### Software’s Used

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of the Software</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Serif Draw</td>
</tr>
<tr>
<td>2</td>
<td>Chem Draw</td>
</tr>
<tr>
<td>3</td>
<td>Ms Excel</td>
</tr>
<tr>
<td>4</td>
<td>Paint</td>
</tr>
</tbody>
</table>
Publications


CHAPTER 1

Introduction
CHAPTER 2

Gold Nanoparticles as a Probe for the Detection of Degradation of Cardiac Troponin I: *In-vivo* Study
CHAPTER 3

Estimation of Time since Death using Cardiac Troponin I in Case of Death due to Asphyxia and Cardio-toxicity of Acebutolol
CHAPTER 4

Study of Degradation Pattern of Cardiac Troponin I using Gold Nanoparticle Conjugates for the Estimation of Time since Death
CHAPTER 5

Summary & Recommendation for Future Work
APPENDIX A
APPENDIX B
APPENDIX C
APPENDIX D
This is to certify the below projects have been approved by IAEC.

<table>
<thead>
<tr>
<th>Project No*</th>
<th>Title</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>KBIPER/2011/235</td>
<td>Estimation of Time Since Death using Cardiac Troponin after drug Poisoning</td>
<td>FSL Ashima 30 Rats</td>
</tr>
</tbody>
</table>

(Dr. Gaurang B. Shah)  
Chairman, IAEC  
Date: 11th June 2011

(Dr. Ramtej Jayram Verma)  
CPCSEA Nominee
An overview of methods used for estimation of time since death

Ashima Mathur a & Y.K. Agrawal a

a Gujarat Forensic Sciences University, Institute of Research and Development, Gandhinagar, Gujarat, India

Available online: 16 Aug 2011

To cite this article: Ashima Mathur & Y.K. Agrawal (2011): An overview of methods used for estimation of time since death, Australian Journal of Forensic Sciences, DOI:10.1080/00450618.2011.568970

To link to this article: http://dx.doi.org/10.1080/00450618.2011.568970

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.tandfonline.com/page/terms-and-conditions

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan, sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.
The correct determination of time since death is an important goal in medico legal investigation. Recent advances in the methods for estimating time since death have enabled us to determine post-mortem interval more precisely. Since the 1850s, scientists have been working on different methods to determine post-mortem interval. Earlier methods were based on body cooling, rigor mortis, changes in the eye, putrefaction, supravital reactions, and contents of stomach. These methods were relatively crude and only an approximate time could be estimated. Due to the lack of precision the focus has now been shifted to biochemical methods. The biochemical methods are based on systematic pathophysiological changes and are found to be more accurate since the effect of external conditions is less. This review describes the various methods used for estimating time since death.

Keywords: postmortem interval; rigor mortis; algor mortis; livor mortis; biochemical methods

1. Introduction and definition

Estimation of time since death is an integral part of medico-legal investigations. Post-mortem Interval is defined as ‘amount of time that has elapsed since the death of the decedent’. The key goal of estimating time since death at the scene of crime is to have a preliminary idea of the time of assault and for narrowing the field of suspects. A precise estimation of post-mortem interval is important for criminal law as it validates the witness’s statement, limits the number of suspects and assesses their alibis.

Eyewitnesses and scientific techniques are the two sources for determination of time since death. Eyewitnesses play an important role in the criminal justice system since it is a readily accepted form of evidence. However, eyewitness testimony is considered the most fragile and unreliable evidence by the criminal justice system, as it tends to convict innocent individuals in high proportions. The chief reasons for making eyewitness evidence as a delicate piece of information is its uncontrollable dependence upon factors such as age, health, personal bias, perception problems, discussions with other witnesses, stress, etc. Thus, eyewitness testimony should be used in conjunction with other evidences in the court of law.

After death, many changes begin to take place in the body due to physical, metabolic, autolysis, physiochemical and biochemical process. These changes
progress in an orderly manner until the body disintegrates. The measurement of these changes along with time is used for estimating time since death\(^1,2\). The physical changes, such as algor mortis, rigor mortis, livor mortis, and putrefaction, form the main basis of estimation of time since death. These changes in the body are affected by external conditions and thus the chance of producing erroneous results is high. Emphasis has been placed on the development of new techniques based on biochemical changes since these techniques are neither altered nor contaminated easily and rapidly\(^3,4\).

At present, a combination of physical (conventional methods) and biochemical methods along with witnesses are used for determining time since death, since the use of only one method produces erroneous results\(^3\).

2. Physical methods

2.1. Algor mortis

Algor mortis refers to the gradual decrease in body temperature after death. It is one of the useful indicators for estimating time since death during the first 24 hours after death\(^3–5\). During life, a balance is maintained between heat loss and heat production. After death, however, the heat production ceases and body heat is lost to the environment. The body temperature falls steadily until it matches the environmental temperature. This cooling of body temperature is mainly a physical process and the influence of biological processes is relatively low. The rate of fall of body temperature with time is used for determining time of death. Different body sites have been used for measuring the temperature such as the abdominal skin surface, axilla, rectum, ear and nostril. However, rectum is the most commonly used site for measuring the temperature\(^5–7\).

Rainy\(^8\) reported for the first time, the relationship between rate of cooling and the temperature drop. The temperature drop curve was determined according to the Newton’s cooling coefficient. Rectal temperatures of 100 patients were measured and readings were taken at periodic intervals of time of the first 2.5 days after death. The rate of cooling per hour at different intervals of time was calculated. A ‘plateau’ period was observed after death, in which the body does not cool. An inert body, which has a low thermal conductivity, has such a plateau during its first cooling phase. The post-mortem cooling curve has a sigmoid or S-shape, comprising an initial plateau followed by rapid and slow cooling phases. The post-mortem temperature plateau generally lasts half to one hour but may persist as long as three to five hours.

Temperature of the skin was also measured for determining time of death but it was never of use because the effect of external conditions was high, resulting in erroneous results\(^6,9\). Fiddes et al.\(^10\) developed a percentage method to represent the fall in body temperature after death. Patten\(^10\) presented a single standardized curve and explained it theoretically. The standardized curve corresponded to the Newton-rule but did not include the post-mortal temperature plateau\(^1,10\). Sellier et al.\(^11\) considered the human body as an infinitely long cylinder to describe the decrease in body temperature. He used the data of De Saram et al.\(^12\) and proved that a decrease in body temperature is dependent upon body radius. The temperature gradient and condition of peripheral cooling could also be determined by this method. Marshall et al.\(^13\) gave the two exponential terms showing that the rate of cooling in the initial period is slow and does not follow Newton’s law. According to them, the slow rate of
A decrease in temperature is due to metabolism and production of heat, and the influence of body surface tissues. Henssge documented a 'nomogram method’ for assessing time of death from body temperature. A nomogram is a two-dimensional diagram used for graphical computation of a function based on the coordinate system. In it, the deep rectal temperature is measured and a normal temperature at death of 37.2°C is assumed. The nomogram method is based upon a formula that approximates the sigmoid-shaped cooling curve. This formula has two exponential terms within it. The first constant describes the post-mortem plateau and the second constant expresses the exponential drop of the temperature after the plateau according to Newton’s law of cooling. The two constants were found to be significantly related in that bodies with a low rate of cooling also had a longer plateau phase than bodies with a high rate of cooling. Since a large number of different environmental factors influence the rate of cooling, Henssge conducted experiments and derived empiric corrective factors to correctly determine time since death.

The commonly used site for measuring temperature is the rectum but scientists have worked on other sites, such as skin, outer ear, brain, and eyeball. Algor mortis is one of the useful parameters for determining time since death. However, the rates of cooling established are valid for only a particular climatic region and are not applicable everywhere. The rates are valid only in cool or temperate climates because hot summer seasons or tropical temperatures slow down the loss of heat and, in some regions, can even raise post-mortem temperatures due to rapid putrefaction. Variables such as the size of the body, amount of subcutaneous adipose tissue, existence of clothing and coverings, air currents and humidity, and the medium where the body remained after death, which affect the post-mortem cooling, should be considered while estimating time since death using algor mortis.

2.2. Rigor mortis

Rigor mortis is a physio-chemical change that causes stiffening of the body after death. There are several reports of the use of rigor mortis for estimating time since death. Death is immediately followed by total muscular relaxation termed as ‘Primary Muscular Flaccidity’ which is followed by muscular stiffening – ‘rigor mortis’. After a period of time (36 hours) rigor mortis gradually fades off and is followed by ‘Secondary Muscular Flaccidity’. The primary reason for the development of rigor mortis is the loss of adenosine triphosphate from the anoxic tissue. Rigor mortis starts to develop 2–4 hours after death and develops fully by 6 to 12 hours and gradually dissipates until approximately 72 hours after death. It has been found that post-mortem muscle proteolysis is responsible for the relaxation following rigor mortis. Nysten was the first to describe the sequence of spread of rigor mortis. Classically, rigor is said to develop sequentially beginning from eyelids, jaw and neck followed by the limbs. The joints of the body become fixed when the rigor is fully developed, and the state of flexion of these joints depends upon the position of the trunk and limbs at the time of death. If the body is in the supine position then the large joints of the limbs become slightly flexed during the development of rigor. The joints of the fingers and toes are often markedly flexed due to the shortening of the muscles of the forearms and legs. Shapiro has suggested that rigor mortis develops more rapidly in small masses of muscles than in larger...
Consequently, differences in the sizes of the joints, and in the muscles that control them, determine the development of joint fixation by rigor and produce the observed pattern of progression in the body. Kobayashi et al.\textsuperscript{23} contradicted Shapiro’s hypothesis and concluded that the muscle volume did not influence either the progress or the resolution of rigor mortis. The rigor mortis phase is not the best time for the pathologist to determine the cause of death, because several changes take place in the internal muscles, such as the heart and the ocular muscles, which can be misleading.

The factors that interfere with the onset and duration of rigor mortis are temperature, existing ante-mortem pathologies, age, body muscular mass, presence of infections, temperature, climatic conditions and the degree of muscular activity immediately before death\textsuperscript{7,22,24–27}.

2.3. Supravital reactions

Supravital reactions of the tissue have been used for estimating time since death. They are the reactions that occur in the body after somatic death\textsuperscript{29–32}. Klein et al.\textsuperscript{29} and Krause et al.\textsuperscript{29} have statistically confirmed death time estimations by examining a large number of cases using supravital reactions. In the experiment, needle electrodes were inserted into the nasal part of the upper eyelid, and the muscle was stimulated using constant current rectangular impulses. The muscular reaction was graded concerning the intensity and spread of movement distant to areas from the electrode. Grellner et al.\textsuperscript{30} demonstrated that interleukin-1 alpha and N-formylmethionyl leucylphenylalanine were potential inducers of supravital reaction. Rosenthal et al.\textsuperscript{29} used the ‘animal electricity’ for determining time since death. Nevertheless, this presumption became reality only another 100 years later when Prokop\textsuperscript{6} made the suggestion in 1960. Henssge et al. measured the maximum force of reaction by a sensitive force transducer in response to a definitive stimulation and showed the decrease of the maximum force in proportion to the postmortem interval\textsuperscript{29,33}.

Madea et al.\textsuperscript{33} stated that with increasing post-mortem interval the maximum force after excitation with the same current intensity decreases and the relaxation time increases due to the fact that the muscle contraction becomes weaker. The relaxation time also shows an exponential correlation with the maximum force. Jones et al.\textsuperscript{34} demonstrated the correlation between the post-mortem interval and the delay rate of muscular response, by stimulating the peripheral muscles directly with electricity.

Supravital reactions are affected by the presence of drugs, diseases, temperature and climate. These reactions can be used for estimating post-mortem interval only after a few hours since death. It is of no use in cases when the bodies are burned or damaged due to other reason.

2.4. Livor mortis

Livor mortis or lividity is the settling of blood in the lower portion of the body, resulting in a dark purple discoloration of the skin. As the heart is no longer agitating the blood, red blood cells sink by the action of gravity. The process begins immediately after the circulation stops. The discoloration does not occur in body areas that are in contact with the ground because the capillaries get
Lividity develops in all bodies under the influence of gravity because the blood remains liquid rather than coagulating throughout the vascular system. After 30–60 minutes since death, the blood becomes permanently incoagulable. This is due to the release of fibrinolysins, especially from small vessels and from serous surfaces such as pleura. This incoagulability of blood is independent of the cause of death. In some cases, due to infections, this fibrinolytic effect fails to develop, explaining the presence of abundant clots in the heart and large caliber vessels. In some cases of sudden death, the blood remains spontaneously coagulable only during a brief period immediately following death, but then it becomes completely free from fibrinogen and will never clot again. Shapiro et al.\textsuperscript{7} stated that this fluidity of the blood is not dependent on the cause of death and the mechanism of death, although it has been cited that the blood remains liquid longer in asphyxia deaths. The colour and distribution of post-mortem lividity are important in medico-legal investigations and can be used for estimating cause of death such as carbon monoxide (CO) poisoning, cyanide intoxication and death from hypothermia. Typically, lividity has a purple or reddish-purple coloration, although in some cases variation in colour is observed\textsuperscript{1,3,35}.

Lividity starts appearing as dull red patches after 20–30 minutes from the time of death. In the succeeding hours, these red patches coalesce together to form larger areas of red-purple discoloration\textsuperscript{22}. After about 10–12 hours, the lividity becomes ‘fixed’ and repositioning the body, e.g. from the prone to the supine position, will result in a dual pattern of lividity since the primary distribution will not fade completely. In some cases, it has been observed that fading of the primary pattern of lividity occurs and there is subsequent development of a secondary pattern of lividity. This is due to the early movement of the body and is found to be more complete if the body is moved within the first six hours after death, than at a later period\textsuperscript{36}. Even after 24 hours, moving the body will result in a secondary pattern of lividity developing.

Lividity attains its maximum intensity at around 12 hours post-mortem, but there is some variation in descriptions of when it first appears, and when it is well developed. Lividity ordinarily becomes perceptible within 1/2 to 4 hours after death, is well developed within the next 3 or 4 hours, and attains its maximum degree between 8 and 12 hours post-mortem\textsuperscript{37}.

### 2.5. Post-mortem decomposition

Post-mortem decomposition or putrefaction is the destruction of the soft tissues of the body by the action of bacteria and enzymes. Tissue breakdown occurs as a result of the action of endogenous enzymes and this process is known as autolysis. Putrefaction results in the gradual dissolution of the tissues into gases, liquids and salts. The main changes that can be recognized in the tissues undergoing putrefaction are changes in colour, the evolution of gases, and liquefaction\textsuperscript{1,6}.

Putrefaction depends upon factors such as environmental temperature, body habits, diseases, obesity, poisoning, presence of drugs and infections\textsuperscript{22}. Putrefaction is optimal at temperatures ranging from 70–100°F and is retarded when the temperature falls below 50°F or when it exceeds 100°F\textsuperscript{1,6}.

The first visible sign of putrefaction is a greenish discoloration of the skin of the anterior abdominal wall. This most commonly begins in the right iliac fossa, i.e. over the area of the caecum. The discoloration, due to sulph-haemoglobin formation,
spreads to involve the entire anterior abdominal wall, and then the flanks, chest, limbs and face. The superficial veins of the skin become visible as a purple-brown network. This is often referred as ‘marbling’\(^{1,36}\). Subsequently, skin blisters varying in size from less than 1 cm to between 10 and 20 cm in diameter develop. These blisters are filled with fluid and putrid gases. They burst on the slightest contact leaving the same slippery, pink base that underlies skin-slip. Putrid gas formation also occurs in the stomach and intestines causing the abdomen to distend and become tense. Gas formation within the tissues causes swelling of the body. The gases produced include hydrogen sulphide, methane, carbon dioxide, ammonia, hydrogen and mercaptans. The putrid gases that are under pressure escape out as the body mass of decomposing tissues collapses\(^7\).

Under average conditions in a temperate climate, the earliest putrefactive changes involving the anterior abdominal wall occur between 36 and 72 hours after death. Progression to gas formation occurs after about one week. The temperature of the body after death is the most important factor generally determining the rate of putrefaction. If it is maintained above 26°C after death then putrefactive changes become obvious within 24 hours and gas formation will be seen in about 2–3 days. The putrefactive changes that have taken place up to this time are relatively rapid when contrasted with the terminal decay of the body. When the putrefactive juices have drained away and the soft tissues have shrunk, the speed of decay is appreciably reduced\(^36\).

3. Biochemical methods

Methods have been proposed in the last 60 years for the determination of time since death by biochemical means. Body fluids such as blood, spinal fluid, aqueous humour and vitreous humour of the eye show chemical changes immediately or shortly after death. These changes progress in a fairly orderly fashion until the body disintegrates. Each change has its own time factor or rate. The determination of chemical changes helps to ascertain time since death more precisely. Variation in these biochemical profiles may be observed due to factors such as pre-existing diseases or disorders, cause of death, survival period, environmental factors and also the properties of the analyte under investigation\(^38–44\).

The chloride concentration in blood has been studied for estimating time since death. The chloride concentration in blood decreases with the increasing post-mortem interval\(^31,38\). Jetter\(^38\) reported that the chloride concentration falls through intra-cellular shift at the rate of 80–90 mEq/ L per day. Later Schleyer\(^31\) stated that the rate of fall was between 0.25 and 1 mEq/L per hour. Querido\(^40,41\) developed a double logarithmic relationship between plasma chloride concentration and post-mortem interval.

Earlier studies were mainly carried out on blood and cerebro spinal fluid. But over time vitreous humour became the most studied material for estimating time since death. This was mainly due to the fact that vitreous humour is topographically isolated and well protected and thus the autolytic changes proceed slower compared with blood and cerebro spinal fluid. The most studied parameter in vitreous humour is potassium. An increase in the concentration of potassium in vitreous humor occurs after death\(^45–49\). Aggarwal et al.\(^45\) worked on the relationship between the potassium levels of vitreous humour collected separately from each eye and the increasing time since death was found using flame photometry. He observed that the
vitreous humour potassium concentration increased in a linear fashion with increasing time since death, and this increase in the level was independent of factors such as age, sex, environmental temperature and humidity. It was also observed that there was no effect of other parameters, such as age, sex, temperature and humidity, on the levels of vitreous potassium.

Zhou et al. developed an analytical method for the determination of potassium in vitreous humour by low-pressure ion chromatography. They developed a linear correlation equation for potassium concentration in the vitreous humour and post-mortem interval.

Experiment was conducted with bird muscles to estimate time since death. Observations were made that only a percentage of non-protein nitrogen on total soluble protein, asparatic amino transferase activity and creatinine concentration showed significant correlation with time after death. Aspartic amino transferase was negatively correlated and non-protein nitrogen percentage and creatinine were positively correlated with post-mortem interval. The stronger correlation was found for creatinine.

Dokgoz et al. studied the cellular changes of leukocytes and observed that degeneration of neutrophils can be used for estimating time since death. Identifiable degeneration of neutrophils was first examined at 6 h after death and up to 120 h.

Bansal et al. established a relationship between the ratio of post-mortem serum sodium and potassium concentrations and time since death. Flame photometry was used for estimating sodium and potassium concentration in post-mortem blood. A highly significant relationship exists between logarithm of serum sodium as well as potassium concentration, logarithm of their ratio and the logarithm of the time since death.

Extensive experimental work has been carried out on the protein degradation during putrefaction in various organs such as the brain, liver, heart. It was observed that brain decomposition resulted in reproducible concentration changes of known metabolites and decay products.

Cardiac troponin is considered as a powerful biomarker for myocardial infarction and drug-induced cardio toxicity. Troponin is a muscle protein and forms a regulatory protein complex together with tropomyosin, which controls the interaction of actin and myosin. Along with calcium ions it controls muscular contraction. It has been reported that elevated post-mortem blood and pericardial cardiac Troponin T levels may depend on the severity of myocardial damage from various causes of death, including hyperthermia, methamphetamine abuse and carbon monoxide poisoning. Cardiac troponin measurement is found useful for investigating the severity of myocardial damage from various causes in medico-legal autopsy when pathological findings and post-mortem interference are taken into consideration. Maeda et al. studied cardiac troponin I and creatine kinase MB in the blood and pericardial fluid from medico-legal autopsy cases with regard to the cause of death. It is established that elevated cardiac troponin I and creatine kinase MB levels in blood and pericardial fluid are related to ischemic, hypoxic and/or cytotoxic myocardial damage, which are characteristic of the cause of death.

Alberto et al. studied the degradation pattern of cardiac troponin I for estimating time since death. He concluded that there is direct correlation between the disintegration of cardiac troponin I with post-mortem interval.

Welner et al. studied if positive immunoreactions to various antigens like insulin, thyroglobulin and calcitonin can be used for determining time since death. It
was found that all three antigens mentioned above showed positive staining initially and, after a period, gave negative staining. This is because the tertiary structure of the antigen undergoes changes with increasing time interval and thus, due to protein denaturation, staining becomes negative.

The degradation pattern of Deoxy Ribonucleic Acid (DNA) has been used for estimation of post-mortem interval\textsuperscript{62,63}. But it is no longer considered as a reliable source for estimating time since death as the influence of external factors is very high\textsuperscript{60}.

4. Conclusion

The ever-increasing crime rate is demanding fast and sensitive methods for determining time since death. A considerable amount of work has been carried out by scientists to accurately determine time since death. Algor mortis, rigor mortis, supravital reactions, and post-mortem decomposition have been a routine tool for the estimation of post-mortem interval for many years. The results of conventional methods are not precise and accurate. The recent developments of biochemical methods have completely changed the face of estimation of post-mortem interval. The biochemical methods are more sensitive, systematic and less prone to errors. The conventional methods are affected by conditions such as environmental temperature, humidity, position of body, presence of infections or diseases. This disadvantage has been overcome by the use of biochemical methods since such methods investigate the pathophysiological changes occurring in the body.

Biochemical methods have been developed today but few of them are being incorporated into daily course work. The main reasons for this are the high cost involved in carrying out the experiments, sparse availability of highly technical professionals, time-consuming methodology and lack of standardized procedures.

A more statistical-based approach is required for developing techniques, which is a difficult task and requires a high degree of cooperation between laboratories. A universally accessible database comprising population studies can solve this problem. Many formulae are available to estimate time since death. Typically used tests are the correlation test, linear regression, Bayesian model and likelihood ratio. The latter is considered as the best documented, most developed and is of a high evidential value for forensic purposes. Other approaches can also be employed. However, higher accuracy in estimating postmortem interval is obtained by using analyte concentrations as the independent variable and time since death as the dependent variable. The efficiency of different methods should be tested and validated before using them in routine case work.

Despite several advancements in the techniques for determination of post-mortem interval, the accuracy of these methods still leaves room for improvement. The development of more reliable techniques will not only be an achievement in the forensic science field but will also prove to be beneficial in the court of law as it would reduce the period of trial and the number of wrong convictions.

References
Estimation of DNA Using Gold Nanoparticle Probe

Amitkumar Lad*, Ashima Mathur, and Y. K. Agrawal

Institute of Research and Development, Gujarat Forensic Sciences University, Near Police Bhavan, Sector 18A, Gandhinagar, India

The present study describes the estimation of DNA damage by using gold nanoparticles (AuNPs) as probe. DNA damage has been studied in various organisms by a number of methods. These methods have several limitations like time, cost and require sample preparation. Thus, we have developed a simple, cost effective method for estimation of DNA. AuNPs were used to measure pure and degraded DNA in a mixture by UV-spectroscopy and spectrofluorometry. It is interesting to note that the absorption of AuNPs increases, while fluorescence intensity decreases on addition of increasing amount of degraded DNA. The study suggests that in both spectroscopic determinations, the results obtained for DNA degradation patterns were in linear range, which will definitely prove to be an effective tool in molecular biology to point out the nature of DNA damage, quantify damage and repair processess.

Keywords: DNA, AuNPs, UV Spectroscopy, Spectrofluorometer.

1. INTRODUCTION

DNA is a natural product, indeed the natural product of the paramount importance in understanding the mechanism of genetic processes, of cell growth and differentiation, of ageing and senescence.1,3 DNA damage has been studied in a variety of organisms such as bacteria, cyanobacteria, phytoplankton, macroalgae, plants, animals and humans.1 DNA damage often alters the coding property of the bases/or results in profound effects on the DNA structure. Therefore DNA damage can lead to cytotoxicity (cell death) and genotoxicity (mutagenesis).2 At present there are several methods such as PCR, comet, halo, TUNEL assay, HPLC-Electrospray tandem mass spectrometry, FISH (Fluorescence in situ hybridization), FCM (Flow cytometry), annexin V labeling, immunological assays Gas chromatography-mass spectrometry, electrochemical methods etc. that are commonly used to detect DNA damage in various organisms but have some or the other limitations. Thus, it is clear that there is a need to combine the features of different detection methods and to develop a unique strategy that can localize damage in genome, point out the nature of damage and quantify damage and repair processes. This will be helpful in developing repair strategies and will also provide better insight into the process of carcinogenesis and ageing.1,3

In recent years tremendous advances have been made in the area of application of nanoparticle in bioanalysis. Nanoparticles are characterized by having one of their dimensions in nanometer range. This size regime is of great importance since it is in the ‘nano-regime’ which can be tuned by varying the size.5,6 The properties of these materials are in-between that of individual molecule and bulk material. These NPs can be composed of a variety of materials including noble metals (e.g., Au,5 Ag,6,7 Pt,8 Pd,9 semiconductors (e.g., CdSe, CdS, ZnS,8,13 TiO2,11 PbS,12 InP,12 Si,13), magnetic compounds (e.g., Fe3O4,14 Co,15 CoFe2O4, FePt,9 CoPt and their combinations (core-shell NPs and other composite nanostructures).

Out of all these AuNPs are the most studied nanoparticles owing to its diversified optical properties. Gold nanoparticles are been used to detect and study biomolecules such as DNA, proteins, enzymes.16–19 The AuNPs are functionalized with specific groups that would attach specifically to target molecules although use of unmodified AuNPs for probing DNA sequences is known.16–21 Recently, Par Sandstrom and his group have reported the mechanism for adsorption of DNA to AuNPs. They suggest that the attraction is an ion-induced dipole interaction.22,23 Thus, this strategy was applied in this experiment. Figure 1 show the strategy used in this study. Citrated capped AuNPs were prepared and then different concentration of pure and degraded DNA were added. This was followed by spectroscopic measurement which
showed significant peak observation in UV-visible spectroscopy and Spectrofluorometry.

2. EXPERIMENTAL DETAILS

2.1. Chemicals

Highly polymerized calf thymus DNA (MP Biomedicals, US) was used in this study. DNA dilutions were prepared in Phosphate buffer pH 7. Phosphate buffer was prepared by dissolving 0.1 M disodium hydrogen phosphate in water and adjusting the pH by adding 0.1 M HCl/period. HAuCl₄ and Tri-sodium citrate were used to prepare AuNPs. All chemicals were purchased from E-Merck (Mumbai, India), SRL (Mumbai, India) and were all of analytical reagent grade.

2.2. Characterization and Measurement

Fluorescence intensity was measured by Jasco FP-6500 spectrofluorometer (Jasco, Japan) at a scan rate of 200 nm/min. An excitation and emission bandwidth of 20 nm and 10 nm was used respectively. UV spectra were obtained on a JASCO V-670 spectrophotometer (Jasco, Japan). Particle size of AuNPs was carried out by Malvern Zeta-sizer (Model- The Zetasizer Nano ZS, UK).

2.3. Preparation of Pure DNA and Degraded DNA Samples

20 ppm DNA solution was prepared in phosphate buffer pH 7. The degraded DNA sample was prepared by heating the pure DNA for 5 min at 70 °C in water-bath. Sample A, B, C, D were prepared by varying the concentration of pure and degraded DNA in appropriate ratio (A-4:1, B-3:2, C-2:3, D-1:4) respectively.

2.4. Citrate-Capped AuNPs Synthesis and Modification

AuNPs were prepared by citrate reduction of HAuCl₄ with slight modifications in the documented methods. 𝑝1–2 Briefly, a solution of sodium citrate (10 mL, 38.8 mM) was added rapidly to the vigorously stirred boiling chloroauric acid (100 mL, 1 mM). The solution turned red in approximately 5 min. Next, the solution was boiled continuously for 10 min and stirred for another 15 min with the heater switched off. The mixture was cooled to room temperature. Finally, a deep red, monodisperse “naked” AuNPs were obtained and this solution was used as stock solution. AuNPs solution as prepared was diluted (double distilled water) to 2× and was used for further studies. The sizes of the NPs were verified by Malvern Zeta-sizer.

3. RESULTS AND DISCUSSION

3.1. Characterization AuNPs

The particle size characterization was carried out by Malvern zeta-sizer. This instrument allows the measurement of particle size distributions in the range 0.6 nm to 10 μm. The average particle size of AuNPs was 14 nm. Figure 2 shows the particle size distribution of AuNPs. Particles were ranging from 25.92 nm (83.7%), 0.7579 nm (10%), and 3.039 nm (3.3%).

3.2. DNA Degradation Patterns by UV-Spectroscopy

DNA degradation studies using AuNPs were studied by UV-spectroscopy. From Figure 3, the absorbance of pure AuNPs was found to be 528 nm, while on addition of pure DNA to AuNPs, the peak height of absorbance decreased with shifting at 530. This suggest that the adsorption of DNA to AuNPs. dsDNA has a stable double-helix geometry that always presents the negatively charged phosphate backbone, while AuNPs also presents negative charge. So they have different abilities to be adsorbed on the negatively charged surface of AuNPs in solution. Recently, Par Sandstrom and his group have reported the mechanism for adsorption of DNA to AuNPs. dsDNA has a stable double-helix geometry that always presents the negatively charged phosphate backbone, while AuNPs also presents negative charge. So they have different abilities to be adsorbed on the negatively charged surface of AuNPs in solution. Recently, Par Sandstrom and his group have reported the mechanism for adsorption of DNA to AuNPs. They suggest that the attraction is an ion-induced dipole interaction. So, in our study, it has been observed that the decrease in absorbance was due to ion induced interaction of pure DNA adsorbed on AuNPs. As shown in Figure 3, the absorbance of AuNPs was increased from A to D. D contains higher amount of degraded DNA and less amount of
pure DNA. As the order C to A, the amount of degraded DNA decreases and the amount of pure DNA increases. Thus, the absorbance increases from A to D, as less DNA was available to adsorb on AuNPs. The spectra of completely degraded DNA show significant spectral observation. The absorbance peak of A, B, C, and D was found to be 529 nm, while pure AuNPs shows peak at 528 nm. The AuNPs + DNA shows absorbance peak at 530 nm. So, the shifting of peak further suggests the adsorption of DNA to AuNPs. The absorbance of completely degraded DNA was higher than that of AuNPs + DNA, but lower than A to D spectra. This was due to the dilution of concentration of AuNPs and additionally no DNA was available for adsorption on AuNPs. Thus, the spectra was obtained in between AuNPs + pure DNA and A to D.

3.3. DNA Degradation Patterns by Spectrofluorometry

DNA degradation studies performed by spectrofluorometer shows significant observations in fluorescence intensity. Due to the ion induced dipole interaction between the negative AuNPs and dsDNA, the dsDNA would dissociate from the surface of AuNPs resulting in aggregation or the change of fluorescence. Normally, fluorescence detection was considered much more sensitive compared with absorption detection. However, in this case, similar detection limits were obtained using the same DNA probes but different detection methods. There might be two possible reasons:

(1) high fluorescence background; and
(2) small enhancement of fluorescence. The fluorescence intensity of pure AuNPs was shown in Figure 4.

On addition of pure DNA to AuNPs, the fluorescence intensity was increased. The reason behind this was adsorption of DNA to AuNPs, which forms agglomerates. Thus, fluorescence intensity of AuNPs + DNA was increased as compared to pure AuNPs. From Figure 4, it was clear that as the amount of pure DNA decreases, the fluorescence intensity also decreases. So, the fluorescence intensity decreased.
Fig. 4. Fluorescence emission AuNPs. Excitation(Ex) at 450 nm yield a fluorescence emission(Em) peak at 531 nm. (Ratio of pure and degraded DNA samples, A-4:1, B-3:2, C-2:3, D-1:4.)

from A to D. Finally, completely degraded DNA shows intensity peak below the A to D. So, no DNA was available to adsorb on AuNPs.

### 4. CONCLUSION

AuNPs provide excellent capability for determination of various studies such as DNA hybridization and detection, molecular interaction studies etc. Therefore AuNPs were prepared and used for the estimation of DNA degradation. The method provided a new way of estimation of DNA in solution with ability to distinguish between the nature of DNA that is pure and degraded DNA. This optical method is simple, fast, and inexpensive for DNA degradation studies. We believe that our study provides better understanding of the DNA degradation pattern and will benefit further improvement of AuNPs probe for DNA damage in the future.