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Optical nanobiosensor: A new analytical tool for monitoring carboplatin–DNA interaction *in vitro*

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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) phenomenon. Paracetamol, an analgesic agent, was used as controlled drug in this study. The DNA labeled AuNPs, exposed to carboplatin, a binding event among the DNA and carboplatin takes place, resulting in a conformational change within the biosensor complex which decreases the distance among the fluorescent molecules or the fluorescent/quencher molecules. As the carboplatin interact with DNA, an increase in fluorescence intensity was observed. 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 limit up to 0.45 μg/ml. This suggests that the developed optical nanobiosensor was ideal for monitoring Drug–DNA interaction studies while performing combinatorial synthesis for new drug development.

1. 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 damage which leads to loss of some functions of DNA, even to apoptosis of the cell and further induces cancer [1]. Tremendous amount of work has been done to investigate the relationship between DNA damage and cancer using 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 interactions [2,3]. But, till date no such nanobiosensor has been reported which can monitor drug–DNA interaction. Here, we have developed an optical nanobiosensor based on gold nanoparticles labeled with DNA. Carboplatin is a second generation cytotoxic drug exhibiting significant anti-cancer activity. The anti-tumor effect is due to the interaction with DNA via intrastrand and interstrand cross-links. This leads to DNA damage and adduct formation, which may contribute to toxic effects [1]. Paracetamol, an analgesic drug, was used as controlled drug in this study. It is a toxicity free drug and does not damage DNA. Our study shows significant observations in fluorescent intensity amongst carboplatin and paracetamol.

Nanotechnology is playing an increasingly important role in the advancement of biosensors. The performance of biosensors is being improved by using nanomaterials for their construction [4]. Nanoparticles play a key role in adsorption of biomolecules due to their large specific surface area and high surface free energy. Gold nnoparticles 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 [5]. 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 [7]. Numerous research work has been done to study DNA detection and DNA hybridization assays using gold nanoparticles and quantum dots [6–13]. Thus, combination of nanomaterials and biomolecules is of considerable interest in nanobiotechnology. Fig. 1 shows the schematic representation of development of optical nanobiosensor. DNA labeled AuNPs when exposed to drug solution show decreased FRET.

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 in the vicinity. In general, the energy transfers from the

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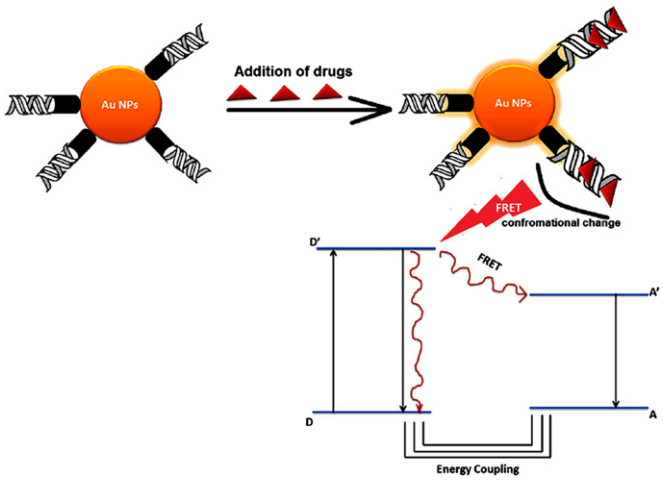

Fig. 1. Schematic representation of optical nanobiosensor. AuNPs were labeled with DNA and exposed to drug solution. Fluorescence enhancement observed on binding of DNA to drug via FRET. Solid and wavy arrows indicate the radiative and nonradiative processes, respectively.

donor to the acceptor [14]. 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 occurred resulting in the 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 (Fig. 1) [15]. 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 in the case of this experiment.

2. Experimental

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. $HAuCl_4$ and Tri-sodium citrate were used to prepare AuNPs. All chemicals were purchased from E-Merck (Mumbai, India), SRL (Mumbai, India) and were all 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.

2.2. Characterization and measurement

Fluorescence intensity was measured by Jasco FP-6500 spectroflurometer (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 Zeta-sizer (Model—The Zetasizer Nano ZS, UK).

2.3. Citrate-capped AuNPs synthesis and modification

AuNPs were prepared by citrate reduction of $HAuCl_4$ according to documented methods with slight modifications [16–17]. A 25 ml aqueous solution containing of 1 mM $HAuCl_4$ was brought to a vigorous boil with stirring in a round bottom flask; a 2.5 ml, 38.8 mM 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 and stirred continuously. Finally, a deep red, monodisperse "naked" AuNPs was obtained and this solution was used as the stock solution. The sizes of the AuNPs were verified by Malvern Zeta-sizer.

2.4. DNA labeled AuNPs

Citrate capped AuNPs were labeled with dsDNA (double stranded DNA). Twenty mg/ml DNA solution was prepared in phosphate buffer having pH 7. AuNPs solution formerly prepared was diluted (by double-distilled water) to 2×2 ml of diluted AuNPs was added to 2 ml of DNA solution, and the solution was stirred at 150 rpm for 3 min, which was then used for carboplatin–DNA and paracetamol–DNA interaction studies.

3. Results and discussion

3.1. Characterization of gold nanoparticles

Fig. 2 shows the SEM image of AuNPs. The particles were predominantly spherical in shape with diameter ranging 20 ± 5 nm. Larger particles with diameter 40 ± 10 nm were also obtained.

The particle size characterization was determined by Malvern zeta-sizer. This instrument allows the measurement of particle size distributions in the range 0.6 nm–10 μm. The average particle size of AuNPs was 14 nm. Fig. 3(A) shows the particle size distribution of AuNPs. Particles were in range of 25.92 nm (83.7%), 0.7579 nm (10%), and 3.039 nm (3.3%). From Malvern zeta sizer and SEM analysis, it is clear that most of the particles were ranging 20 ± 5 nm and suggested as donor particles in the experiment.

3.2. Evaluation of DNA labeled AuNPs

dsDNA has a stable double-helix geometry that always presents the negatively charged phosphate backbone [7]. So they have

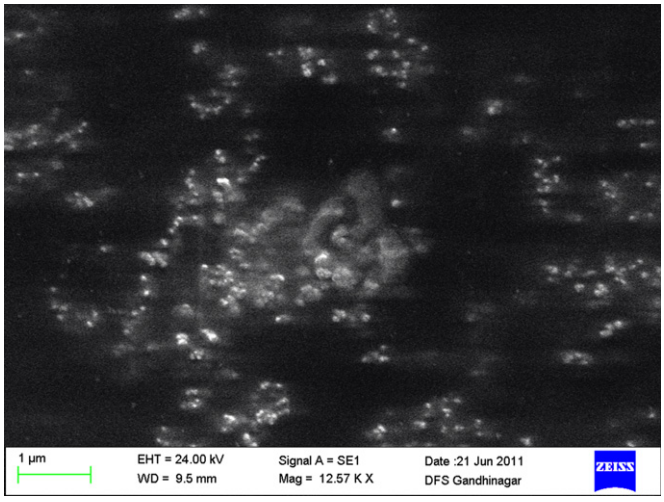

Fig. 2. SEM image of AuNPs.

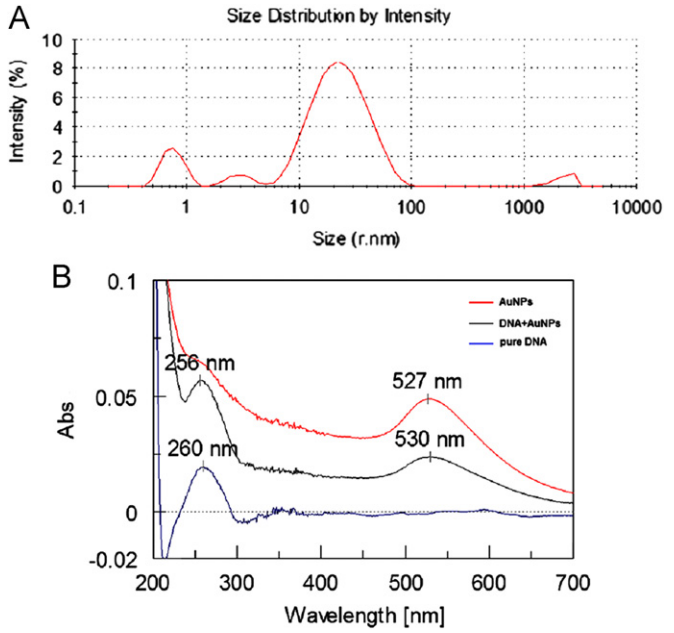

Fig. 3. (A) Average particle size distribution of AuNPs; (B) UV–vis spectra of AuNPs obtained at 527 nm, while the intensity peak decreased and shifted at 530 on binding with DNA. (A) Size distribution of AuNPs (Z-average-14 nm) and (B) UV–visible spectra of AuNPs.

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 suggested 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. At a certain distance, the ion-induced dipole interaction takes over, resulting in a net attractive force [18–19]. As shown in Fig. 3(B) it was clearly seen that the DNA adsorbs to the AuNPs. The peak of DNA and AuNPs were shifted. In addition, the intensity of AuNPs was decreased, while in case of DNA it was elevated which confirms the adsorption of DNA to citrate-capped AuNPs. The pure DNA gives absorbance peak at 260 nm, while AuNPs give peak at 527 nm. The peaks of DNA labeled AuNPs are 256 and 530, which show the hypsochromic and bathochromic shift respectively. Thus, the shifting and the change in absorbance peak confirm the absorption of DNA to the citrate capped AuNPs.

3.3. Carboplatin–DNA interaction by optical nanobiosensor

Hundred μg/ml concentration of carboplatin solution was prepared by dissolving it in distilled water. Five microliters to 25 μL carboplatin was added to DNA labeled AuNPs and fluorescent measurement was carried out by spectrofluorometer. Bathochromic shift of the absorption of the AuNPs as shown in Fig. 3(B) 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 (Fig. 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 as shown in Fig. 4 is of bare AuNPs, while spectra b are of DNA labeled AuNPs. Spectra of

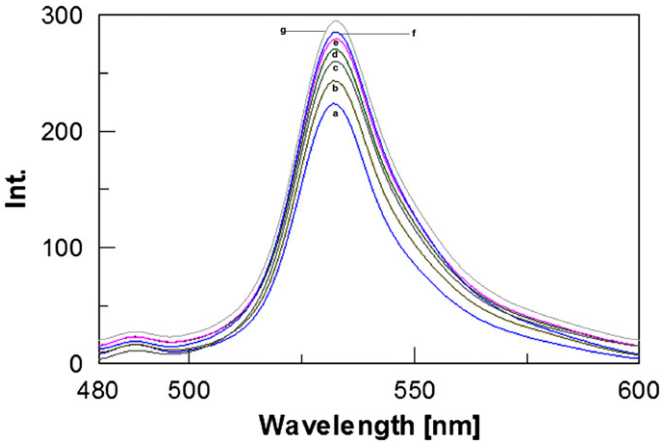

Fig. 4. Fluorescence emission of DNA labeled AuNPs. Excitation (Ex) at 450 nm yield a fluorescence emission (Em) peak at 531 nm. [(a): bare AuNPs, (b): DNA–AuNPs, after addition of increasing amount of carboplatin to DNA–AuNPs, i.e., (c): 5 μL, (d): 10 μL, (e): 15 μL, (f): 20 μL, (g): 25 μL].

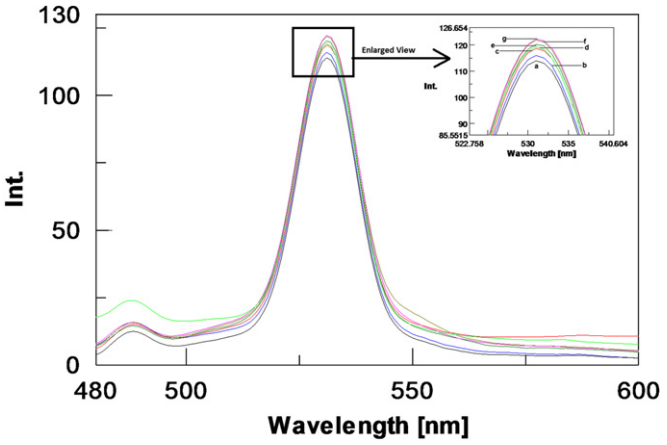

Fig. 5. Fluorescence emission of DNA labeled AuNPs. Excitation (Ex) at 450 nm yields a fluorescence emission (Em) peak at 531 nm. [(a): bare AuNPs, (b): DNA–AuNPs, after addition of increasing amount of paracetamol to DNA–AuNPs, i.e., (c): 5 μL, (d): 10 μL, (e): 15 μL, (f): 20 μL, (g): 25 μL].

DNA labeled AuNPs show 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 the 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 cause 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.

3.4. Paracetamol–DNA interaction by optical nanobiosensor.

Hundred μg/ml concentration of paracetamol solution was prepared by dissolving it in distilled water. Five microliter to 25 μL aliquots of paracetamol were added to DNA labeled AuNPs and fluorescent measurement was carried out by spectrofluorometer. From Fig. 5, it is clearly observed that the increase in fluorescent intensity was very less as compared to carboplatin.

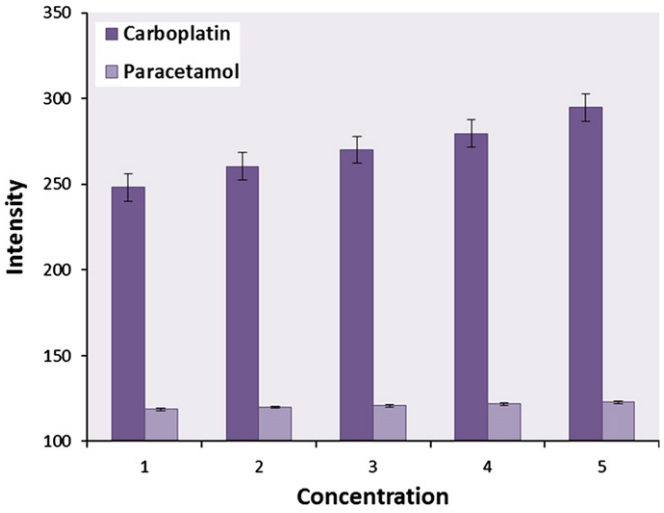

Fig. 6. Comparative study of carboplatin–DNA and paracetamol–DNA interaction by optical Nanobiosensor at 100 μg/ml.

Paracetamol is not an intercalative drug and is reported as a non-toxic drug. However, few studies have reported that paracetamol shows marginal DNA interaction in vitro studies [20–22]. 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.

3.5. Sensitivity and selectivity of optical nanobiosensor

To evaluate the sensitivity and selectivity of this optical nanobiosensor, 100 μg/ml drug concentrations ranging from 5 to 25 μL were used. Fig. 6 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. While, the fluorescence intensity of paracetamol–DNA interaction increased slightly, this suggested that a very weak interaction is occurring. 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.

3.6. Performance of nanobiosensor

The comparisons of analytical performances for determining carboplatin–DNA and paracetamol–DNA interaction are given in Table 1. For carboplatin, the values of correlation coefficient (R^2), slope and intercept were found to be as 0.9929, 2.2541 and 237.04, respectively. Limit of detection (LOD) and limit of quantification (LOQ) were found as 0.450 μg/ml and 0.450 μg/ml respectively. For paracetamol, the values of correlation coefficient (R^2), slope and intercept were found to be as 0.9965, 0.2066 and 117.67, respectively. Limit of detection (LOD) and limit of quantification (LOQ) were found as 1.435 and 4.349 respectively.

Table 1
Comparison of the analytical performance for determination of carboplatin–DNA and paracetamol–DNA interactions.

Parameters	Fluorescence intensity of carboplatin	Fluorescence intensity of paracetamol
Regression equation (Y)	2.2541	0.2066
Slope (b)	237.04	117.67
Intercept (c)		
Correlation coefficient (r)	0.9929	0.9965
Limit of detection[a] (μg/ml)	0.450	1.435
Limit of quantitation[b] (μg/ml)	1.35	4.349

[a] Limit of detection = 3.3 SD/slope.
[b] Limit of quantitation = 10 SD/slope.

4. Conclusion

In conclusion, we have developed an optical nanobiosensor for monitoring Drug–DNA interaction. It is based on the adaptive characteristic modulation of fluorescence enhancing efficiency among drug and DNA labeled AuNPs in aqueous solution. In fact, we can observe DNA damage 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 the briskness, simplicity, low cost and above all, an insight to the monitoring Drug–DNA interaction and toxicological studies in near future.

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