

A
Thesis
On
**“Detection of Melamine in Milk Using Gold
Nanoparticles”**

Submitted in the fulfilment of the partial requirements for the award of degree of

Master of Technology
In
Material Science
(2015-2017)

Submitted by

Amandeep Kaur
(601502001)

Under the Supervision of

Dr. Bhupendra kumar Chudasama
(Associate Professor)



SCHOOL OF PHYSICS AND MATERIALS SCIENCE
THAPAR UNIVERSITY
PATIALA – 147004
July 2017

A grateful heart is a beginning of greatness

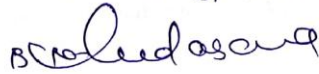
I dedicate this thesis to my parents. I am really thankful for providing me the best education and inspiring me so that I can accomplish my dreams.

CERTIFICATE

I hereby certify that the work which is being presented in the dissertation entitled "**Detection of Melamine in Milk Using Gold Nanoparticles**" in partial fulfilment of requirement for the degree of M. Tech (Material Science) submitted in the Department of (SPMS) at THAPAR UNIVERSITY, PATIALA is an authentic record of my own carried out during a period from "Jan 2017 to July 2017" under the supervision of **Dr. Bhupendra Kumar Chudasama**. The matter presented in this dissertation has not submitted by me in any other University/ Institute for the granting of M. Tech degree.

I, hereby, further declared that in case of legal dispute in relation to my M. Tech Dissertation, I will be solely responsible for the same.

Date: 15th July, 2017.



Dr. Bhupendra Kumar Chudasama

(Associate Professor)

School of Physics and Material Science

Thapar University, Patiala-147004.

Amandeep Kaur,
Signature of Candidate

(Amandeep Kaur)

ACKNOWLEDGMENT

First of all I want to thank God for giving me this beautiful life. This is only God gift that I am getting everything I want. I am studying because GOD has written this is my fate. Without luck nothing is possible. Thank you God for giving me everything. It gives me great pleasure in expressing my gratitude to all those people who have supported me and had their contributions in making this thesis able to be done. First and foremost, I must acknowledge and thank. The Almighty God for his blessings, securing and guiding me throughout this period.

I would like to place on record my deep sense of gratitude to my Supervisor Dr. B.N Chudasama Associate Prof. of School of Physics and Material Science, Thapar University, Patiala, India for his energizing guidance, continuous inspiration and supervision throughout the course of present work and giving me the freedom to pursue topics of my interest and providing me with exactly the amount of structure needed to ensure my success. You are not simply taught me how to succeed as a student, but rather how to be independent researcher. Thank you so much for all the academic, professional and personal advice that you have given me.

I express my sincere gratitude Ms. Parveer Kaur, Ms. Navjot and and Ms. Purnima Sharma, PhD Scholar at Thapar University Patiala, India, for their generous guidance, help and useful suggestions. You have taught me how to do the research work. I would like to convey my sincere gratitude towards my Friend Ms Barinder Kaur for her kind support, timely help, valuable discussion and encouragement.

The completion of this dissertation would not have been possible without the boundless encouragement my intellectual and personal growth. From you all, I have learned to take pride in my work and to enjoy the simple pleasure of a job well done. Thank you for everything that you have given me.

Amandeep Kaur

LIST OF FIGURES

Figure	Title	Page No.
Figure 1.1	Dimension of different objects in the length scale.	1
Figure 1.2	Schematic representation of top-down and bottom-up approach.	3
Figure 1.3	Schematic representation of Plasmon oscillation for a sphere.	5
Figure 3.1	Schematic representation of mechanism of synthesis of gold nanoparticles.	21
Figure 3.2	Representation Synthesis of Spherical Gold Nanoparticles.	22
Figure 3.3	UV-Visible spectra of GNPs shows SPR peak at 520 nm.	24
Figure 3.4	Hydrodynamic Particle size distribution of GNPs	25
Figure 3.5	(a) UV-Visible spectra of Melamine, (b) Calibration curve of melamine.	26
Figure 3.6	UV-Visible spectra and absorbance (P_2/P_1) with time of Melamine reacts with gold nanoparticles by 1:1 v/v.	27
Figure 3.7	Hydrodynamic size distributions of Melamine reacts with gold Nanoparticles by 1:1 v/v.	28
Figure 3.8	UV-Visible spectra of Melamine react with GNPs in v/v of 1:2.	29
Figure 3.9	Hydrodynamic Size Distribution of 2mM Melamine reacts with GNPs (1:2)	30
Figure 3.10	UV-Visible Spectra of Melamine reacts with GNPs v/v is 1:5.	31
Figure 3.11	UV-Visible spectra of Melamine react with GNPs v/v 1:5	32
Figure 3.12	Hydrodynamic Size Distribution of Melamine after Reaction with GNPs of v/v 1:5.	33
Figure 3.13	Standard curve of Melamine reacts with GNPs of v/v 1:5 at nM.	34
Figure 3.14	UV-Visible spectra of GNPs-infant formula Powder	34

LIST OF TABLES

Table	Title	Page No.
Table 1.1	Difference between the different detection methods for melamine	8
Table 2.1	various methods for synthesis of GNPs	12-14
Table 2.2	various methods for synthesis of GNPs	15-17

Abstract

Melamine is a chemical compound generally used in milk based products. It is a highly toxic compound and not permitted to be use with food. Excessive intake of melamine can cause several side-effects that can lead to death in infants. Therefore, melamine detection in milk products has become essential. Existing methods of melamine detection have limited detection and they are complex and time consuming. In this thesis a simple colorimetric method for the detection of melamine is developed by using gold nanoparticle based surface Plasmon resonance. Gold Nanoparticles have tendency to agglomerate in the presence of melamine results into shift in Plasmon band of melamine. Effect of concentration of gold Nanoparticles on detection limit of melamine was evaluated. The melamine concentration measured in the tested sample of infant formula powder is 39nM which is well below the FDA limit of ppm. The method is rapid, simple, cost effective and highly reproducible.

Table of Contents

Chapter 1 Introduction

1.1 What are nanoparticles?	1-2
1.2 Historical Background of Gold	2
1.3 Nanoparticle synthesis strategies	2
1.3.1 Chemical Methods	3
1.3.2 Physical methods	3
1.4 Gold Nanoparticles	4
1.4.1 Gold Nanostructures in Sensors	4
1.4.2 Surface Plasmon resonance	5-6
1.5 Food Adulteration	6
1.5.1 Intentional Adulteration	6
1.5.2 Unintentional Adulteration	6
1.5.3 Natural Adulteration	7
1.6 Melamine	7
1.6.1 Toxicity of Melamine	7
1.7 Methods of Melamine Detection	7
1.7.1 Colorimetric Detection Method	7- 8
References	9-11

Chapter 2 Literature Survey

2.1 Introduction	12
2.2 Methods for synthesis of spherical GNPs.	12-14

2.3 Detection of Milk Adulteration	15-17
References	18-19
Chapter 3 Experimental Techniques, Results & Discussions	
3.1 Introduction	20
3.2 Chemical Reagents	20
3.3 Instrumentation	20
3.4 Stock Solution Preparation	20
3.5 Turkevich Method for gold nanoparticles Synthesis	20-22
3.6 Detection of Melamine	
3.6.1 Detection of Melamine using Spherical Gold Nanoparticles	22
3.6.2 Sample preparation for Detection of Infant Formula Powder	22
3.6.3 Detection of Melamine in Infant Formula	23
3.7 Characterization Techniques	
3.7.1 UV-Visible Spectroscopy	23
3.7.2 Dynamic Light Scattering	23-24
3.8 Results and Discussion	
3.8.1 Characterization of Gold Nanoparticles	24
3.8.1.1 UV-Visible spectroscopy	24
3.8.1.2 Dynamic Light Scattering	25
3.8.2 Melamine measurement based on colorimetric detection	25- 34
3.8.3 Detection of Melamine in Infant Formula Powder	34-35
References	36
Conclusion	37

1. 1. What are Nanoparticles?

Nanoscience and nanotechnology are new and revolutionary advancements in the field of Science and technology which are sprouting at very fast rate. It is actually motivated by the desire to manufacture substances with new and enhanced properties so that the materials become able to impact virtually all spheres of the physical, chemical, biological and health sciences. Initially ‘nano’ word was derived from the Greek word ‘nanos’, which stands for dwarf or extremely small. Mathematically, a nanometer is equals to 10^{-9} m which would probably be billionth of a meter. The size of the nanoparticles usually falls under the range of 1-100 nm in all the three mediums whether they are dispersed in all forms of matter. Nanoparticles are consisted of number of atoms or molecules which are bonded together and intermediate in the sizes ranging from individual atoms to aggregates which are large enough for the formation of bulk material.^(1,2) *Figure [1.1]* depicts the dimensionality of different systems. The materials at the nanoscale regime illustrate the behavior which is in-between of an atom or molecule system and a microscopic solid because whenever the size of nanoparticles is found maximum but less than bulk particles.

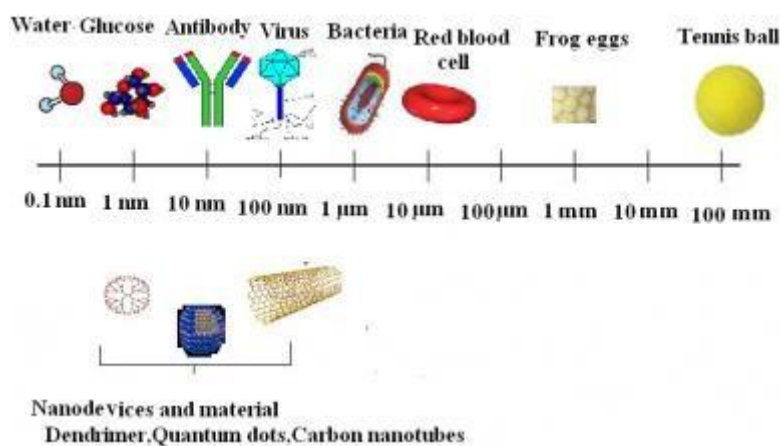


Figure 1.1:- Dimension of different objects in the length scale as shown by Allhoff

Lin P and Moore “John Wiley and Sons” (2010) 3-5.

These differences are the results of two important phenomenon. First, whenever the size of a particle is abridged, its surface-to-volume ratio increases, which means that in comparison to its bulk size, the number of atoms at the surface of the particle increases. A cluster having a

radius of 1 nm is consisted of near about 25 atoms while most of the atoms are on its surface. So, now the properties of the material become dominated by the surface of the structure properties, which were earlier certained by the bulk lattice's molecular structure. Second, at the nanometer scale regime the motion of the electrons and its properties become susceptible to the size of the particles. This special effect is called the quantum size effect ⁽³⁾ (QSE) which is specific to the small size of particles resulting in opens up the prospect to craft materials and devices with completely new properties.

1. 2. Historical background of Gold

The extraction of the gold was started in Bulgaria in near about 5th millennium B.C. but the gold in 'soluble' form was appeared in Egypt and China around the 5th century B.C.⁽⁴⁾ The use of soluble gold was done in number of diseases like heart problems, dysentery, epilepsy tumors and for the diagnosis of syphilis to reveal its magnificent curative powers until the middle ages. The gold solution's optical properties have fascinated interest since the romans began to utilize them as colorants in glasses, which were known as 'Purple of Cassius'. Michael Faraday started the scientific research on gold solution as a revolutionary work.⁽⁵⁾ Faraday, in 1857, formed deep red solutions of colloidal gold by using reduction method. For this purpose, an aqueous solution of chloroaurate (AuCl_4^-) was reduced using phosphorus in CS_2 . Thin films optical properties were investigated by him which were prepared from dried colloidal solutions and upon mechanical compression, he observed the reversible change in color of the films. In 1861, the term 'colloid' was coined by shortly thereafter by Graham. In 1908, G. Mie ⁽⁶⁾ became able to elucidate the red color of the gold nanoparticles in solution. In fact, Mie got motivated to pertain the general theory of light extinction to small particles by realizing that the color variation of colloidal gold particles occur with size. His idea has led to the expansion of new meadow of cluster science and has become the subject of immense investigation as the time progresses.

1.3. Nanoparticle synthesis strategies

The nanoparticles are formed by using two different approaches : 'top-down' and 'bottom-up'.⁽⁷⁾ Under the top-down approach, the nanomaterials are formed by breaking bigger materials into small and fine particles, while under the bottom-up approach, nanomaterials are created by the assemblage of atoms or molecules. In the production of metal colloids, the size or shape of nanoparticles can be controlled by the adjusting the concentration ratio of the

chemicals making the nanoparticle to that of the selected capping material. This kind of production is known as the ‘bottom-up’ method. In ‘top-down’ method, lithographic or laser ablation-condensation techniques are used to machine down the bulk materials into the nanometer length scale. A schematic presentation of the ‘top-down’ (physical method) and ‘bottom-up’ (chemical method) approaches is shown in *Figure [1.2]*. From the viewpoint of mass production of metal nanoparticles, the physical methods are less effective than the chemical ones.

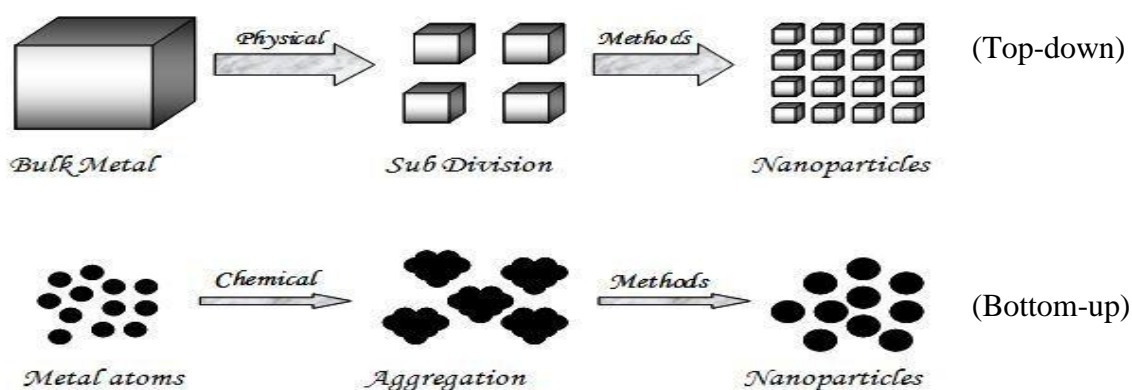


Figure 1.2:- Schematic representation of top-down and bottom-up approach.

1.3.1. Chemical methods

By using chemical routes, metallic nanoparticles can be formed by using iterative (single-step) and non-iterative growth (seed-mediated) methods. Chemical reduction is most commonly used synthesis method. It generally consists of a soluble metal salt, a reducing agent and a stabilizing agent. For this purpose, commonly used reducing agents are citrate, sodium borohydride ^(8, 9) and alcohols ⁽¹⁰⁾. The role of the stabilizing agent is to cap the particle and prevents its further growth or aggregation. The seeded-growth procedure (non-iterative) is another commonly used technique. Variation in the ratio of metal salt to seed can manipulate the size of material. To avoid secondary nucleation, a one step seeding method is less effective than step by step enlargement of particle and therefore to control nanoparticle size distribution. ⁽¹¹⁻¹³⁾

1.3.2. Physical methods

UV-irradiation, ⁽¹⁴⁻¹⁸⁾ near-IR laser irradiation, ⁽¹⁹⁻²¹⁾ thermolysis ⁽²²⁾ or sonolysis, ⁽²³⁻²⁵⁾ radiolysis ⁽²⁶⁻²⁷⁾ etc. methods have been adopted under physical methods for production of metal nanoparticles in a variety of environments. Recently, there has been growing interest in

preparing metal nanoparticles by using lithographic techniques⁽²⁸⁻³²⁾ Nanosphere lithography (NSL), originally known as ‘natural lithography’, is an inexpensive nanofabrication technique which comes under the second generation innovation technique and is now being employed in laboratories around the world.

1.4. Gold Nanoparticles

In nanotechnology, Gold based nanostructures come under imperative research subjects. Recently, the tremendous research on gold nanostructures has been emerging and its advanced applications have been reported. Gold is an old material used by ancient Egyptians and Chinese in the 5th or 4th century B.C.⁽³³⁾ The other evidences are also available which shows that ancient Romans also used gold colloids to stain glass red or mauve. However, Michael Faraday, in the 1850s⁽³⁴⁾, was the first scientist to report literature of gold nanoparticles (GNPs). The surface functionality, morphology, stability and solubility of GNPs can be controlled by using various synthetic routes⁽³³⁻³⁸⁾.

1.4.1 Gold Nanostructures in Sensors

GNPs, within diameter of 1-100 nm, have been reported to be extensively used in biological and chemical sensors due to their excellent chemical and physical properties. Due to their unique optical properties, GNPs catch the incredible attention from different field of science and especially in advancement of sensors. With increase in the particle size, the solution of spherical GNP shows a range of vibrant colors and in ancient time they were used to stain glass. When the particles are excited with the incident light, on the surface of GNPs, there is collective oscillation of electrons occurs which in further results in the intense color caused by the strong absorption and scattering of light⁽³⁹⁾. This observable fact is called as surface plasmon resonance (SPR) which is greatly dependent on size and shape of the particles. So, the SPR peak can be tuned by manipulating the size of GNPs particles. Along with size and the shape, the SPR peak is also sensitive to many other factors like protective ligand, refractive index of solvent, and temperature. The interparticle distance particularly shows great influence on SPR. That is why, the red-shifting and the broadening of the peak is experienced when the GNPs particles get aggregated via analyte binding. The change in the color in aggregated GNPs from red to blue is considered as the principle of colorimetric sensing.

1.4.2. Surface plasmon resonance

A strong absorption band in UV-visible region is exhibited by Metal nanoparticles, which can be the result of the surface plasmon resonance of the metal clusters. Under the irradiation of light, a small spherical metallic nanoparticle exhibits conduction of electrons to oscillate coherently which is caused by the oscillating electric field. This is schematically presented in *Figure [1.3]*. When the electron cloud is displaced relative to the nuclei, a restoring force arises from Coulomb attraction between electrons and nuclei that results in oscillation of the electron cloud relative to the nuclear framework. The oscillation frequency is determined by four factors: the density of electrons, the effective electron mass, shape and size of the charge distribution.

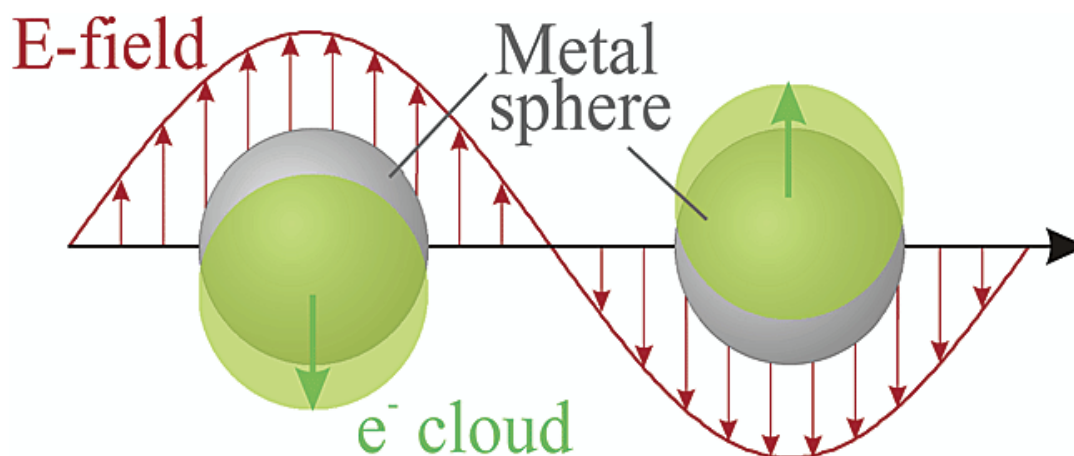


Figure 1.3:- Schematic representation of Plasmon oscillation for a sphere, showing the displacement of the conduction electron charge cloud relative to the nuclei as a result of electromagnetic field as shown by Kelly et al. *J. Phys. Chem. B* 2003, 107, 668.

This collective oscillation of the electrons is called the dipole plasmon resonance of the particle. Higher modes of plasmon excitation can occur, such as the quadrupole mode where half of the electron cloud moves parallel to the applied field and half moves anti-parallel.

The width and frequency of the surface plasmon absorption depend on the shape and size of the metal nanoparticle as well as on its surrounding medium and of the dielectric constant of the metal itself. ⁽⁴¹⁻⁴³⁾ The reason why the noble metals have historically fascinated scientists dating back as early as Faraday ⁽⁵⁾ is due to strong Plasmon resonance and its shifting to the visible part of electromagnetic spectrum for noble metals (gold, copper and silver).

Most of the other transition metals show only a wide and weakly resolved absorption band in the ultraviolet.⁽⁴⁴⁾ This difference is the result of the interband excitation, plasmon transition and the Drude free-electron model can be used to well approximate the electrons in conduction-band of the noble metals.⁽⁴⁵⁾ According to this model, the electron in conduction-band are treated as they are independent from the ionic background and can move “freely,” whereas the ions play the role of scattering centers. This in turn gives the electrons in the noble metals a higher polarizability, thereby shifting the plasmon resonance to lower frequencies and also giving rise to a sharp bandwidth.

1.5. Food Adulteration

Food is composed of carbohydrates, water, fats, proteins and many other things which can be drunk or eaten by humans, including animals, for nutrition or pleasure. In other words, food can be considered as the basic need for every living being and very important aspect of life. But now day’s foods are affected by number of adulterants. Adulteration is a substance resulting in the reduction of the vital importance of food. It is the subtraction or addition of any unwanted material from or to food which influence the quality, natural composition as well as the nutritive value of the original food substance. It is not easy for the consumer to notice the adulteration degree. Food adulteration can either be intentional, unintentional or natural.⁽⁴⁶⁾

1.5.1. Intentional Adulteration

Intentional adulteration can be defined as the removing or adding substances to changing the existing natural properties of food knowingly. It can be the substances added in food to improve its appearance, flavor, texture and storage properties. Some examples of this adulteration are melamine addition to infant formula and pet food which falsify the level of protein content, coloured leaves and iron fillings to tea causes appendicitis and small intestine problems and oxytocin a hormone used for faster growth of pumpkin, watermelon, brinjal, gourds, cucumber can leads to brain damage.

1.5.2. Unintentional Adulteration

Unintentional adulteration is the accreditation to carelessness, lack of facilities for maintaining food quality or ignorance. This adulteration results from insect and pesticide residues or microorganisms entering the food right from the farm through other stages leading to the customer causes disruption of internal metabolism of cells.

1.5.3. Natural adulteration

It occurs due to the presence of certain organic compounds, chemicals or radicals which are naturally occurring in foods and are injurious to health and are not added to the foods.

1.6 Melamine

Melamine $C_3H_6N_6$ (2, 4, 6-triamino-1, 3, 5-triazine) is a triazine heterocyclic organic compound. It is produced from urea releasing cyanuric acid which is used in the fabrication of different resins, flame retardants, fertilizer and other products. This organic compound is commonly found in milk and the products based on milk. Melamine can be hydrolyzed to formulate the insoluble crystals used in kidneys and subsequent tissue injury, bladder cancer etc. The main purpose was to boost the earnings by watering the milk and then addition of melamine to boost the apparent protein content in milk because of nitrogen-rich organic base of melamine. ⁽⁴⁷⁻⁴⁹⁾

1.6.1 Toxicity of Melamine

The main toxic effects of dietary exposure to melamine in mice and rats were inflammatory reactions, calculi formation and hyperplasia in the urinary bladder ⁽⁵⁰⁾, Melamine crystalluria has been reported in dogs. ⁽⁵¹⁾

Melamine cyanurate exhibits very low solubility and it can be hypothesized that it can result in the development of melamine cyanurate crystals in the kidney. It is assumed that cyanuric acid and melamine are absorbed in the GI tract, distributed systemically and precipitate in the renal tubules leading to progressive tubular blockage and degeneration. ⁽⁵²⁾

1.7 Methods of Melamine detection

Till date different kinds of methods have been engaged for detection of melamine in milk based product like NIR ⁽⁵³⁾, Spectrometry, ⁽⁵⁴⁾ Chromatography ⁽⁵⁵⁾, Capillary electrophoresis ⁽⁵⁶⁾ etc. These methods have need of complex procedure and they are very time consuming, costly and laborious.

1.7.1 Colorimetric Detection Method

Surface stabilization of gold nanoparticles is the basic principle behind the colorimetric detection of melamine. Gold nanoparticles are delicately and finely dispersed in the aqueous solution formed by using citrate reduction method. Gold nanoparticles experience red wine color and show a strong SPR peak at 523 nm ⁽⁵⁷⁾. There is a relation between the position of

the peak of the SPR band and the distance between the particles. Combination of melamine and gold nanoparticles consequences in the aggregation of the gold nanoparticles caused by the strong attraction between melamine and gold chloride ions. Red shift of SPR peak of gold nanoparticles occur that will change the change in color of AuNPs colloid from red wine to purple is due to the aggregation in melamine and gold nanoparticles ^(57, 58). This change in the color of colloid would act as mark or the signature of the presence or absence of the melamine and thus provide a sensitive visual detection of melamine. This technique exhibits the limit of detection (LOD) 0. 4ppm. The whole process got finished in less than 1 hour from commencement of sample to final result from spectrophotometer.

Table [1.1] describes the difference between the different detection methods for melamine.

NIR	Spectrometric	Chromatography	Capillary electrophoresis	Colorimetric
Low cost	Rapid & Cost effective	Instruments are relatively cheap to maintain and use.	Efficient and cost-effective, minimum sample and reagent consumption	Visible color changes by naked eye
Easy sample preparation	Easy sample preparation	Derivative reagent is expensive much more time consuming	If coupled with UV detection, the detection sensitivity and reproducibility would become a problem	Simple and rapid
High limit of detection (LOD) Below 1ppm.	Low LOD 0.033ppm.	Low LOD 0.035ppm	Low LOD 0.054ppm.	High LOD 0.0025ppm

References:

1. U. Kreibig, M. Vollmer, "Optical Properties of Metal Clusters", Springer: Berlin **1995**.
2. G. Schmid, "Clusters and Colloids – From Theory to Applications", VCH, Weinheim, **1994**.
3. R. Kubo, "J. Phys. Soc. Jpn", 17(**1962**) 975.
4. M.C Daniel, D. Astruc, "Chem. Rev." 104(**2004**) 293-346.
5. M. Faraday, "Philos. Trans. R. Soc. London" 147(**1857**) 145-181.
6. G. Mie, "Ann.Phys." 25(**1908**) 377-445.
7. N. Toshima, T. Yonezawa, "New J. Chem." 22(**1998**) 1179-1201.
8. K.K Caswell, C.M Bender, C. J Murphy, "NanoLetters" 3(**2003**) 667-669.
9. N. Pradhan, N.R Jana, T.Pal, "Surface Sci. Tech." 16(**2001**) 188.
10. A. J Henglein, "Phys. Chem." 97(**1993**) 5457-5471.
11. T.K Sau, A. Pal, N. R Jana, Z. L Wang, T. J Pal, "Nanoparticle Res." 3(**2001**) 257-261.
12. T. K Sau, C. J Murphy, "Langmuir" 20(**2004**) 6414-6420.
13. C. J Johnson, E. Dujardin, S. A Davis, C, J Murphy, S. J Mann, "Mater. Chem." 12(**2002**) 1765-1770.
14. A. Pal, "Talanta" 46(**1998**) 583.
15. Z. Huang, G. Mills, B. Hajek, "J. Phys. Chem." 97(**1993**) 11542-11550.
16. S. Mossmer, J.P Spatz, M. Moller, T. Aberle, J. Schmidt, W. Burchard, "Macromolecules" 33(**2000**), 4791-4798.
17. S. Meltzer, R. Resch, B. E Koel, M. E Thompson, A. Madhukar, "Langmuir" 17(**2001**) 1713-1718.
18. K. Mallick, Z. L Wang, T. Pal, "J. Photochem. Photobiol." 140(**2001**) 75.
19. Y. Zhou, C. Y Wang, Y.R Zhu, Z.Y Chen, "Chem. Mater. " 11(**1999**) 2310-2312.
20. Y. Niidome, A. Hori, T. Sato, S. Yamada, "Chem. Lett." 29(**2000**) 310-311.
21. J. A Reed, A. Cook, D.J Halaas, P. Parazolli, A. Robinson, T.J Matula, F. Griezer, "Ultrason. Sonochem." 10(**2003**) 285.
22. M. Nakamoto, M. Yamamoto, M. Fukusumi, "Chem. Commun." (**2002**) 1622-1623.
23. W. Chen, W.P Cai, C.H Liang, L.D Zhang, "Mater. Res. Bull." 36(**2001**) 335-342.
24. W. Chen, W. Cai, L. Zhang, G. Wang, L. Zhang, "J. Colloid Surf. Sci." 238(**2001**) 291-295.
25. V. G. Pol, A. Gedanken, Calderro-Moreno, "J. Chem. Mater." 15(**2003**) 1111-1118.
26. A. Henglein, D. Meisel, "Langmuir" 14(**1998**) 7392-7396.

27. A. Dawson, P.V Kamat, "J. Phys. Chem. B" 104(2000) 11842-11846.
28. J. Belloni, M. Treguer, H. Remita, R. de Keyzer, "Nature Materials" 402(1999) 865-867.
29. K. Balaji, R.P Kulkarni, A. Majumdar, "Nanoletters" 4(2004) 1521.
30. R. J. Barsotti, M.S O'Connell, F. Stellacci, "Langmuir" 20(2004) 4795.
31. A. J Haes, S. Zou, G.C Schatz, R.P van Duyne, "J. Phys. Chem. B" 108(2004) 6961-6968.
32. P. M. Mendes, S. Jacke, K. Critchley, J. Plaza, Y. Chen, K. Nikitin, R.E Palmer, J.A Preece, S.D Evans, D. Fitzmaurice, "Langmuir" 20(2004) 3766-3768.
33. M. C. Daniel, D. Astruc, "Chem. Rev." 104(2003) 293-346.
34. M. Faraday, "Philos. Trans. R. Soc. Lond." 147(1857) 145-181.
35. M. Grzelczak, J. Perez-Juste, P. Mulvaney, L.M Liz-Marzan, "Chem. Soc. Rev." 37(2008)1783-1791.
36. H. Feng, Y. Yang, Y. You, G. Li, J. Guo, T. Yu, Z. Shen, T. Wu, B. Xing, "Chem. Commun." 15(2009) 1984-1986.
37. K. N. Thakkar, S.S Mhatre, R.Y Parikh, "Nanomed. Nanotechnol. Biol. Med." 6(2010) 257-262.
38. K. Saha, S. S. Agasti, C. Kim, X. Li, V.M Rotello, "Chem. Rev." 112(2012) 2739-2779.
39. P. K. Jain, K. S. Lee, I.H El-Sayed, M.A El-Sayed, "J. Phys. Chem. B" 110(2006) 7238-7248.
40. Z. Yuanchao, Wendy Chu , Alireza Dibaji Foroushani , Hongbin Wang , Da Li , Jingquan Liu , Colin J. Barrow , Xin Wang , and Wenrong Yang, "A Review, Materials" 7(2014) 5169-5201.
41. M. Kerker, "New York: Academic" (1969).
42. C. F. Bohren, D.R Huffman, "New York: Wiley" (1983).
43. G. C. Papavassiliou, "Prog. Solid State Chem." 12(1979) 185.
44. J. A. Creighton, D.G Eadon, "J. Chem. Soc. Faraday Trans." 87(1991) 3881-3891.
45. N. W. Ashcroft, N.D Mermin, "Solid State Physics. Philadelphia: Saunders College" (1976).
46. S. Manasha, M. Janani, "IJRFM" 6(2016) 2331.
47. F. Wei, R. Lam, S. Cheng, S. Lu, D. Ho, and N. Li, "Applied Physics Letters", 96(2010) 133702.
48. W. Chen, H. Deng, L. Hong, Z. Q. Wu, S. Wang, A. L. Liu, X. H. Lin, and X. H. Xia, "Analyst", 137(2012) 5382.

49. C. W. Kim, J. W. Yun, I. H. Bae, J. S. Lee, J. H. Kang, K. M. Joo, H. J. Jeong, J. H. Chung, Y. H. Park, and K. M. Lim, "Chem. Res. Toxicol", 2(2010) 220.
50. R. L. Melnick et al., "Toxicol Appl Pharmacol" 72(1984) 292-303.
51. E. Bingham, B. Cofrissen, C. H. Powell, "Patty's Toxicology Volumes 195th ed. John Wiley & Sons. New York, N.Y." (2001) 1335.
52. Dobson et al., "Toxicological Sciences Advanced publication" (2008).
53. R. M. Balabin, S. V. Smirnov, "Talanta" 85 (2011) 562–568.
54. L. He, Y. Liu, M. Lin, J. Awika, "Sens. & Instrumen. Food Qual." 02 (2008) 66–71
55. G. Venkatasami, John Jr R. Sowa, "Anal. Chim. Acta" 665 (2010) 227–230.
56. J. Xia, N. Zhou, Y. Liu, Bo Chen, Y. Wuc, S. Yao, "Food Control" 21(2010) 912–918.
57. F. Wei, R. Lam, S. Cheng, S. Lu, D. Ho, and N. Li, "Appl. Phys. Lett." 96(2010) 133702.
58. G. M. Gadd, "Microbiology" 156(2010) 609.

2.1 Introduction

During last few decades, there has been an enormous progress in the research area focusing on the synthesis and application of gold nanoparticles. The survey described in this chapter helps us to understand the state of art of synthesis of GNPs and colorimetric detection of melamine in milk using gold nanoparticles.

To have the thorough knowledge of the experimental work, finding the gaps in literature regarding synthesis, detection method and to know the state of art of gold nanoparticles large number of research papers has been referred as follows:

Gold Nanospheres are synthesized by various methods like physical, chemical and biological. The most commonly used method is citrate reduction method. It was established more than 50 years (Described by Turkevich in 1951 and Fren's in 1973) and remains the most frequently used method in which the metallic gold converted into nano-particulate gold.

2.2 Methods for synthesis of Spherical GNPs.

Table 2.1:- various methods for synthesis of GNPs

Author name	Method	Results
Nikhil R. Jana et al., "Adv. Materials" 13(2001) 1389-1392.	Seed-Mediated Growth ⁽¹⁾	<ul style="list-style-type: none"> • Spherical gold Nanoparticles produced using gold seeds (3-4nm) in the presence of surfactant template. • The seed increases the growth rate. The particle size is controlled by varying the ratio of seed to metal salt. • Due to slow addition of reducing agent (ascorbic acid) seed obtained nanoparticles with poor homogeneity.
Toshio Sakai & PaschalisAlexandris,	Block copolymer ⁽²⁾	<ul style="list-style-type: none"> • The synthesis involves minimum number of components, economic and environmentally

“American Chem. Society” (2004).		<p>benign</p> <ul style="list-style-type: none"> • It proceeds fast to complete. It results in a “ready-to-use” product. • Particle size cannot be varied by varying the ratio between gold and reducing agent.
Hussain et al. “J. AM. CHEM. SOC.”127(2005) 16398-16399.	Thiol capping ligand ⁽³⁾	<ul style="list-style-type: none"> • The particle size depends upon the concentration of the stabilizing agent and the particles are readily obtainable in both non-aqueous and aqueous solutions. • Monodisperse spherical gold nanoparticles (1-4nm) can be synthesized by this method, which is used in limited applications only.
Kimling et al. “J. Phys. Chem. B” 110 (2006) 15700-15707.	By reduction of ascorbic acid and citrate ⁽⁴⁾	<ul style="list-style-type: none"> • Gold nanoparticles can be produced in range from 9-120 nm size with defined size distribution. • Thermal activation is essential for reaction initiation. • Primary & secondary clustering leading to polycrystallites.
Haiss et al. “Anal. Chem.” 79(2007) 4215-4221.	Sodium citrate reduction ⁽⁵⁾	<ul style="list-style-type: none"> • Gold nanoparticles with size range of 5-100 nm were synthesized in this method. Hence Surface Plasmon Resonance can be tuned into entire electromagnetic spectrum.
Bastus et al. “Anal. Chem.” 79(2007) 4215-4221.	Seeded growth ⁽⁶⁾ .	<ul style="list-style-type: none"> • Spherical shaped 200nm GNPs are obtained with narrow size distribution. • High yield of particles obtained by controlling the pH and temperature. Obtained particles are to be additional functionalized with various molecules. • The process of secondary nucleation happens during the homogeneous growth.
Klavs F.	Segmented Flow	<ul style="list-style-type: none"> • Fast reduction yields gold nuclei, which produce

<p>Jensen et al., “Langmuir” 28(2012) 7007–7013.</p>	<p>Microfluidic Platform ⁽⁷⁾.</p>	<p>by agglomeration, and it is controlled by the interaction of the nuclei with local flow. Hence to control the nanoparticles size and size distribution, a careful choice of continuous and dispersed phase is necessary</p>
<p>Ahmad et al., “World Journal of Nano Science and Engineering” 3 (2013) 62-68.</p>	<p>Tannic acid⁽⁸⁾.</p>	<ul style="list-style-type: none"> • Small size was obtained from 1 mM gold chloride concentration. Higher gold chloride concentrations have significant impact on size of gold nanoparticles with tannic acid as reducing agent. • For various tannic acid and chlorauric and tannic acid molar ratios, GNPs ranging from 17.2-36 nm. • Small saturation was observed at higher concentration of gold chloride solution.
<p>Evelina Polievkova et al., “Int J Nanomedicine” 9 (2014) 4007– 4021.</p>	<p>Green method ⁽⁹⁾.</p>	<ul style="list-style-type: none"> • Used for various biomedical applications including in-vitro & in-vivo bioimaging techniques,
<p>Thanh Ngo et al., “Adv. Nat. Sci.: Nanosci. Nanotechnol” 7 (2016) 035016.</p>	<p>Microwave heating ⁽¹⁰⁾.</p>	<ul style="list-style-type: none"> • The higher yield and narrower distribution of gold nanoparticles were obtained (18-22nm). Stability for gold nanoparticles was about 1 month.

2.2 Detection of Milk Adulteration

Table 2.2:- Literature on Detection of Milk Adulteration

Reference	Method	Results
Roman M. Balabin, Sergey V. Smirnov, "Talanta" 85 (2011) 562–568	MIR/NIR ⁽¹¹⁾ .	<ul style="list-style-type: none"> • Broad range of applicability to various dairy products. • Low cost, quick, robust and sensitive method for infant formula, milk powder and liquid milk analysis. • Limit of detection (LOD) is below 1ppm.
Gopalakrishnan Venkatasami et al., "Analytica Chimica Acta" 665(2010) 227–230	HPLC ⁽¹²⁾ .	<ul style="list-style-type: none"> • Detection of melamine in dry and liquid infant formula. • The separation is performed at room temperature. • The limit of detection (LOD) is 0.03ppm.
Jingen Xia et al., "Food Control" 21 (2010) 912–918	Capillary zone electrophoresis ⁽¹³⁾ .	<ul style="list-style-type: none"> • Economical, good separation, easy operation and short analysis time. • The optimized method established good performance precision, accuracy and enough sensitivity. • The limit of detection is 0.055 ppm
Kelong Ai et al., "American Chemical Society" 2009.	Hydrogen-Bonding (Visual Detection) ⁽¹⁴⁾ .	<ul style="list-style-type: none"> • The method does not require complex and costly instruments, which simplifies operations and reduces the cost. • The limit of detection concentration is as low as 0.0025 ppm.
Li Li et al., "Food Chemistry Analytical	Visual detection as colorimetric probe ⁽¹⁵⁾ .	<ul style="list-style-type: none"> □ This method of low cost, simple, rapid, visual colorimetric & operated at room temperature □ The Limit of detection of melamine is 0.4 ppm

Methods” 122(2010) 895-900.		
Fang Wei et al., “Applied physics letters” 96(2010) 133-702.	Colorimetric ⁽¹⁶⁾ .	<ul style="list-style-type: none"> • Simple and accurate approach toward gold nanoparticle mediated detection of melamine based on a precipitation-based readout and dual color. • The sensitivity was 0.04 ppm
Liangqia Guo et al., “Talanta” 82(2010) 1654-1658.	Visual detection ⁽¹⁷⁾ .	<ul style="list-style-type: none"> • A realistic, rapid, field-portable colorimetric method • The detection limit in liquid milk and infant formula is 1.0 and 4.2 ppm by naked eyes. It detects as low as 2.5 ppm of melamine in infant formula and 0.15 ppm of melamine in liquid milk.
Hong Chi et al., “Analyst, Royal Society of Chemistry” 135 (2010) 1070-1089.	colorimetric visualization ⁽¹⁸⁾	<ul style="list-style-type: none"> • A simple, sensitive and reliable reliable colorimetric visualization method. • Detection of melamine below 0.025 ppm
Ansoon Kim et al., “American Chemical Society” 84(2012) 9303–9309	Surface Enhanced Raman Scattering ⁽¹⁹⁾ .	<ul style="list-style-type: none"> • A simple, less time consuming, cost effective & rapid colorimetric detection of melamine. • Limit of detection (LOD) of the melamine was 100 ppb in infant formula and 120 ppt in water.
Hai-bo Xing et al., “Food Anal. Methods” 6	Colorimetric Detection ⁽²⁰⁾ .	<ul style="list-style-type: none"> • A rapid, cheap, visual, facile colorimetric detection of melamine. • The limit of detection for melamine is below 7 ppm.

(2013):1441–1447		
Naven Kumar et al., “Analytical Biochemistry” 456(2014) 43-49.	Colorimetric detection ⁽²¹⁾ .	<ul style="list-style-type: none"> Extremely sensitive and, limit of detection is below a concentration of 0.05 ppm
Jia-ying Xin et al., “Food chemistry”, 174 (2015) 473–479	Colorimetric detection ⁽²²⁾ .	<ul style="list-style-type: none"> A simple and rapid field-portable method. A linear correlation was established between the melamine concentration and absorbance ranging from 3.97×10^{-6} M to 3.90×10^{-7} M. The detection limit was as low as 2.38×10^{-7} M.
Keke Chang et al., “ PLoS ONE” 12(5):e0177131	LSPR via optical fibers ⁽²³⁾ .	<ul style="list-style-type: none"> The biosensing system with simple, economical and is well-proven for the melamine detection Limit of detection is 33nM.

References:

1. N. R. Jana, Catherine J. Murphy, "Adv. Materials" 13(2001) 1389-1392.
2. T. Sakai, Paschalis Alexandridis, "American Chem. Society" (2004).
3. I. Hussain, Mathias Brust et al., "J.AM. CHEM. SOC." 127(2005) 16398-16399.
4. J. Kimling, M. Maier, B. Okenve, V. Kotaidis, H. Ballot, and A. Plech, "J. Phys. Chem. B" 110(2006) 15700-15707.
5. W. Haiss, Nguyen T. K. Thanh, Jenny Aveyard and David G. Fernig, "Anal. Chem." 79(2007) 4215-4221.
6. N. G. Bastús, Joan Comenge and Víctor Puentes, "Langmuir" 27(17) (2011) 11098–11105.
7. V. S. Cabeza, Simon Kuhn, Amol A. Kulkarni, Klavs F. Jensen, "Langmuir" 28(2012) 7007–7013.
8. T Ahmad, W Khan, "World Journal of Nano Science and Engineering" 3(2013) 62-68.
9. P Elia, Raya Zach, Sharon Hazan, Sofiya Kolusheva, Ze'ev Porat, and Yehuda Zeiri, "Int J Nanomedicine" 9(2014) 4007–4021.
10. VO Ke Thanh Ngo, Dang Giang Nguyen, Trong Phat Huynh and Quang Vinh Lam, "Adv. Nat. Sci.: Nanosci. Nanotechnol" 7(2016) 035016.
11. R. M. Balabin, Sergey V. Smirnov, "Talanta" 85(2011) 562–568.
12. G. Venkatasami, John R. Sowa Jr., "Analytica Chimica Acta" 665(2010) 227–230.
13. J. Xia, Naiyuan Zhou, Yujun Liu, Bo Chen, Yongning Wuc., Shouzhuo Yao, "Food Control" 21(2010) 912–918.
14. K. Ai, yanlan Liu & Lehui Lu, "American Chemical Society" (2009).
15. Li Li, Baoxin Li, Di Chen & Lihui Mao, "Food Chemistry Analytical Methods" 122(2010) 895-900.
16. F. Wei, Robert Lam, Stacy Cheng, Steven Lu, Dean Ho & Na Li, "Applied physics letters" 96(2010) 133-702.
17. L. Guo, Jianhai Zhonga, Jinmei Wua, Feng Fu Fua, Guonan Chena, Xiaoyan Zhengc & Song Linc, "Talanta" 82(2010) 1654-1658.
18. H. Chi, Bianhua Liu, Guijian Guan, Zhongping Zhang & Ming-yong Han Analyst, "Royal Society of Chemistry" 135(2010) 1070-1089.
19. A. Kim, Steven J. Barcelo, R. Stanley Williams, and Zhiyong Li, "American Chemical Society" 84(2012) 9303–9309
20. Hai-bo Xing, Yuan-gen Wu, Shen-shan Zhan & Pei Zhou, "Food Anal. Methods" 6(2013) 1441–1447.

21. N. Kumar, Raman Seth, Harish Kumar, “Analytical Biochemistry” 456(2014) 43-49.
22. Jia-ying Xin, Lan-xuan Zhang, Yan Wang, “Food chemistry” 174(2015) 473–479.
23. K. Chang, S. Wang, H. Zhang, et al., “ PLoS ONE” 12(5): e0177131.

23.1 Introduction

This chapter outlines the synthesis method of spherical gold nanoparticles, detection of melamine in milk using gold nanoparticles, the basic theories & principles for the experimental techniques like UV-Visible spectroscopy and dynamic light scattering which are further used for characterization of gold nanoparticles and for the detection of melamine.

23.2 Chemical Reagents

HAuCl₄ was bought from Sigma-Aldrich; Sodium citrate tri-basic dehydrates was purchased from Sigma-Aldrich, Chloroform was purchased from SDFCL, Melamine purchased from SDFCL and Infant formula was purchased from Nestle. All solvents were used without any further purification. Millipore ($\rho=18.2 \text{ M}\Omega/\text{cm}$) water was used in all experiments.

23.3 Instrumentation

Magnetic stirrer with hot plate, ultrasonic bath, pH meter, UV-Visible-NIR Spectrophotometer, DLS (Particle size analyzer), Vortex Mixer Remi CM101 PLUS, Mega 17R Centrifuge Machine, were used.

23.4 Stock solution preparation

Stock solution was prepared by using 1gm of chloroauric acid with 1mM, dissolved in 250 mL DI water with concentration 10 mM. Further 1 mL of stock solution was diluted to 100 mL to get 1mM concentration for this experiment. For trisodium citrate: 0.05 g (1%) of Na₃C₆H₅O₇.2H₂O was dissolved in 5 mL of water.

23.5 Turkevich Method for gold nanoparticle synthesis

One of the most popular in-situ synthesis of gold nanospheres technique is pioneered by J.Turkevich in 1951. It involves the reduction of solvated aurochloric acid by sodium citrate^(1, 2). Citrate can be used as both stabilising and reducing agent in this method.

Synthesis of gold Nanoparticles was done with citrate reduction method. To synthesize gold nanoparticles, 50 mL, 1.0 mM HAuCl₄ Solution was added to beaker. The beaker was kept on the magnetic stirrer & the yellow solution in beaker brought to the boil at 100 °C. 5 mL of 1% trisodium citrate dehydrate was added to boiling solution and continue to

boil for another 10 min. Preheating of the solution not only reduces the reaction time but also helps in controlling the distribution of nanoparticles. The solution then turns to light yellow from yellow and then to colorless. The change in color of the HAuCl_4 solution is ascribed to the rapid reduction of Au^{3+} to Au^0 . After few min, the color of the yellow solution turned to pale red indicating the commencement of formation of gold nanoparticles. Citrate initially act as reducing agent to convert Au^{3+} ions to Au^0 to form particles and then act as capping agent by adsorbing to Nanoparticles surface & repelling particles from each other, preventing further aggregation. Schematic representation of the reaction mechanism of gold nanoparticles synthesis is shown in *Figure [3.1]*

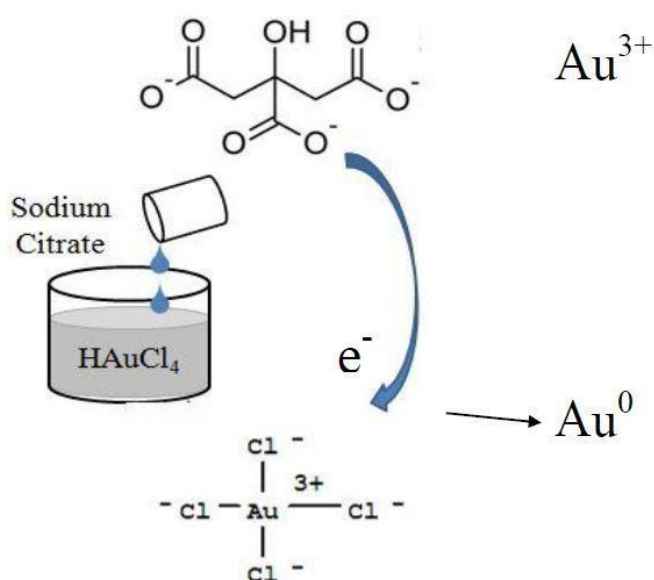


Figure 3.1:- Schematic representation of mechanism of synthesis of gold nanoparticles.

The solution was then cooled to room temperature under continuous stirring and the wine red solution of spherical GNPs was obtained. The wine red solution of GNPs was stored at 4°C before use. The whole process is shown in *Figure [3.2]*.

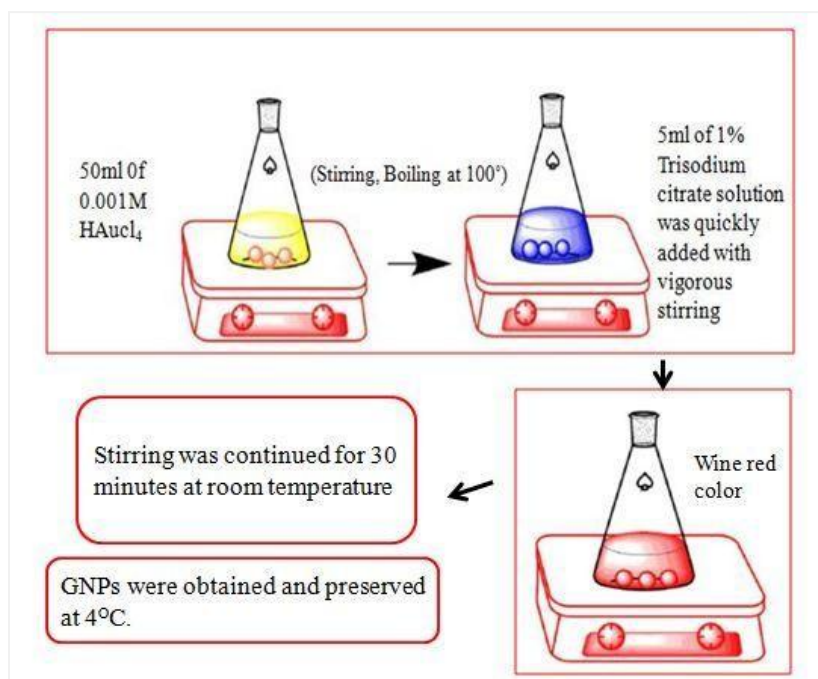


Figure 3.2:- Representation Synthesis of Spherical Gold Nanoparticles.

23.6 Detection of Melamine

23.6.1 Detection of Melamine using Spherical Gold Nanoparticles:

For detection of melamine, 1mL, 2mM stock solution (made by dissolving 25.22mg of melamine in 100 mL of water) of various concentrations (0.2 mM, 2 μ M, 20 μ M, 0.2 μ M, 2 nM, 4 nM, 6 nM, 8 nM, 10 nM, 20 nM) was taken and added into 1mL, 2mL and 5mL of GNPs solution. The mixture was then allowed to react for 3- 5 min at room temperature. The absorption spectra of each solution were recorded and the concentration of melamine was quantified on the basis of absorption ratio of spherical gold nanoparticles specific wavelength (λ_{\max}) before and after the addition of melamine.

23.6.2 Sample preparation for Detection of Infant Formula Powder

For the detection of melamine in infant formula, 0.08 gm of infant formula powder was dissolved in 4 mL of water in a centrifuge tube. After that 1.2 mL of 10% trichloroacetic acid (0.12 gm) and 1.5 mL chloroform was added into the milk solution. The whole sample was vortexed for at least one minute and then ultrasonically treated for 15 min.

This mixture was then centrifuged at 13000 rpm for 10 min to separate supernatant from pellet. 5mL of 20 nM melamine solution was added into the final mixture to determine the detection limit.

23.6.3 Detection of melamine in infant formula

For the detection of melamine, 1 mL of infant formula was added to 5 mL gold Nanoparticles solution. The mixture was then allowed to react at room temperature for 3-5 min. Finally, the absorption spectra of mixture were measured and concentration of melamine was determined.

23.7 Characterization techniques

23.7.1 UV-Visible spectroscopy

Spectroscopy is called as the study of interaction of electromagnetic radiation with matter. Electromagnetic waves are consisted of discrete packets of energy which are called as photons. These waves are consisted of an oscillating electric field (E) and oscillating magnetic fields (M) which are perpendicular or can be said as at 90 degree to each other. UV spectroscopy is a kind of absorption spectroscopy which uses light to be absorbed by the molecules. Upon the absorption of the UV-visible radiation, the excitation of the electrons occurs from the ground state to higher energy state.

Principle: UV spectroscopy follows the Beer-Lambert law, which states that: Upon the passage of a beam of monochromatic light through a solution of an absorbing substance, the amount of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution⁽³⁾. The Beer-Lambert law's expression is-

$$A = \log (I_0/I) = Ecl$$

Where, A is absorbance of the sample. I_0 = Intensity of light incident upon sample cell

I = Intensity of light transmitted through the sample cell

E = molar absorptivity

C = molar concentration of solute

L = length of sample cell,

23.7.2 Dynamic Light Scattering

Dynamic light scattering works on the phenomenon of scattering of light from the particles or molecules and analysing their Brownian motion and in that way measuring the size of the particles⁽⁴⁾. The motion of the particles caused by the interaction of particle with solvent molecules is called as Brownian motion. The velocity related with Brownian motion can be

stated as translation diffusion co- efficient. The translation diffusion co-efficient examines the size of the particles by using Stokes-Einstein equation ⁽⁵⁾

$$d(H) = \frac{KT}{3\pi\eta D}$$

Where, d (H) = hydrodynamic diameter, K = Boltzmann's constant, D=Diffusion co-efficient, η = viscosity and T = absolute temperature.

3.8 Results and Discussion

3.8.1 Characterization of Gold nanoparticles

GNPs present different optical and physical properties, which are dependent upon their shape, size, surface structure and agglomeration state. When handling GNPs, especially when functionalizing their surface with chemical groups or biomolecules, it is often necessary to characterize them, e.g., to evaluate the result of surface modification.

3.8.1.1 UV Visible Spectroscopy

The UV-Vis absorption spectra of GNPs were recorded on Shimadzu made double beam spectrometer (UV-2600).

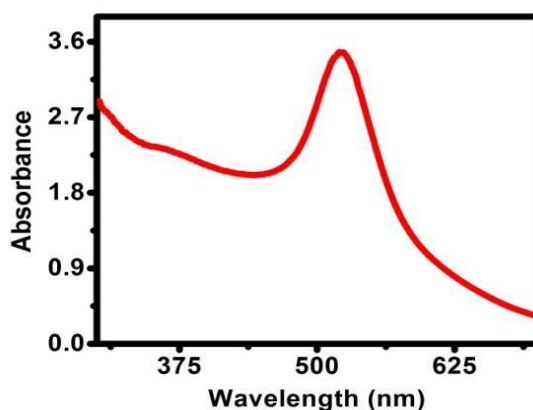


Figure 3.3:- UV-Visible spectra of GNPs shows SPR peak at 520 nm.

Measurements were performed at room temperature. Surface plasmon resonance of GNPs was observed at 520 nm *Figure [3.3]*. Single SPR peak at 520 nm shows the GNPs formed have the spherical morphology.

3.8.1.2 Dynamic light scattering

The hydrodynamic size of particle is obtained by fitting the size distribution histogram with lognormal particle size distribution function as shown in *Figure [3.4]* for as-synthesized GNPs. The mean hydrodynamic size of Gold nanoparticles is 20 nm with polydispersity of 0.16.

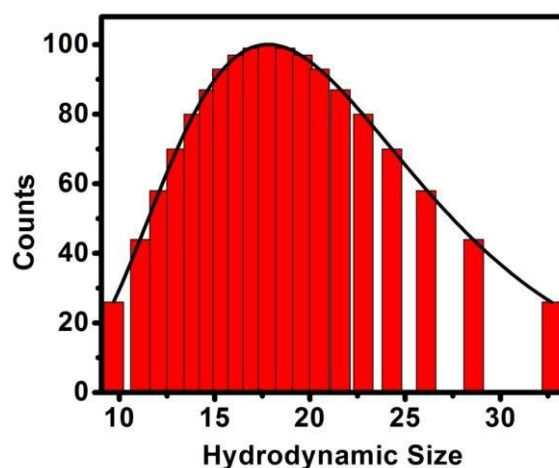


Figure 3.4:- Hydrodynamic Particle size distribution of GNPs

3.8.2 Melamine measurement based on colorimetric detection

UV-Visible spectra of melamine of known concentration were recorded for the calibration curve. The absorbance decreases with the decrease in melamine concentration *Figure [3.5a]*. UV-Visible spectra of melamine samples ranging from 2 μM to 10 μM were recorded. On the basis of these spectra, calibration curve of melamine was obtained *Figure [3.5b]*.

Gold nanoparticles in aqueous solution are stable because of repulsive forces between citrate ions on their surface, which prevent them from aggregating. Upon mixing melamine solution with the GNPs solution, aggregation of gold nanoparticles took place which causes red shift in plasmon resonance peak of GNPs. Color of sample is also changed visually from wine red to purple because of the aggregation of GNPs. Melamine molecule is consisted of three exocyclic amino groups and a three-nitrogen hybrid ring. When these three amino and ring groups of melamine strongly attach to the surface of gold nanoparticles, ligand exchange (with citrate ions) decreases the electrostatic repulsion between individual gold nanoparticles, are responsible for the aggregation of gold nanoparticles ⁽⁶⁾.

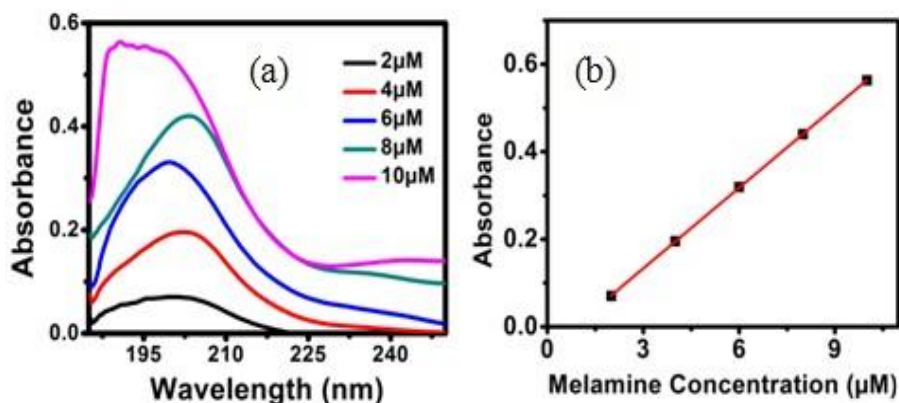


Figure 3.5:- (a) UV-Visible spectra of Melamine, (b) Calibration curve of melamine.

In order to further validate the above detection mechanism, a series of UV-Visible spectra of gold nanoparticles are measured by UV-visible spectrophotometer with different concentrations of melamine. The relationship between absorption spectra and reaction time was investigated by adding 1 mL, 2 mL and 5 mL of GNPs solution into 1ml of different concentrations of melamine from 2 mM to 2 nM.

Figure [3.6] shows that the aggregation of Melamine with GNPs in 1:1 v/v. As shown in *Figure [3.3]*, the absorption spectra of gold nanoparticles solution have maximum absorption peaks at 520 nm. The maximum absorption peak of gold nanoparticles decrease upon adding melamine with concentration of 0.2mM and 0.02mM and new absorption peak at about 1.38 (689 nm) decreases to 0.9 (709 nm) and 1.5 (685 nm) decreases to 1.15 (702 nm) at 10 and 90 min as shown in figure 3.6b,c. Further, when 2 mM melamine is added into the gold nanoparticles solution, a new absorption peak at about 1.16 (715 nm) appears within 0-3 min and it decreases to 0.4 (724 nm) in *Figure [3.6a]*. At the same time, the color of the solution changed from wine-red to dark purple and finally to light purple progressively. The absorption peaks of different concentration decreases with time, whereas the absorption ratio (P_2 -New peak shift at right side/ P_1 - Max. absorption peak decreases) as the time progresses *Figure [3.6]*.

The absorption ratio increases from 1.1 to 1.3 in 2 mM, 0.1 to 1.2 in 0.2 mM and 0.45 to 1 in 0.02 mM concentrations as shown in a, b, c with time.

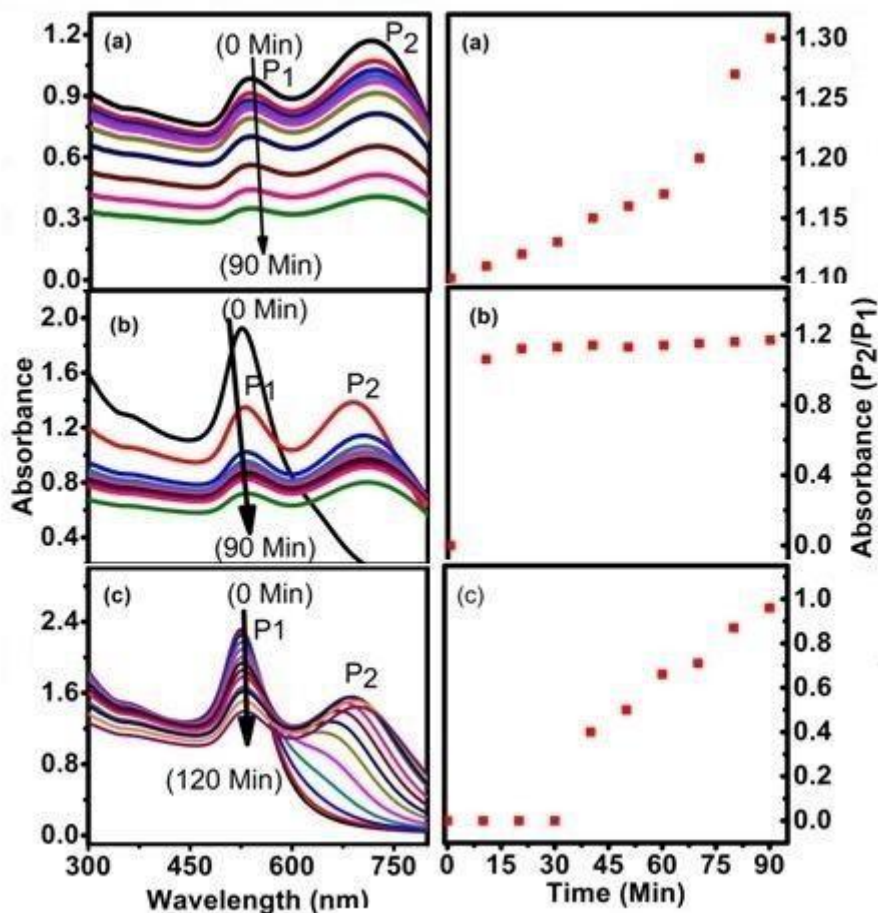


Figure 3.6:- UV-Visible spectra and absorbance (P_2/P_1) with time of Melamine reacts with gold nanoparticles by 1:1 v/v. (a) 1 mL, 2×10^{-3} M, (b) 1 mL, 2×10^{-4} M and (c) 1 mL, 2×10^{-5} M concentration of melamine with 1 mL gold nanoparticles.

The particle size data obtained from DLS analysis and given in Figure [3.7], from the DLS data in Figure [3.7], it is observed that with the decreasing concentrations of Melamine, diameters of the synthesized nanoparticles increases. This particle size increase is due to the formation of nanoparticles clusters in the solution, as evidenced from a broadening absorption band in the UV-Vis spectra.

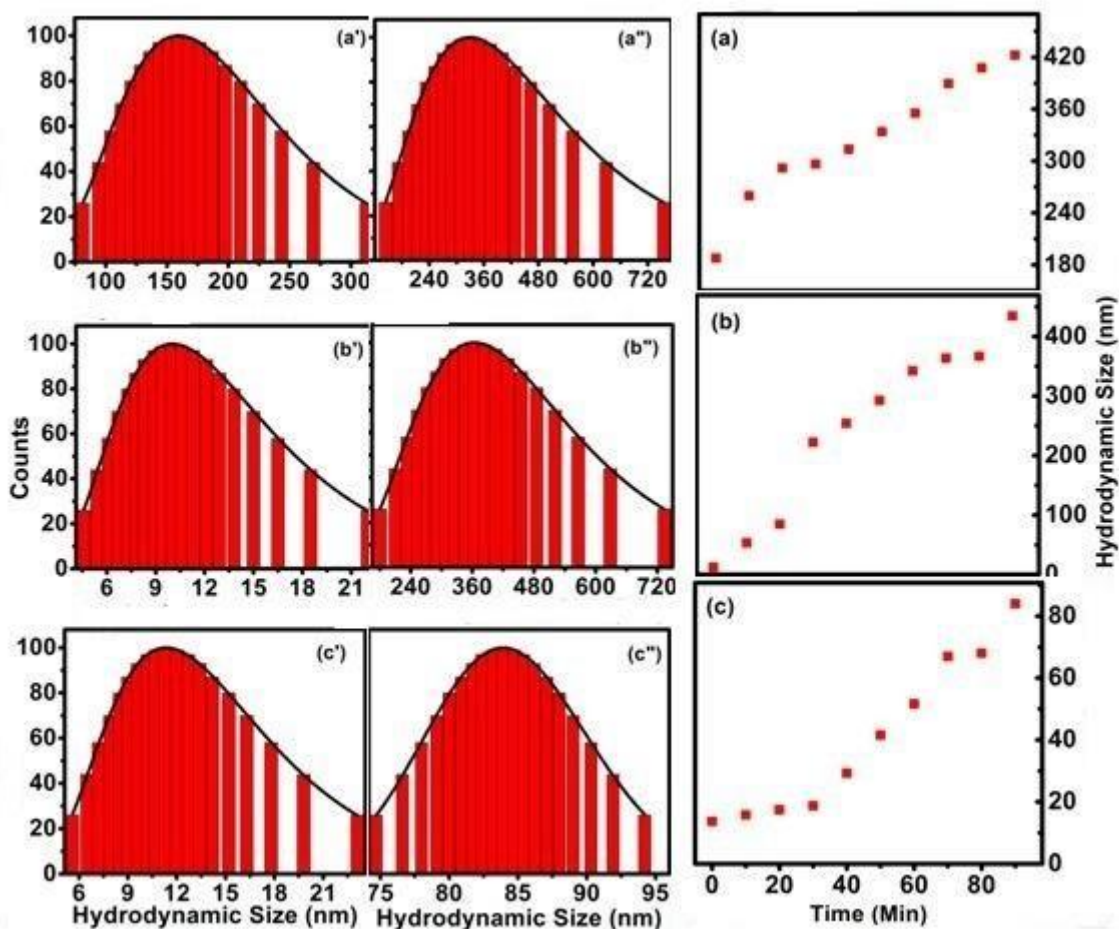


Figure 3.7:- Hydrodynamic size distribution of Melamine reacts with gold nanoparticles by 1:1 v/v. (a) 1 mL, $2 \times 10^{-3} M$, (b) 1 mL, $2 \times 10^{-4} M$, (c) 1 mL, $2 \times 10^{-5} M$, concentration of melamine with 2 mL gold nanoparticles. (a') at 0 min & (a'') at 90 min. (b') at 0 min & (b'') at 90 min & (c') at 0 min & (c'') at 90 min.

Figure [3.8] represents the UV spectra of Melamine with GNPs in 1:2 v/v. When melamine with the concentration from 2 mM to 2 μM was added into GNPs solution the maximum absorption peak of solution decreases. Figure [3.8] shows the absorption spectra as a function of reaction time.

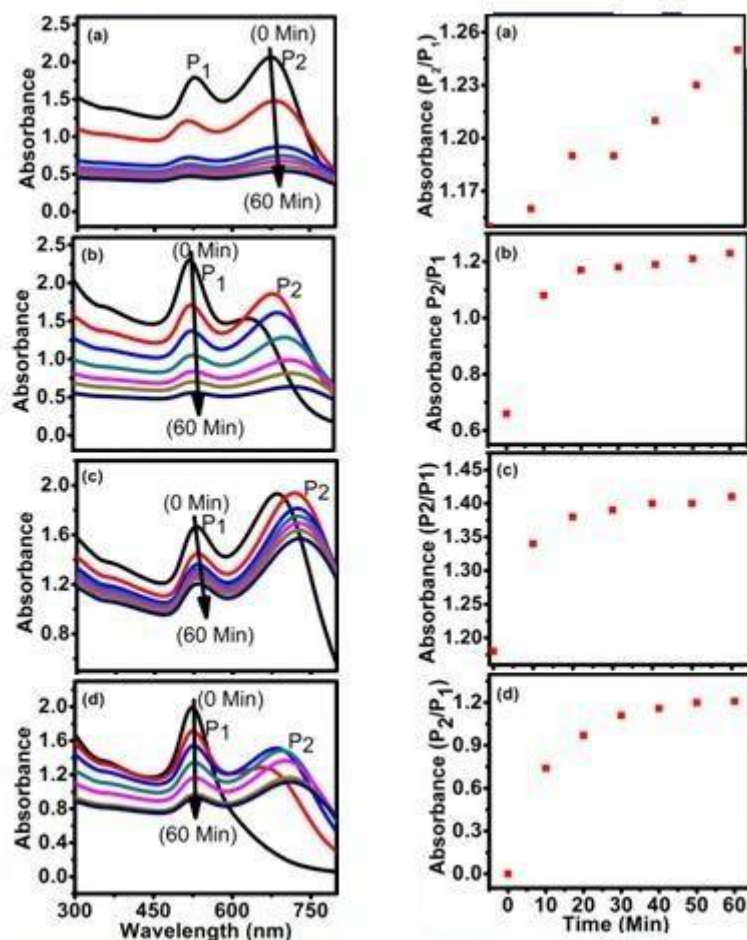


Figure 3.8:- UV-Visible spectra of Melamine react with GNPs in v/v of 1:2. (a) 1 mL, 2×10^{-3} M, (b) 1 mL, 2×10^{-4} M (c) 1 mL, 2×10^{-5} M and (d) 2×10^{-6} M concentration of Melamine with 2 mL GNPs.

From the DLS data in *Figure [3.9]*, it is observed that the hydrodynamic diameters of synthesized nanoparticles vary from 281 nm to 427 nm with 0-90 min, at 1 mL of 2 mM concentration of Melamine with 2 mL GNPs.

It was further observed that rate of aggregation decreases with the decrease in concentration of melamine and also affects by the change in ratio of GNPs to melamine. From *Figure [3.6]*, it was observed that aggregation took place only for 2 mM to 0.02 mM melamine when we use melamine to GNPs in 1:1 v/v. but when we increase the volume of GNPs to 2, the aggregation was observed for 2 mM to 2 μ M as shown in *Figure [3.8]*.

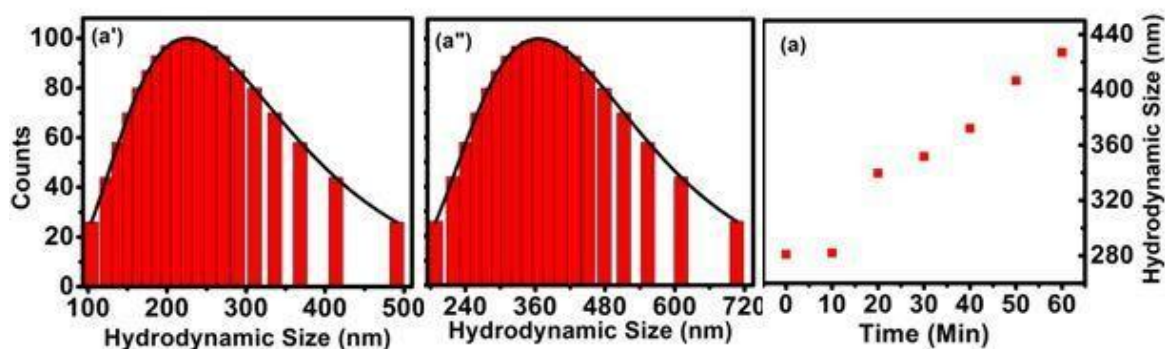


Figure 3.9:- Hydrodynamic Size Distribution of 2mM Melamine reacts with GNPs (1:2) (a) 1 mL, 2×10^{-3} M, concentration of melamine with 2 mL gold nanoparticles. (a') at 0 min & (a'') at 90 min.

Figure [3.10] and [3.11] shows the absorption spectra and reaction time of absorption ratios of Melamine aggregation with GNPs in 1:5 v/v. It was observed that when melamine with the concentration from 2 mM to 2 nM added into gold nanoparticles solution, the maximum absorption peak of solution decreases as shown in Figure [3.10] and Figure [3.11]

Similarly, Figure [3.11] (a) shows the decrease in absorption maxima (2 nM) and formation of new absorption peak at about 1.63 (707 nm) at 175 min and decrement in new absorption peak at about 1.54(745 nm) after 210 minutes was observed. The absorption ratio increases with time from 0 to 1.03.

[Figure 3.11] (b) shows decrease in absorption maxima peak (10 nM) and formation of new absorption peak at about 1.21 (691 nm) after 30 min and decrement in new absorption peak at about 0.76 (708 nm) after 100 min. The absorption ratio increases with time from 0 to 1.18.

[Figure 3.11] (c) shows decrease in absorption maxima peak (20 nM) and formation of new absorption peak at about 1.67 (722 nm) after 30 minutes and decrement in new absorption peak at about 1.15(721 nm) after 90 minutes. Growth was recorded after every 10 min. The absorption ratio increases with time from 0 to 1.2.

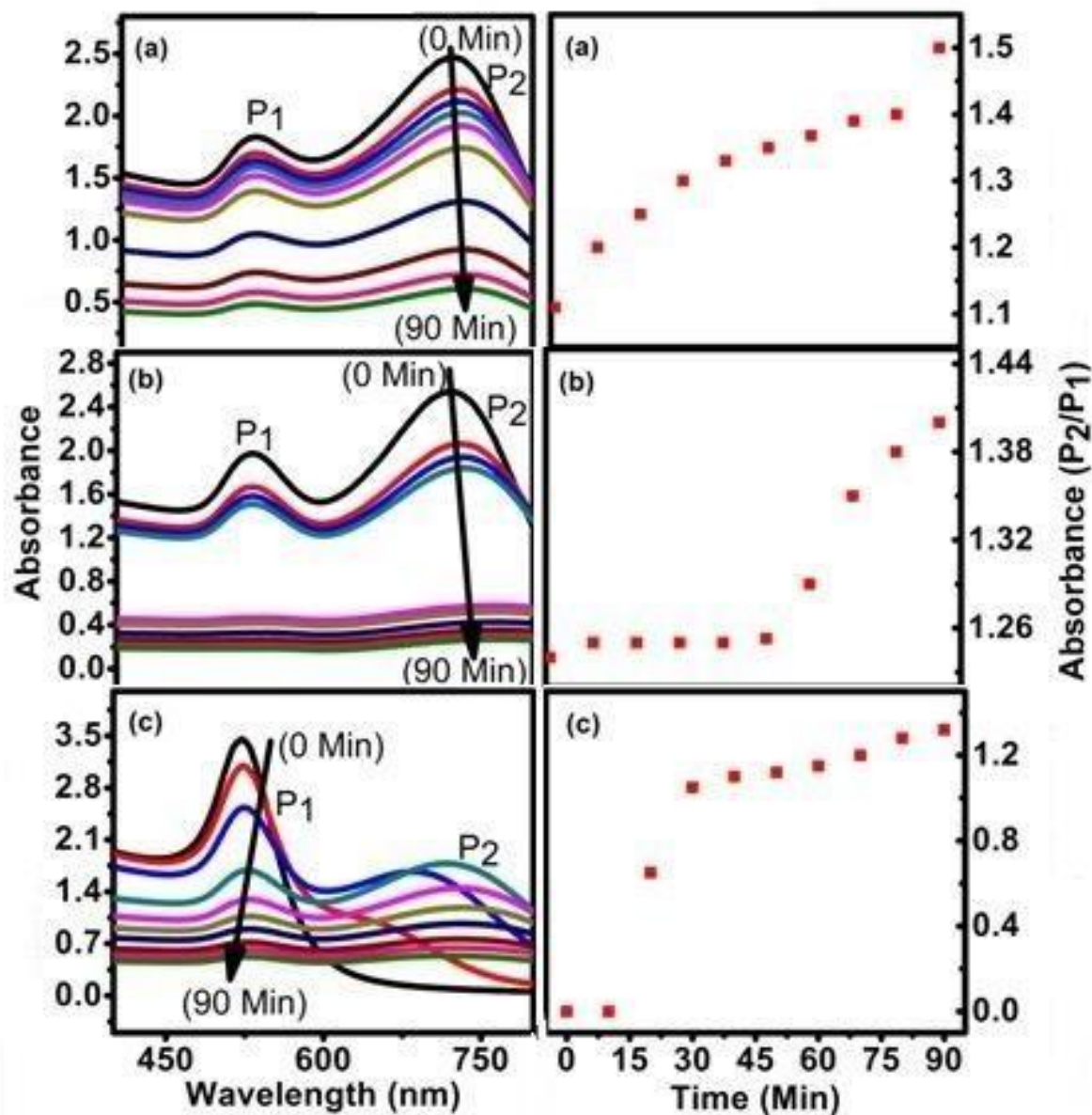


Figure 3.10:- UV-Visible Spectra of Melamine reacts with GNPs v/v is 1:5. (a) $1 \text{ mL}, 2 \times 10^{-3} \text{ M}$, (b) $1 \text{ mL}, 2 \times 10^{-6} \text{ M}$ (c) $1 \text{ mL}, 2 \times 10^{-7} \text{ M}$ concentration of Melamine with 5 mL GNPs.

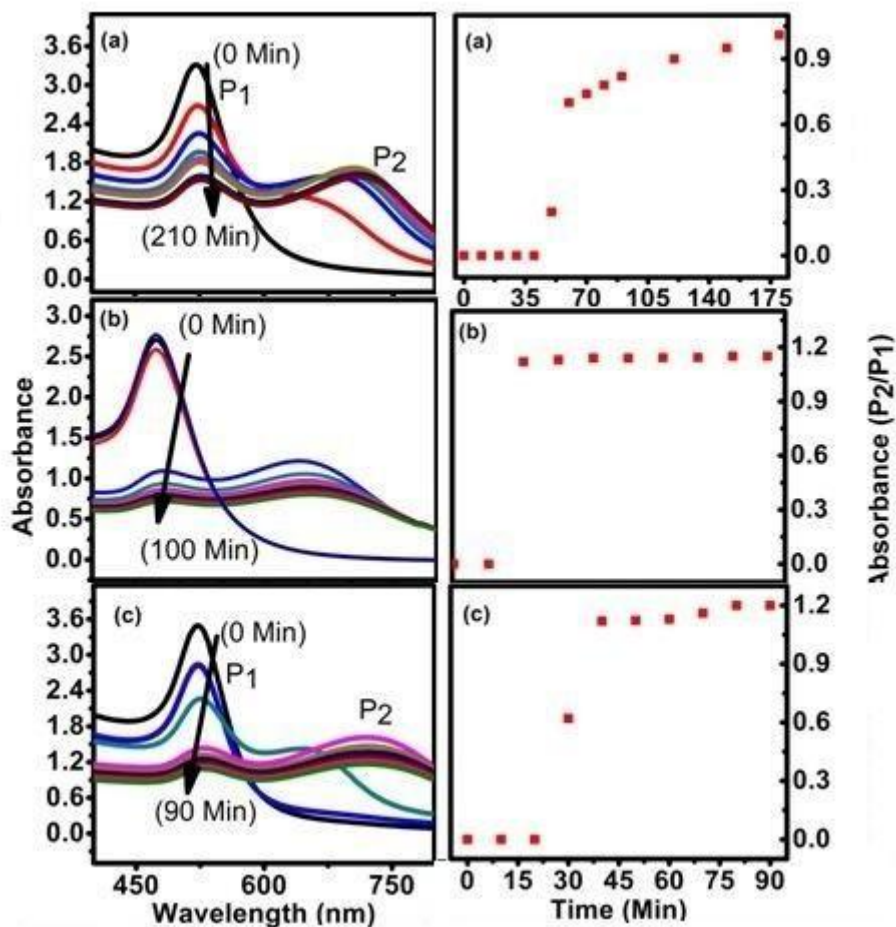


Figure 3.11:- UV-Visible spectra of Melamine react with GNPs v/v 1:5. (a) 1 mL, 2 nM, (b) 1 mL, 10 nM and (c) 1 mL, 20 nM Concentration of Melamine with 5ml GNPs.

DLS data in Figure [3.12] showed that the hydrodynamic size of the gold nanoparticles change with time from (a) 250 nm to 431.53 nm, (b) 234.7 nm to 406.33 nm and (c) 11.73nm to 46.15 nm, respectively with the decreasing concentrations of Melamine. Where a, b, c are the 1 mL of 20 nM, 10 nM and 2 nM Concentrations of melamine with 5 mL GNPs.

So, we took the benefit of above production method of GNPs to form a simple colorimetric assay for monitoring melamine. Quantitative analysis was performed on the addition of different concentrations of melamine into the growth solution and then observing the absorption peak and the change in the color of the system. The absorbance characteristic of the plasmon of the GNPs was decreasing with increase in the concentration of melamine, while the absorbance maxima got slightly red-shifted absorbance maxima were used to quantify the concentration of melamine in this system.

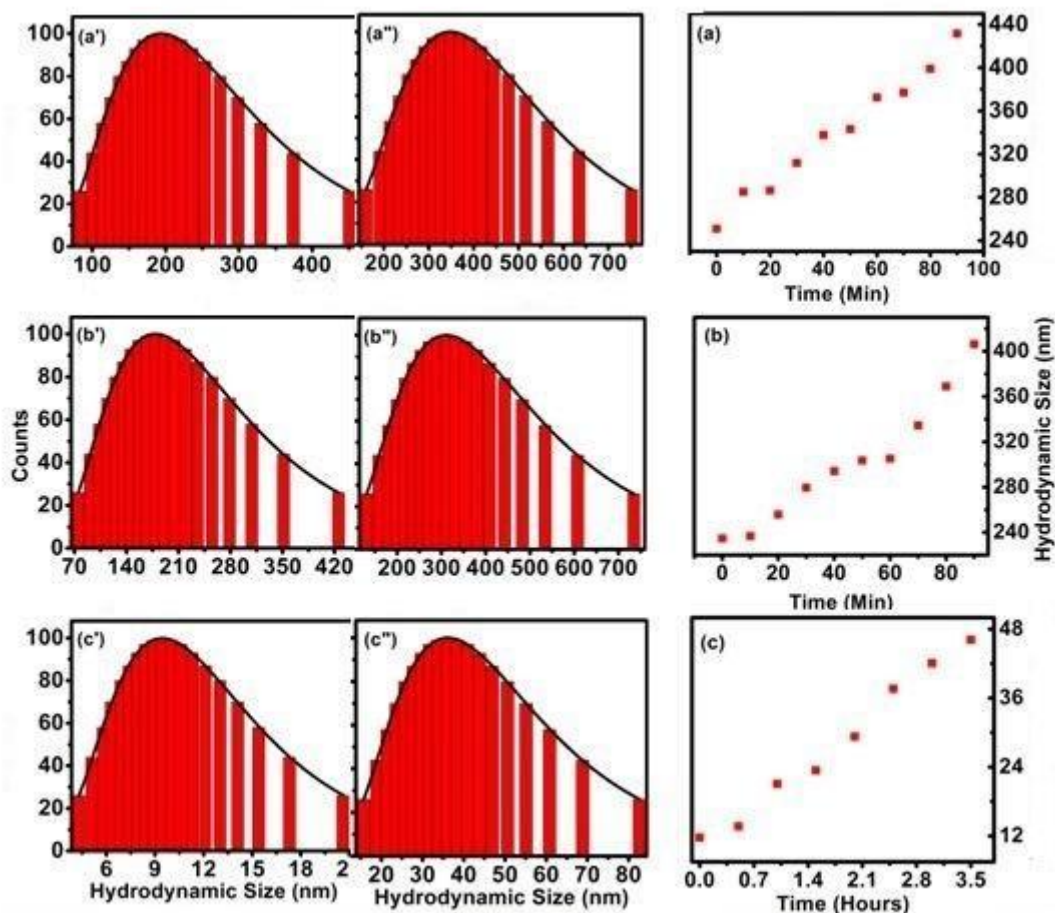


Figure 3.12:- Hydrodynamic Size Distribution of Melamine after Reaction with GNPs of v/v 1:5. (a) 1 mL, 20 nM, (b) 1 mL, 10 nM and (c) 1 mL, 2 nM Concentration of Melamine with 5ml GNPs. (a') 1 mL, 20 nM at 0 min & (a'') at 90 min, (b') 1 mL, 10 nM at 0 min & (b'') at 90 min and (c') 1 mL, 2 nM at 0 min & (c'') at 90 min.

As the average hydrodynamic particle size of AuNPs was 20 nm, color changes occur at different hydrodynamic diameters of approximately 13-434 nm. Results have indicated that aggregate size and the inter-particle distances are the parameters for colorimetric change. With increasing or decreasing the inter-particle distance than average particle diameters, color will change from red or purple.

Standard solutions of melamine were prepared by different nM melamine concentration dissolved in water. To detect the melamine in standard melamine solution gold nanoparticles were used under the above optimized conditions, and the calibration curve was drawn by plotting the absorbance ratio (P_2/P_1) against the melamine concentration *Figure [3.13]*. The calibration curve was linear with a concentration range of 2 nM to 20 nM with the regression

equation $y = 0.02x + 0.55$ and R^2 of 0.998. The detection limit is 31.5nM as calculated from the formula $3\alpha / \text{slope}$, where α is the standard deviation (0.21) and slope is the slope (0.02) of the fitting straight line.

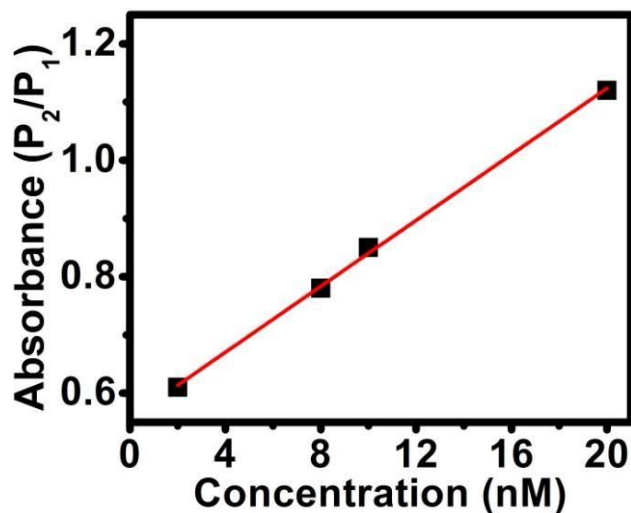


Figure 3.13:- Standard curve of Melamine reacts with GNPs of v/v 1:5 at nM.

3.8.3 Detection of Melamine in Infant Formula Powder

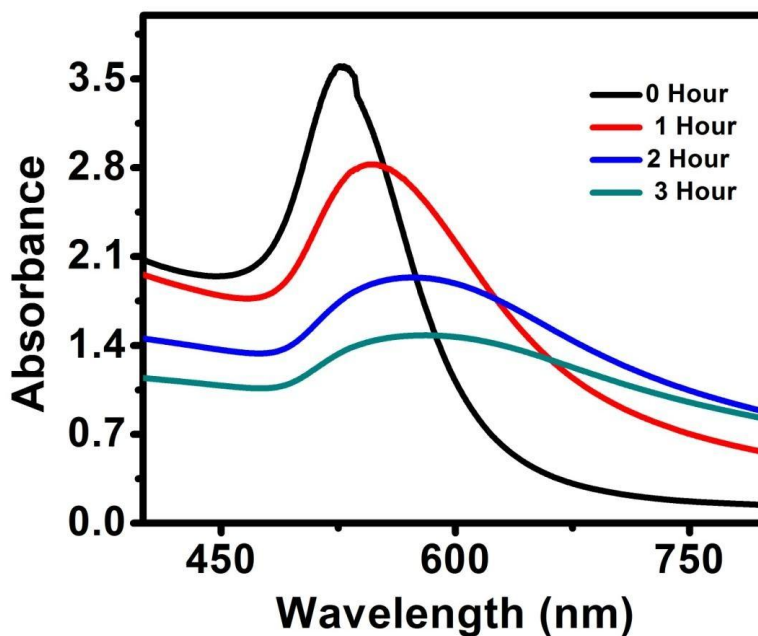


Figure 3.14:- UV-Visible spectra of GNPs-infant formula Powder

In infant formula powder, the concentration of melamine is determined by UV-Visible spectra of GNPs-infant formula Powder Melamine induced aggregation of gold nanoparticles.

Figure [3.14] shows shifting in the SPR peak in sample containing gold nanoparticles and infant formula powder. Upon the addition of gold nanoparticles in infant formula powder, the color of the melamine present in the infant formula powder show a change from wine red to deep red. In case of infant formula powder the absorbance peak around 3.59 (527 nm) and decreases slightly after 3 hours, a new absorbance peak around 1.4 (583nm) develops due to the aggregation in melamine presence. Thus melamine can be identified by measuring absorption ratio at these wavelengths in raw infant formula powder. Using *Figure [3.13]*, the concentration of melamine present in the infant formula powder was determined from standard solution of melamine. The concentration is estimated to be **39 nM** in tested sample.

References:

1. X. Ji, X. Song, J. Li, Y. Bai, W. Yang, X. Peng, “Journal of the American Chemical Society” 129(2007) 13939-13948.
2. S. Kumar, K. S. Gandhi, R. Kumar, “Industrial & Engineering Chemistry Research” 46(2007) 3128-3136.
3. Aman Thakur, “Principle, working and applications of UV-Visible Spectroscopy” (2011).
4. W.I. Goldberg, “American Journal of Physics” 67(1999) 1152.
5. W. Schärt, “Light scattering from polymer solutions and Nanoparticles dispersions” Springer (2007).
6. H. Chi, B. Liu, G. Guan, Z. Zhang, M.Y. Han, “Analyst” 135(2010) 1070–1075.

Conclusion

- Spherical gold nanoparticle with average particle size of 20 nm was synthesized by chemical reduction method.
- As-synthesized nanoparticles were used for colorimetric detection of melamine. The limit of detection depends on the ratio of concentration/volume of gold to melamine. Limit of detection increases with increase in melamine to gold v/v ratio from 1:1 to 1:5. LOD was 0.02 mM, which increases to 31.5 nM.
- Melamine was also detected in commercially available infant formula. The melamine concentration measured in the tested sample is 39nM, which is well below the FDA limit of 1ppm.