91667
0.5	1469.65	384
1	470.5833333	308.48333
2	152.8166667	419.05
3	132.85	177.68333
24	161.7666667	338.73333

Table 12: Z-Average size & Global kcps of DOPA (conc- 40 µg/ml) coated Iron oxide nanoparticles

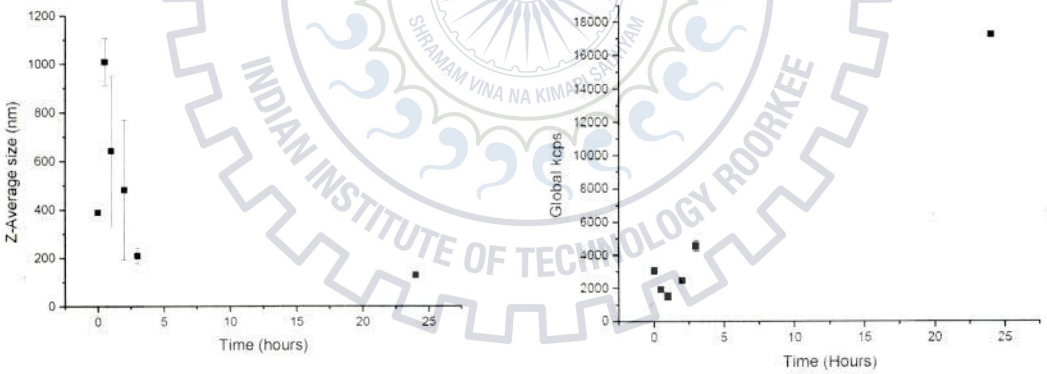


GRAPH 15: Z-Avg size & Global kcps of DOPA (40µg/ml) coated Fe3O4 nanoparticles

c) **Initial concentration of DOPA 20 µg/ml**

Time	Average size(in nm)	Global kcps
0	389.3	340.91667
0.5	1010.38	215.43333
1	643.0833333	167.65
2	482.7833333	275.08333
3	210.1666667	342.78333
24	129.2166667	217.56667

Table 13: Z-Average size & Global kcps of DOPA (conc- 20 µg/ml) coated Iron oxide nanoparticles



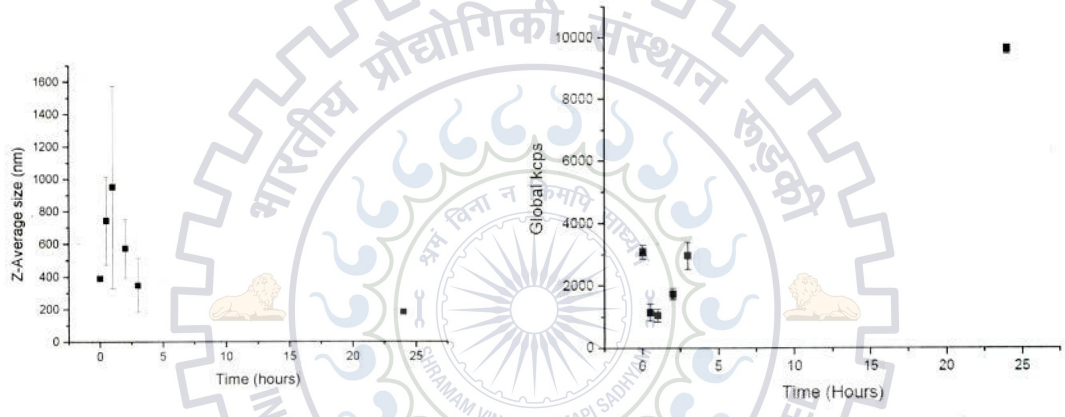
GRAPH 16:Z-Avg size & Global kcps of DOPA (20µg/ml) coated Fe3O4 nanoparticles

d) **Initial concentration of DOPA 10 µg/ml**

Time	Average size(in nm)	Global kcps
0	389.3	340.91667
0.5	744.4666667	321.33333
1	952.0833333	207.06667
2	574.45	193

3	348.0333333	215.16667
24	182.9333333	121.41667

Table 14: Z-Average size & Global kcps of DOPA (conc- 10 µg/ml) coated Iron oxide nanoparticles

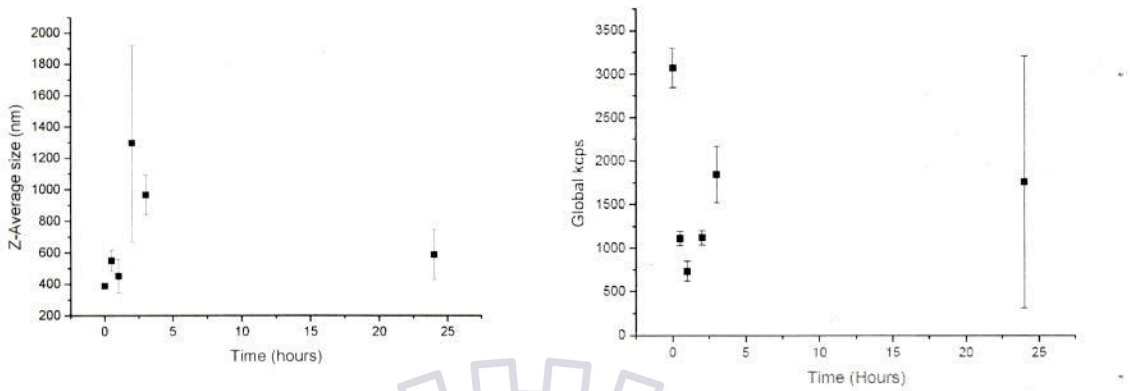


GRAPH 17: Z-Avg size & Global kcps of DOPA (10µg/ml) coated Fe3O4 nanoparticles

e) **Initial concentration of DOPA 5 µg/ml**

Time	Average size(in nm)	Global kcps
0	389.3	340.91667
0.5	550.35	123.51667
1	452.6333333	148.26667
2	1296.6	225.3
3	966.3666667	204.86667
24	590.2333333	335.38333

Table 15: Z-Average size 7 Global kcps of DOPA (conc- 5 µg/ml) coated Iron oxide nanoparticles



GRAPH 18:Z-Avg size & Global kcps of DOPA (5µg/ml) coated Fe3O4 nanoparticles

Within 24 hours there was a decrease in the size of Z-Average size for that samples. The reason might be either the nanoparticles are not being coated and sedimenting down or PVA coating on nanoparticles is degrading with time and being replaced by P-DOPA coating. To find out the facts the global kcps data has been analyzed which has shown that an increasing trend.This proves that size of nanoparticles are increasing with time proving that PVA coating is being replaced by P-DOPA.

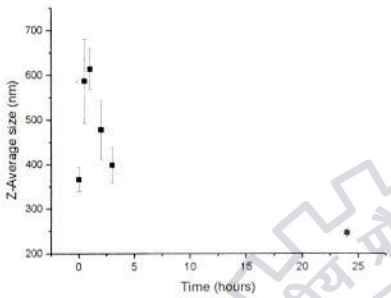
4.6 Control experiments of Iron oxide nanoparticles

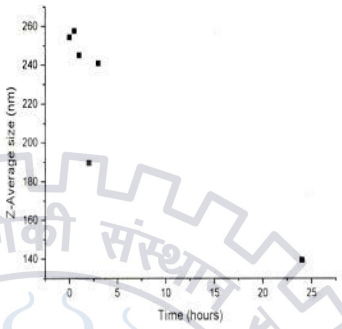
Control experiment of PVA coated iron oxide nanoparticles in Tris and Di-water has been conducted to prove that the Z-Average size trend observed in the experiments in section 4.4 don't reflect that on Iron oxide nanoparticles without DOPA.15ml Tris buffer (pH-8.5) was taken and 5 µl of PVA coated Iron oxide nanoparticles (conc-5mg/ml) were added. Similarly 15ml Di water was taken and 5 µl of PVA coated Iron oxide nanoparticles (conc-5mg/ml) were added Samples were taken out at specified time intervals to perform DLS.

Time	Average size(in nm)
0	366.7
0.5	587.2333333
1	613.9333333
2	478.6666667
3	399.0666667
24	246.6333333

Time	Average size(in nm)
0	254.4
0.5	257.68333
1	245.16667
2	189.73333
3	240.9
24	139.51667

Table 16: Z-Avg size Iron oxide nps in TrisTable 17: Z-Avg size Iron oxide nps in Di water





GRAPH 19:Z-Avg size of Fe3O4 nps in Tris GRAPH 20: Z-Avg size of Fe3O4 nps in Di water

Aggregation is observed with PVA coated nanoaprticles in Tris indicating the degradation of PVA coating on Fe3O4 nanoparticles. The Z-average size of nanoaprticles in Di-water is quiet stable within 24 hours.

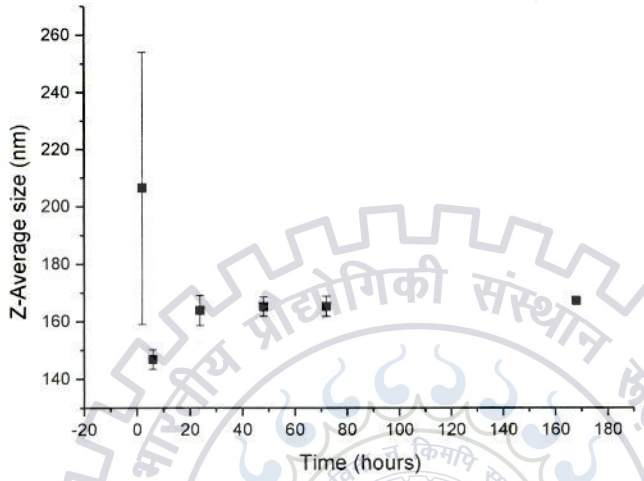
4.7 Stability studies

Stability of coated nanoparticles is one of the feature that has to be verified for storing the coated samples. Hence to identify PVA coated iron oxide nanoparticles stability coating experiment was extended for 1 week and DLS measurements were taken at specified tiem intervals.15ml DOPA solution (conc- 40 µg/ml, 10µg/ml) were prepared in tris buffer (pH-8.5, conc 10mM) and 5 µl of PVA coated iron oxide nanoparticles (conc-5mg/ml) were added. Samples were taken out at specified time intervals to perform DLS.

a) Results for dopa solution with concentration(40µg/ml)

Time(in hours)	Z-Average size(in nm)
2	206.56
6	146.75
24	163.86
48	165.2
72	165.38
1 week	167.36

Table 18: Z-Avg size of DOPA(conc 40µg/ml) coated iron oxide nps(Coating time 1 week)

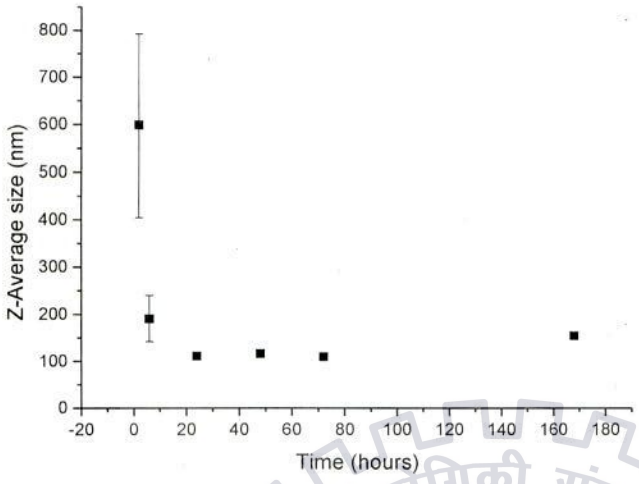


GRAPH 21: Z-Avg size of DOPA (conc 40µg/ml) coated iron oxide nps(Coating time 1 week)

b) Results for DOPA solution with concentration(10µg/ml)

Time(in hours)	Z-Average size(in nm)
2	598.76
6	191
24	111.15
48	116.4
72	109.5
1 week	155.6

Table 19: Z-Avg size of DOPA (conc 10µg/ml) coated iron oxide nps(Coating time 1 week)



GRAPH 22: Z-Avg size of DOPA (conc 10µg/ml) coated iron oxide nps(Coating time 1 week)

The P-DOPA coating onto PVA coated iron oxide nanoparticles is found to be quiet stable with both the concentrations for 168 hours.

4.8 Shear adhesion test

Pellet preparation

Calcium hydroxide pellets were prepared using the hydraulic press of FTIRby applying a pressure of 0.5 tons for 1 minute uniformly on all thesamples.
Dimensions of pellet were measured using Vernier Calipers.

Weight of the pellet(gms)	Pressure(Tons)	Time(sec)	Thickness of pellet(mm)	Diameter of pellet(mm)
0.3	0.5	60	1.9	13.1
0.5	0.5	60	3.8	13.1
0.7	0.5	60	4.4	13.1
0.9	0.5	60	4.9	13.1
1.1	0.5	60	6.6	13.1

Table 20: Details of Calcium hydroxide pellets

Pellet prepared from 0.3 grams mass of Calcium hydroxide has dimensions suitable for performing adhesion test using chatillon tester.With the help of this data Kidney stone pellet(Weight- 0.2 gms, Diametre-13.1mm) was prepared using hydraulic press of FTIR(pressure-1ton,Time-60secs) .

P-DOPA solution (conc -500µg/ml) was added in between the prepared kidney stone pellets

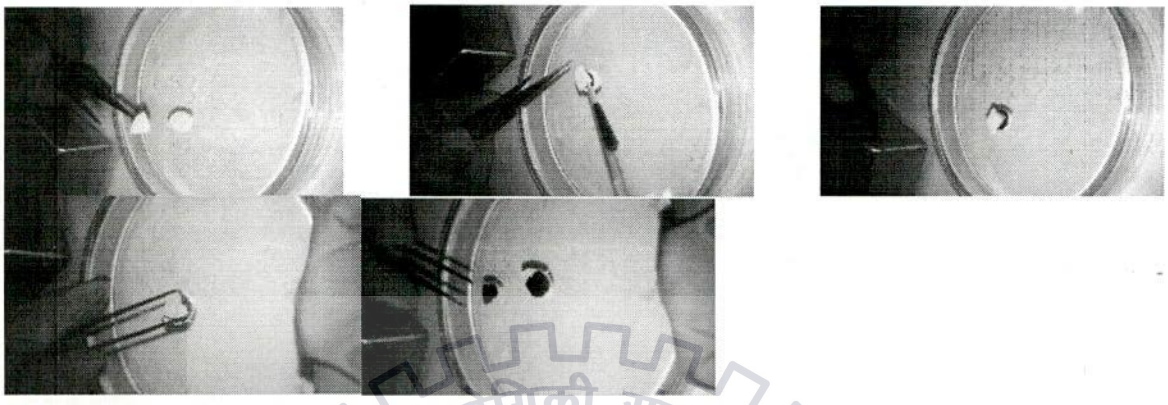


Figure 13: P-DOPA attaching to Kidney stone pellets

- P-DOPA instantly attached to kidney stone pellets as well as Calcium hydroxide pellets indicating a chelation bond between calcium of kidney stones and P-DOPA. But due to P-DOPA has undergone a cohesive failure. Thus could not proceed with the designed Shear adhesion test with Chatillon tester.
- With this test it was successfully proved that P-DOPA chelates to calcium.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Dopamine is undergoing polymerization within 24 hours onto nanoparticles. Also with iron oxide nanoparticles the aggregates of iron oxide nanoparticles formed due to the degradation of PVA in Tris buffer has been broken down by P-DOPA formation thus stabilizing the nanoparticles.

P-DOPA has been proved to chelate with calcium of kidney stones and the adhesive bond formed between P-DOPA and kidney stone is found out to be quiet strong. Polydopamine coating onto nanoaprticles is found out to be quiet stable within one week in Tris buffer. Thus Polydopamine has been proved as the ideal bioadhesive for implementing the novel technique of removing kidney stones that has been discussed in the report.

5.2 Recommendations

- Magnetization studies has to performed on P-DOPA coated iron oxide nanoparticles to identify the effect of P-DOPA coating on magnetization values of nanoparticles.
- Theoretical iron loading efficiency on each kidney stone has to be determined.
- Using the iron loading efficiency values average magnetization value that can be induced into a kidney stone of size range (0.5mm-2mm) for a particular coating thickness of P-DOPA has to found out.
- Feasibility studies has to conducted to determine whether the deduced Average magnetisation values are sufficient enough to pull a kidney stone of size range(0.5mm-2mm) with a magnetic catheter of maximum field strength of 103 milliT.

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