



International Journal of **Biological Chemistry**

ISSN 1819-155X



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Binding Properties of Biosynthesized Gold Nanoparticles with Calf-Thymus DNA *in vitro*

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ABSTRACT

The investigation of drug-DNA interaction is important for understanding the molecular mechanism of drug action. Interaction of ct-DNA with *Alternanthera sessilis*, *Acorus calamus*, *Rhaphidophora aurea*, *Portulaca oleracea* and *Sterculia foetida* mediated AuNPs was monitored by recording the changes in absorption band before and after interaction of ct-DNA and AuNPs by UV-Visible spectroscopy. The interaction of the complex with calf thymus DNA (ct-DNA) showed blue shift and hyperchromism in the UV-Vis spectra indicating groove binding for SF, PAK, MP and AC-AuNPs. The morphology of the DNA-nanoparticle complexes seems to be dependent on the nanoparticles concentration and is significantly different for each plant-mediated nanoparticles.

Key words: Gold nanoparticles, ct-DNA, UV-Visible spectroscopy, intercalation, groove binding

INTRODUCTION

DNA interaction with drug molecules has been an active research area at the boundary line of chemistry and biology. The binding to DNA may occur through weak and strong interactions between the base pairs of DNA and the active sites of the drug molecule (Zhou and Yang, 2006). Generally, there are two well-characterized binding modes for small molecules, including molecular drugs with double-stranded (dsDNA) i.e., covalent (chemical modification of various DNA constituents) and non-covalent (outer electrostatic binding, groove binding or intercalation) (Nowicka *et al.*, 2007). The most common modes by which small molecules bind directly to dsDNA are intercalation and groove binding (Oliveira *et al.*, 2009). The binding of DNA to drug can affect its transcription, replication, the expression of genetic information in cells and thereby influence its physiological function. Drug-DNA interaction studies have been carried out to obtain the effective information and explain the disease mechanism. The different binding studies have always been widely used in designing low toxic drug for clinical use (Arvin *et al.*, 2013).

DNA binding is the critical step for many cytotoxic metal complexes as DNA is the primary intracellular target of antitumor drugs. Coordination compounds offer many binding modes to polynucleotides, including outer-sphere non-covalent binding, metal coordination to nucleobases and phosphate backbone interactions (Komor and Barton, 2013). The potential DNA targeting antitumour drugs can be developed by studying the binding mechanisms of metal complexes to DNA. The clarification on the binding mechanism of these stains to biological macromolecules could help us to understand the biological value and toxicity of them (Wang *et al.*, 2011).

Obviously, metal nanoparticles act as a double-edged sword, either as a toxic or as a therapeutic agent depending on the synthesis environment. This has led to increased interest in understanding

the DNA-nanoparticles interactions. Thus the potent materials are developed by the biological inference of these interactions (De Paoli Lacerda *et al.*, 2010). Gold nanoparticles are known for its promising biomedical applications, such as targeted drug delivery treatments of cancer (Everts *et al.*, 2006; Loo *et al.*, 2005), photothermal and diagnostic systems (Gobin *et al.*, 2007). Hence, these properties of AuNPs emphasize the crucial importance in exploring the DNA binding interactions with biosynthesized AuNPs.

Anthracycline drugs adriamycin and daunorubicin are efficient in the treatment of various human cancers, form strong intercalation complexes with calf thymus DNA (Guin *et al.*, 2011). Calf thymus DNA (ct-DNA) used in the present study, is a low protein and highly polymerized nucleic acid. A facile way to establish interaction between the DNA and the drug is to examine the shifting of the position of the maximum of this band from when the ligand is free in solution to when the ligand is bound with the DNA. It has been assumed that the magnitude of this shifting could be interpreted as an indication of the strength of the interaction between the DNA structure and the ligand considered (Sun *et al.*, 2011; Jaumot and Gargallo, 2012; Wei *et al.*, 2010; Bhadra and Kumar, 2011). The bathochromicity and hypochromicity observed in UV-Vis absorption curve for DNA interaction with square-pyramidal copper (II) complexes with ciprofloxacin and is consistent for the classical intercalation mode of binding (Patel *et al.*, 2012).

In recent years, AuNPs interacting with DNA have been extensively studied for potential applications such as new intercalating agents, gene delivery vectors and smart bio-based nanoparticles. Various techniques that are used to study the binding of drug molecules with DNA includes infrared (IR), Raman, Circular dichroism, UV-Visible, Atomic Force Microscopy (AFM), electrophoresis, mass spectrometry, viscosity measurements, thermal denaturation studies, cyclic, square wave and differential pulse voltammetry, etc. UV-Visible absorption spectroscopy is perhaps the simplest and most commonly employed instrumental technique for studying both the stability of DNA and their interactions with small ligand molecules.

Hence, elucidating the binding mode between AuNPs and DNA provides great help in understanding drug-DNA interactions and validate the pharmacological activities and therapeutic applications of the biosynthesized AuNPs.

MATERIALS AND METHODS

Sonication-assisted synthesis and room temperature was adopted to produce different colours of AuNPs using the selected plant extracts.

Biosynthesis and characterization of gold nanoparticles: Aqueous extracts of *A. sessilis* (4 mL), *P. oleracea* (2 mL), *S. foetida* (5 mL), *A. calamus* extract (2 mL) and *R. aurea* (2 mL) was sonicated in a Digital Ultra Sonic Cleaner LMUC-200 series sonic bath after addition of gold ions (1 mL) to yield rapid formation of AuNPs without agglomeration. The dosage and time were standardized as above after varying the parameters: concentration of gold ions and that of extracts. The biosynthesized gold nanoparticles were characterized by UV (UV-Visible spectrophotometer-Systronics 2202) and TEM (Transmission Electron Microscope-FEI'S Tecnai TM G²) analysis.

In vitro DNA binding studies: Gold nanoparticles synthesized using the aqueous extracts of *Alternanthera sessilis* (PGK), *Acorus calamus* (AC), *Rhaphidophora aurea* (MP), *Portulaca oleracea* (PAK) and *Sterculia foetida* (SF) were investigated for its interaction with Calf thymus DNA by the procedure of Biver *et al.* (2012) and is given below.

Preparation of stock solutions for the study: A stock solution of Calf thymus DNA (ct-DNA, lyophilized sodium salt, highly polymerized) was prepared by dissolving approximately 1 mg ct-DNA fibers in 10 mL TRIS-HCl buffer (10 mM) solution, sonicated and stored for 24 h at 4°C. The solution was kept in an ice-bath to minimize thermal effects caused by sonication. Experiments were performed in TRIS-HCl buffer at pH = 7.4. The concentration of DNA solution is expressed in monomer units, as determined by spectrophotometry at 260 nm using an extinction coefficient of $6600 \text{ M}^{-1} \text{ cm}^{-1}$. DNA solutions were used within 4 days. A solution of ct-DNA in the buffer gave a ratio 1.8-1.9:1 at 260 and 280 nm, indicating the DNA to be free from protein. Aliquots of the five different colours of plant-mediated gold nanoparticles solution (10, 20, 30, 40 and 50 μL) were treated with the constant concentration of DNA (25 μL) solution. The final volumes of samples were incubated at 37°C for 24 h.

UV-Visible evaluation of AuNPs-CT-DNA interaction: Absorbance spectra were recorded using a Biospec Nano (Shimadzu Biotech). The absorbance measurements were performed by keeping the DNA concentration constant ($3 \times 10^{-9} \text{ M}$), while varying the gold nanoparticles concentration as 1.7×10^{-9} , 2.97×10^{-9} , 4.167×10^{-9} , 5.37×10^{-9} and $7 \times 10^{-9} \text{ M}$. The samples were incubated at 25°C for 2 h and the spectra were recorded in the range 200-800 nm.

RESULTS AND DISCUSSION

Different shades (violet, purple and ruby red) of AuNPs were obtained for the varied concentration of the PGK, PAK and MP extracts and gold chloride solution within 10-20 min. A remarkable broadening of peak at 530-570 nm indicates spherical shape particles for PGK and MP-mediated AuNPs (Fig. 1d, e) as confirmed by TEM analysis (Fig. 2d, e). The PAK-AuNPs reveal the presence of bands at 540 and 570 nm, with the difference in their intensity (Fig. 1a). The TEM micrographs also confirm the formation of triangular plate with round edges in PAK and SF-mediated AuNPs of size 20-30 nm (Fig. 2a, b). The SF extract and gold ions produced pink colour AuNPs under sonication after 30 min and two sharp bands at 520 and 570 nm were noted for SF-AuNPs (Fig. 1b). The UV-Visible spectra for blue colour AC-AuNPs (Fig. 1c) showed a broad band at 520-640 nm and sharp one at 720 nm, which reveals the formation of large size nanoparticles. Figure 2c obviously represents the TEM images of tetrapods shape nanogold for blue colour AC-mediated AuNPs.

The interaction of ct-DNA with PGK, AC, MP, PAK and SF-mediated AuNPs was noted for four different time intervals (30, 60, 90 and 120 min) below room temperature. They show a decrease in OD value as time increases and the AuNPs concentration increases. The UV spectral results recorded after 30, 60 and 90 min did not show much difference in the OD values whereas, the values obtained after 2 h is unique and has a specific pattern. Hence, the reliable one was discussed.

Quantization and evaluation of purity of DNA: Quantization of DNA is commonly carried out to determine the average concentrations of DNA present in a mixture, as well as to determine their purity. Spectrophotometric analysis is one such reliable and economical method for evaluating the purity of DNA. The UV spectrum of DNA is recorded at wavelengths 260 and 280 nm and their optical densities are also recorded. The more the light absorbed by the sample, the higher the DNA concentration in the sample. The amount of light and the concentration of DNA are correlated by applying Beer's Lambert's law. At 260 nm, the average extinction coefficient for double-stranded

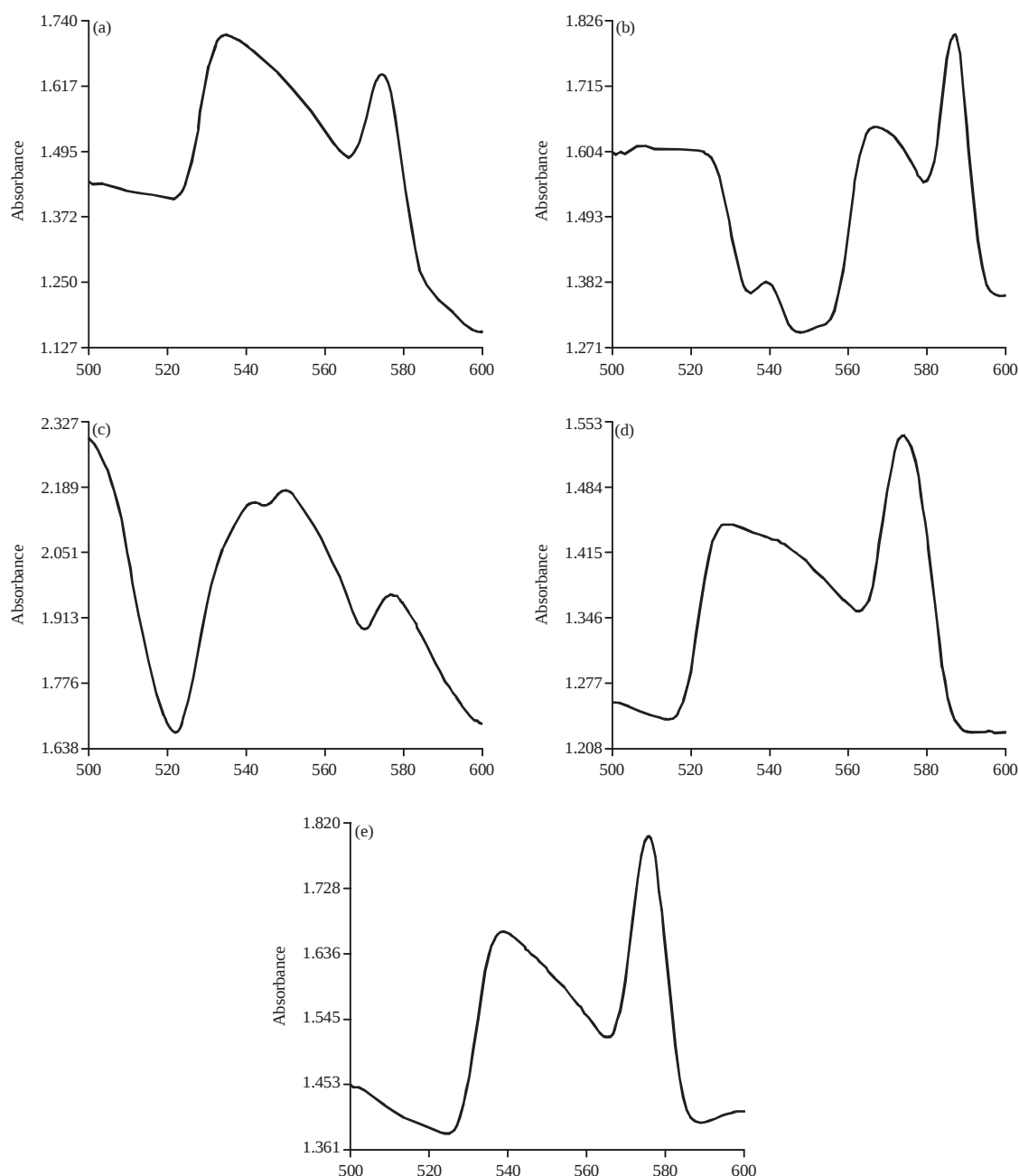


Fig. 1(a-e): UV-visible spectra of (a) PAK-AuNPs, (b) SF-AuNPs, (c) AC-AuNPs, (d) PGK-AuNPs and (e) MP-AuNPs

DNA is $0.020 \mu\text{g mL}^{-1} \text{ cm}^{-1}$ and for single-stranded DNA it is $0.027 \mu\text{g mL}^{-1} \text{ cm}^{-1}$. An absorbance of 1 corresponds to a concentration of $50 \mu\text{g mL}^{-1}$ for double-stranded DNA. This method of calculation is valid for up to an absorbance of at least 2 (Sambrook and Russell, 2001).

A common problem noted in the storing of DNA is its contamination with other molecules. Hence it is very essential to determine the purity of the DNA before studying its interaction with organic molecules of interest. The purity is ascertained by recording the absorbance at 260 and

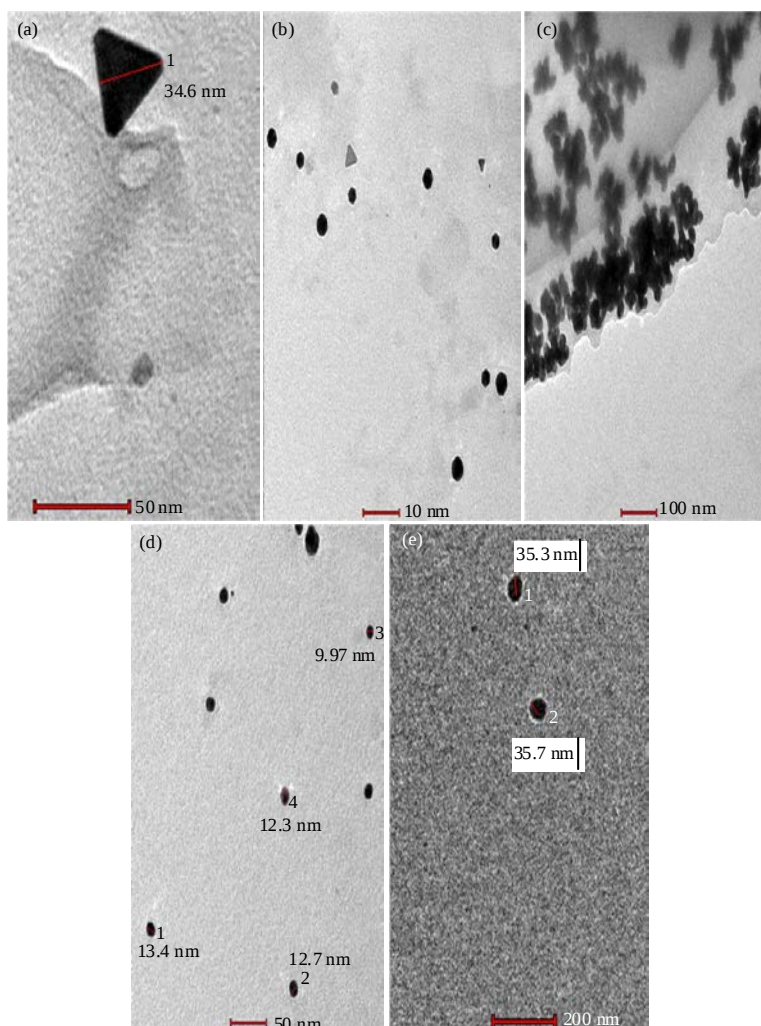


Fig. 2(a-e): TEM micrographs of (a) PAK-AuNPs, (b) SF-AuNPs, (c) AC-AuNPs, (d) PGK-AuNPs and (e) MP-AuNPs

280 nm ($A_{260/280}$). For pure DNA, $A_{260/280}$ is ~ 1.8 . UV-V is spectrophotometric Optical Density (OD) is the most commonly-used technique for estimating chromophore formation and cell concentration in liquid culture. The OD wavelength is often chosen with little thought given to its effect on the quality of the measurement. The optical density value provides the relation of absorption at two wavelengths (Myers *et al.*, 2013). In the present study OD value 1.9 was obtained for DNA for $A_{260/280}$. For studying the interaction of DNA with molecules the absorbance in terms of optical densities is measured. The literature review reveals sparse work with gold nanoparticles and DNA interaction studies. Hence, our area of interest is focused on the interaction of biosynthesized AuNPs with ct-DNA.

UV-Visible evaluation of AuNPs-CT-DNA interaction: The study of drug-DNA interactions could be carried out by UV-Visible absorption spectroscopy by monitoring the changes in the absorption properties of the drug or the DNA molecules. Usually, molecules used as ligands show

an absorption band that can be clearly distinguished in the visible region. A facile way to find the interaction between the DNA and the drug is to examine the shift in the position of the band from when the ligand is free in solution to when the ligand is bound with the DNA. DNA-biosynthesized (PGK, AC, MP, PAK and SF) AuNPs interaction causes a conformation change in the DNA structure during the course of the binding and confirmed by UV-visible spectral results. The results obtained for binding of five biosynthesized AuNPs with DNA revealed the structural changes of CT-DNA, which is reflected from the blue shift in the UV absorption bands.

Figure 3a, b displays the absorption spectra of a competitive binding between PGK-AuNPs and SF-AuNPs with DNA. As shown in Fig. 3a, with increasing concentration of PGK-AuNPs, the maximum absorption between 500-600 nm of the DNA-PGK-AuNPs complex increased but a slight decrease in intensity was observed in the developing band around 520 nm. Compared with PGK-AuNPs, an absorption band at 520 nm of the DNA-SF-AuNPs in the presence of increasing concentrations of SF-AuNPs (Fig. 3b) was observed. The results suggest that PGK-AuNPs intercalated into the double helix of DNA at a concentration above 40 μ L and also shows hyperchromic effect. The absorption spectra of SF-AuNPs-DNA increase with increasing SF-AuNPs concentration. This is a typical hyperchromic effect, which suggests that the DNA double helix structure is damaged after the SF-AuNPs complex bound to DNA through intercalation mode.

These bands are caused by stacking interactions between the bases and the helical suprastructure of the polynucleotide that provides an asymmetric environment for the bases. The AC-AuNPs show slight increase in the absorption band at 520 nm indicating hyperchromic effect (Fig. 3c). An abrupt increase was observed at 550 nm for DNA-MP-AuNPs interaction as the concentration of MP-AuNPs increases (Fig. 3d). This might be probably due to the increasing concentration of AC-AuNPs and MP-AuNPs, which binds with DNA either directly (via intercalative insertion) or indirectly. A steady increase in the absorbance at 550 nm was noted for PAK-AuNPs (Fig. 3e) after interaction with ct-DNA reveals hyperchromism results from the secondary damage of the DNA double helix structure. The hyperchromic effect may also be due to the electrostatic interaction between positively charged gold ions in the mixture and the negatively charged phosphate backbone at the periphery of the double helix ct-DNA.

The causes for the difference in absorbance for sample with AuNPs and without AuNPs are due to the interaction of DNA with the samples. The biosynthesized AuNPs interacts with hydrogen bonds in the nucleic acid, thereby causing a shift from 1.9, the ratio of OD at 260/280, which denotes the purity of DNA. At a very high concentration (50 μ L) of biosynthesized gold nanoparticles, network structures are created possibly through the interconnection of the wrapped species. The morphology of the DNA-AuNPs complexes seems to be dependent on the AuNPs concentration and is significantly different for each plant-mediated nanoparticles. Cisplatin [cis-dichlorodiamine platinum (II)] is a famous covalent binder used as an anticancer drug and makes an intra/inter-strand cross-link through the chloro groups with the nitrogen on the DNA bases (Malina *et al.*, 2000).

The present results of absorption spectra for CT-DNA showed that no major difference was observed in the value of λ_{max} . These spectral characteristics of PGK-AuNPs suggest that the AuNPs has been bound to the base pairs of DNA by intercalation and bathochromism result might be due to the decrease in the energy gap between the highest (HOMO) and the lowest molecular orbitals (LUMO) after the interaction of AuNPs to DNA. The compactness in the structure of either the drug alone and/or DNA after the formation of drug-DNA complex may result in hypochromism. The observable hypochromism and red shift are usually characterized by the non-covalently

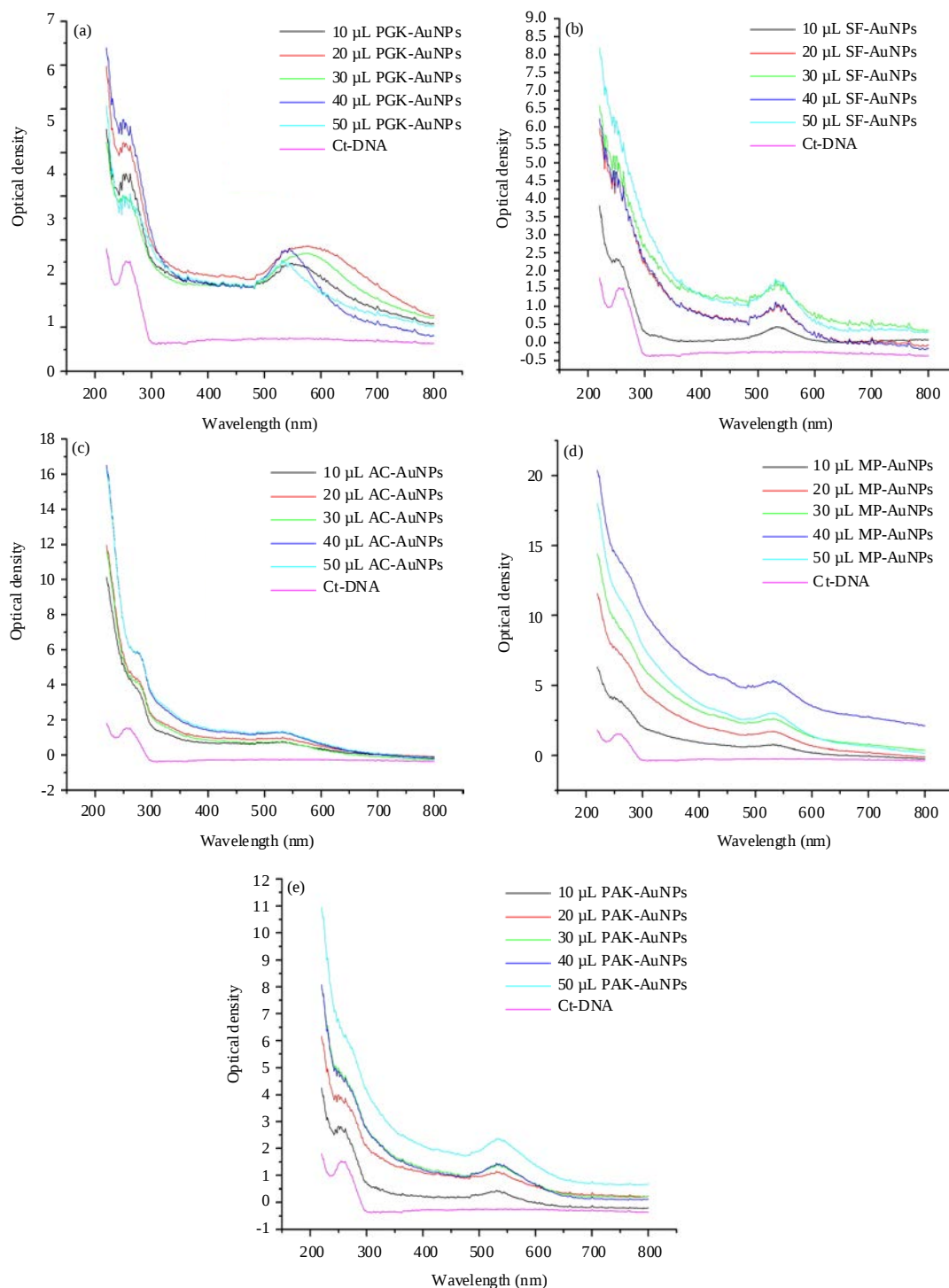


Fig. 3(a-e): Absorbance spectra of (a) PGK and (b) SF-mediated, (c) AC, (d) MP-mediated and (e) PAK-mediated AuNPs with 25 μ L concentration of ct-DNA

intercalative binding of the compound to DNA helix, due to the strong stacking interaction between the aromatic chromophore of the compound and base pairs of DNA (Zhang *et al.*, 2012, 2013).

Despite of the smaller size and lesser quantity of the biosynthesized AuNPs, the Surface Plasmon band of AuNPs is almost detectable with regard to UV-Vis technique. Moreover, the changes in the UV absorption band provide support to the DNA-AuNPs complex formation. The absorption, increased significantly upon addition of biosynthesized AuNPs, indicating interaction of the AuNPs with DNA. The CuL^{2+} can make a contraction in the helix axis of DNA by electrostatic binding to the phosphate group of DNA backbone. Concurrently, partial intercalation act as a “wedge” to pry apart one side of a base pair stack but not fully separate the stack as required by the classical intercalation model. A static bend or kink in the helix, which results in a reduction in helix end-to-end distance of DNA and confirm the hypochromism (Zhou and Yang, 2006).

The capping agents of the biosynthesized AuNPs may be the reason for the intercalative binding with DNA. Comparison of variations in the substituents on the nanoparticles surface shed light on the preferred balance between charge density and hydrophobic bulk (Goodman *et al.*, 2006). The functional groups stabilize the nanoparticles by electrostatic repulsion and can be exploited for the conjugation of other molecules to the particles. The choice of ligand depends on the particle size and the solvent. These ligands can also be used as anchor points for further attachment of biological molecules (DeLong *et al.*, 2010). Thus the charge density on the surface modified or the functional groups anchored to the biosynthesized AuNPs may help in the stacking onto the base pairs of the ct-DNA and pave the way for the interaction.

Doxorubicin is a cationic anticancer drug that binds to double stranded DNA via its minor groove through intercalation at the CpG step in the sequence, 5-TCG. An uncharged anticancer drug Actinomycin D bind by intercalation in the minor groove of DNA with high affinity for the sequence 5-AGCT and related GC sequences (Alexander *et al.*, 2012). A three-step reversible mechanism is proposed for the binding of DNA to gold nanoparticles capped with tiopronin. An external adduct (DNA, AuNPs) was formed in the first step indirectly and groove binding of NPs to the DNA was achieved in the second step (Prado-Gotor and Grueso, 2011). The absorbance titrations of interaction of DMAP-Au NPs with DNA, indicate that a non-covalent interaction between DNA takes place and the external surface of the NPs does take place (Biver *et al.*, 2012). The increase in absorbance (hyperchromism) and a blue shift suggest that the biosynthesized AuNPs strongly binds to CT-DNA due to the insertion into the adjacent base pairs on the DNA duplex, i.e., partial intercalation and groove binding. Similar results were observed for previous literature (Eshkourfu *et al.*, 2011; Zhou *et al.*, 2007). The typical main absorption band of dsDNA is 260 nm and this band disappeared after interaction with SF, PAK, AC and MP AuNPs except PGK-AuNPs. This is a strong indication that there was a DNA conformational transition during incubation with biosynthesized AuNPs which leads to the condensation of the DNA double helix structure. Hence, the smaller size of AuNPs enhances the interaction with DNA even at very low concentration compared to that of standard anticancer drugs. Thus the biosynthesized AuNPs altered the reactivity of DNA of cancer cells and facilitates the drug effects implying molecular structure is directly related to biological activity.

CONCLUSION

In vitro DNA binding studies of the biosynthesized AuNPs was determined from the variation in the shift of absorption band before and after interaction with DNA. Both hypsochromic and hyperchromic shifts were obtained for PGK-AuNPs. The AuNPs intercalate with DNA even at lower concentrations and this trend was changed at higher concentration. As concentration of SF, MP, PAK and AC-AuNPs increases, the absorbance also increases revealed hyperchromic shift,

suggesting that the DNA double helix structure is damaged after the AuNPs bound to DNA through intercalation mode. The study demonstrates biosynthesized AuNPs interact with DNA mainly through groove binding and partial intercalation.

ACKNOWLEDGMENT

The authors would like to acknowledge Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore, Tamil Nadu, for providing research facilities.

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