CHAPTER-5

OPTICAL NANOBIOSENSOR FOR MONITORING CARBOPLATIN-DNA INTERACTION
5.1 ABSTRACT

The interaction of DNA and Carboplatin was studied with DNA labeled gold nanoparticles (AuNPs) based optical nanobiosensor. Carboplatin, a cytotoxic drug is responsible for producing nephrotoxicity at effective dose. Thus, we have developed optical nanobiosensor for monitoring Carboplatin-DNA interaction based on Fluorescence Resonance Energy Transfer (FRET) phenomena. Paracetamol, an analgesic agent was used as controlled drug in this study. The DNA labeled AuNPs, exposed to Carboplatin and Paracetamol, a binding event between the DNA and Carboplatin takes place, resulting in a conformational change within the biosensor complex which decreases the distance between the fluorescent molecules or the fluorescent/quencher molecules. As the Carboplatin interact with DNA, an increase in fluorescence intensity was observed. In case of Paracetamol, slight increase in fluorescence intensity was observed because Paracetamol was reported as non-toxic drug. So, the major difference in increased fluorescence intensity between Carboplatin-DNA and Paracetamol-DNA interaction shows significant observations. Results have demonstrated that Optical sensor is able to rapidly and effectively monitor Carboplatin-DNA interaction with a detection range 0.45µg/ml. This suggests that the developed optical nanobiosensor was an ideal for monitoring Drug-DNA interaction studies while performing combinatorial synthesis for new drug development.
5.2 INTRODUCTION

Recently, much attention has been concentrated on the study of the interaction between the biological macromolecules, such as DNA–DNA, DNA–protein, and protein–protein. Especially for DNA interaction will lead to loss of some functions of DNA, even to apoptosis of the cell and further induces cancer. Many researches have performed relationship between drug-DNA interaction by means of different spectroscopic and chromatographic techniques and cell culture assays. Several papers have been reported on biosensor monitoring Drug-dsDNA, Drug-ssDNA, heavy metal-DNA and Drug-RNA interaction. But, till date no such nanobiosensor have been reported which can monitor drug-DNA interaction. Here, we developed optical nanobiosensor based on gold nanoparticles labeled with DNA. Carboplatin, a second generation cytotoxic drug exhibiting significant anti-cancer activity with less side effects than Cisplatin. The anti-tumor effect is due to interaction with DNA via intrastrand and interstrand cross-links. These leads drug-DNA adduct formation, which may contribute to toxic effects. Paracetamol, an analgesic drug was used as controlled drug in this study. It is toxicity free drug and very weak interacted with DNA. Our study shows significant observations in fluorescent intensity between Carboplatin and Paracetamol.

Nanotechnology is playing an increasingly important role in the advancement of biosensors. The sensitivity and performance of biosensors is being improved by using nanomaterials for their construction. Nanoparticles play a key role in adsorption of biomolecules due to their large specific surface area and high surface free energy. Gold nanoparticles and quantum dots have been widely used due to their optical properties. Recently, regulation of protein-DNA interaction was reported by Jun Fang and her coworkers. Xinbing Zuo and his team developed different DNA probes on AuNPs to compare single stranded DNA and hybridized DNA interaction with Hg$^{2+}$using both absorption and fluorescence detection. Numerous researches have been carried out to study DNA detection and DNA hybridization assays using gold nanoparticles and quantum dots. Thus combination of nanomaterials and biomolecules is of considerable interest in the field of nanobiotechnology. Figure 5.1 shows the schematic
representation of development of optical nanobiosensor. DNA were labeled with AuNPs and exposed to drug solution show decrease FRET.

**Figure 5.1:** Schematic representation of optical nanobiosensor. AuNPs were labeled with DNA and exposed to drug solution. Fluorescence enhancement observed on binding of DNA to drug via FRET. Solid and wavy arrows indicate the radiative and nonradiative processes, respectively.

Fluorescence resonance energy transfer (FRET) assays are often used to identify the interaction of two molecules. One molecule is labeled with a fluorescence acceptor, which is excited only when a molecule—usually a binding partner—bearing a fluorescence donor is nearby. In general, the energy transfers from the donor to the acceptor\(^1\). In 1948, Theodor Förster observed that when the resonating dipole moments of two molecules are identical, which in this case are D and A, an energy coupling between the two could occur resulting in photon-less transfer of energy. In addition, the excited acceptor molecule (A’) returns to the ground state (A) by losing its energy via photon emission (in case, acceptor is a fluorophore), i.e., fluorescence\(^2\) (Figure 5.1). Irrespective of the photo-physical characteristic of the acceptor, i.e., whether it is a chromophore or fluorophore, the energy transfer process is called as Förster resonance.
energy transfer colloquially referred to as fluorescence resonance energy transfer. When the acceptor molecule is non-emitting then the fluorescence intensity is solely due to the donor’s fluorescence, as is the case of this experiment.
5.3 EXPERIMENTAL

5.3.1 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 0.1M disodium hydrogen phosphate in water and adjusting the pH by adding 0.1M HCl. 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. Carboplatin was obtained from Cipla Ltd and used without purification. Paracetamol was obtained from Sun pharmaceuticals. All aqueous solutions were prepared in Milli-Q water from a Millipore purification system and all experiments were done at room temperature.

5.3.2 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 10 nm and 5 nm was used respectively. Excitation and emission wavelength of 450 nm and 480 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 Zetasizer (Model-The Zetasizer Nano ZS, UK).

5.3.3 Citrate-Capped AuNPs synthesis and modification

AuNPs were prepared by citrate reduction of HAuCl₄ according to documented methods with slight modifications. A 25ml aqueous solution containing of 1.0×10⁻³ mol L⁻¹ HAuCl₄ was brought to a vigorous boil with stirring in a round bottom; a 2.5 mL, 38.8×10⁻³ mol L⁻¹ trisodium citrate solution was then added rapidly to the above solution. The mixture solution was heated for another 20 min and color of the solution changed from pale yellow to deep red. Subsequently, the solution was cooled to room temperature while being still stirred continuously. Finally, a deep red, monodisperse
“naked” AuNPs with was obtained and this solution is used as stock solution. The sizes of the AuNPs were verified by Malvern Zeta-sizer.

5.3.4 DNA labeled AuNPs

Citrate capped AuNPs were labeled with dsDNA (double stranded DNA). 20 mg/ml DNA solution were prepared in phosphate buffer 7. AuNPs solution as prepared was diluted (double distilled water) to 2x. 2 ml of diluted AuNPs were added to 2 ml of DNA solution and solution was stir at 150 rpm for 3min. Then, this was used for monitoring Drug-DNA interaction.
Chapter 5

5.4 RESULTS AND DISCUSSION

5.4.1 Characterization of gold nanoparticles

Figure 5.2 illustrates that morphology of AuNPs. The particles were predominantly spherical in shape with diameter ranging from 20±5 nm. Larger particles with diameter 40±10 nm were also obtained.

![Figure 5.2: SEM image of AuNPs](image)

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 5.3 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%).
5.4.2 Evaluation of DNA labeled AuNPs

dsDNA has a stable double-helix geometry that always presents the negatively charged phosphate backbone. 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. The charges of the phosphate groups of DNA may induce a dipole in the highly polarizable gold particles. This mechanism is quite short ranged, and therefore the Coulombic repulsion keeps the species apart at longer distances. One incubation scenario is thus that the ionic strength of the solution needs to be raised in order to screen the long-range repulsion so that DNA and the particles are allowed to approach each other. At a certain distance, the ion-induced dipole interaction takes over, resulting in a net attractive force. As shown in Figure 5.4, it was clearly seen that the DNA adsorbs to the AuNPs. The peak of DNA and AuNPs were shifted. Also, the height of intensity peak was decreased in case of AuNPs, while in case of DNA it was elevated. The pure DNA gives absorbance peak at 260 nm, while AuNPs give peak at 527 nm. The peak of DNA labeled AuNPs are 256 and 530, show the hypsochromic and bathochromic shift. Thus the shifting and the change in height of the intensity peak confirm the absorption of DNA to the citrate capped AuNPs.

Figure 5.3: Average particle size distribution of AuNPs
Figure 5.4: UV-visible spectra of AuNPs obtained at 527 nm, while the intensity peak decreased and shifted at 530 on binding with DNA.

5.4.3 Carboplatin-DNA interaction by optical nanobiosensor

100 µg/ml concentration of Carboplatin solution were prepared by dissolving it in to distilled water. 5 to 25 µL Carboplatin were added to DNA labeled AuNPs and fluorescent measurement was carried out by spectrofluorometer. Bathochromic shift of the absorption of the AuNPs from Fig. 5.4 in the presence of Nucleobases viz., adenine, guanine, thiamine and cytosine bound in the DNA is an obvious consequence of their ground state complexation with AuNPs (Figure 5.1) with a static quenching efficiency. Nucleobases are non-radiating in nature when excited at the AuNPs’ wavelength. Here, we have realized the Nucleobases as acceptor molecules and AuNPs as donor molecules as per the FRET theory. The experiment has been designed to monitor the DNA interaction proportional to the concentration of Carboplatin. As the Carboplatin concentration was incrementally increased (by 5 µL), the interaction of the same with the DNA increased. The first spectrum a shown in Figure 5.5 is of bare AuNPs, while spectra
b is of DNA labeled AuNPs. Spectra of DNA labeled AuNPs shows higher fluorescence intensity than bare AuNPs due to adsorption of DNA to AuNPs. Spectrum from c to g is of increasing concentrations of Carboplatin added to DNA-AuNPs solution. The interaction of the Carboplatin with DNA is seen as conformational change in the DNA structure as the former intercalates with the DNA by breaking the latter’s Hydrogen bonds laterally attached. Furthermore, the hydrogen bonds which are responsible for the compactness of the DNA helix, on breaking causes expansion of the DNA structure. The resultant expansion indicates an increase in distance between the Nucleobases and the AuNP molecule. Therefore, FRET from AuNP to the Nucleobases decreases with reduction in the quenching effect of the colloidal gold which becomes evident from the increase in the fluorescence peak intensity with respect to Carboplatin concentration.

Figure 5.5: Fluorescence emission of DNA labeled AuNPs. Excitation(Ex) at 450 nm yield a fluorescence emission(Em) peak at 531 nm. [a: bare AuNPs, b: DNA-AuNPs, after addition of increasing amount of Carboplatin to DNA-AuNPs, i.e., c:5µL, d:10µL, e:15µL, f:20µL, g:25µL]
5.4.4 Paracetamol-DNA interaction by optical nanobiosensor

100 µg/ml concentration of Paracetamol solution were prepared by dissolving it in to distilled water. 5 to 25 µL aliquots of Paracetamol were added to DNA labeled AuNPs and fluorescent measurement was carried out by spectrofluorometer. From Figure 5.6, it is clearly seen that the increase in fluorescent intensity was very less as compare to Carboplatin. Paracetamol is not an intercalative drug and reported as non-toxic drug. However, few studies have reported that Paracetamol shows marginal DNA interaction in vitro studies\textsuperscript{22-25}. Here, in our studies, a very weak interaction was observed between Paracetamol and DNA. Spectrum from c to g was for varying concentration of Paracetamol added to DNA-AuNPs shows increased fluorescent intensity. This suggests that the binding behavior of Carboplatin is much stronger than Paracetamol.

![Figure 5.6: Fluorescence emission of DNA labeled AuNPs. Excitation(Ex) at 450 nm yield a fluorescence emission(Em) peak at 531 nm. [a: bare AuNPs, b: DNA-AuNPs, after addition of increasing amount of Paracetamol to DNA-AuNPs, i.e., c:5µL, d:10µL, e:15µL, f:20µL, g:25µL](image)]
5.5 SENSITIVITY AND SELECTIVITY OF OPTICAL NANOBIOSENSOR

To evaluate the sensitivity and selectivity of this optical nanobiosensor, 100 µg/mL drug concentrations was used and ranging from 5 to 25 µL. Figure 5.7 shows the average fluorescence intensity of the Carboplatin-DNA and Paracetamol-DNA interaction obtained by collecting data from three independent measurements at the same conditions. The results showed that the fluorescence intensity of Carboplatin-DNA interaction was enhanced along with the increase of Carboplatin concentration because of the increasing aggregation state due to conformational change. (Figure 5.8) While, the fluorescence intensity of Paracetamol-DNA interaction increased slightly suggest that a very weak interaction occurs. Overall, the Carboplatin shows good fluorescence signals on interaction with DNA, while Paracetamol shows a very weak fluorescence signal. Hence, the proposed nanobiosensor was perfect for monitoring Drug-DNA interaction.

![Graph](image)

**Figure 5.7:** Comparative study of Carboplatin-DNA and Paracetamol-DNA interaction by optical Nanobiosensor
Figure 5.8: Comparison of interacting behaviour of Carboplatin and Paracetamol with DNA
5.6 PERFORMANCE OF NANOBIOSENSOR

The comparisons of analytical performances for determining Carboplatin-DNA and Paracetamol-DNA interaction are given in Table 5.1. For Carboplatin, the values of correlation coefficient ($R^2$), slope and intercept were found as 0.9929, 2.25 and 237.04, respectively. (Figure 5.9) Limit of detection (LOD) and limit of quantification (LOQ) were found as 0.45 and 0.1.35 µg/ml respectively.

**Table 5.1**: Comparison of the analytical performance for determination of Carboplatin-DNA and Paracetamol-DNA interaction

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fluorescence intensity of Carboplatin</th>
<th>Fluorescence intensity of Paracetamol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression equation (Y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (b)</td>
<td>2.25</td>
<td>0.20</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>237.04</td>
<td>117.67</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.992</td>
<td>0.996</td>
</tr>
<tr>
<td>LOD$^a$ (µg/mL)</td>
<td>0.45</td>
<td>1.43</td>
</tr>
<tr>
<td>LOQ$^b$ (µg/mL)</td>
<td>1.35</td>
<td>4.34</td>
</tr>
</tbody>
</table>

$^a$LOD =$3.3\times SD/slope$

$^b$LOQ = 10,SD/slope

For Paracetamol, the values of correlation coefficient ($R^2$), slope and intercept were found as 0.996, 0.20 and 117.67, respectively. (Figure 5.10) LOD and LOQ were found as 1.43 and 4.34 respectively.
Figure 5.9: Plots of the concentration of Carboplatin versus the intensity of Carboplatin-DNA interaction

Figure 5.10: Plots of the concentration of Paracetamol versus the intensity of Paracetamol-DNA interaction
5.7 CONCLUSION

In conclusion, we have produced optical nanobiosensor for monitoring Drug-DNA interaction based on its modulation of fluorescence enhancing efficiency between drug and DNA labeled AuNPs in aqueous solution. In fact, we can observe DNA interaction occurring with increased amount of Carboplatin which suggests that Carboplatin intercalates with DNA and slowly interacts with it causing some breaking of the hydrogen bonds. In case of Paracetamol, a very weak interaction was observed. In addition, the present method features with rapidity and saving-time, simplicity, and low cost. The developed method provides a new perspective to the FRET and is expected to be used for the monitoring Drug-DNA interaction and toxicological studies in nearest future.
5.7 REFERENCE


