Chapter 4: Nanomaterial based platform for Escherischia coli Sensing

4.1 Introduction

Enterotoxigenic *Escherichia coli* (ETEC) is distinct pathogen causing secretory diarrhea mostly in developing countries. Such class of bacteria secretes both a heat labile enterotoxin (*Lt*) and heat stable enterotoxin (*St*). *St* enterotoxin (under ETEC group) is responsible for secretory diarrhea. It resembles cholera toxin in its antigenicity [Hedge et al., 2009; Rajkhowa et al., 2009]. Since *Lt* gene shows sensitivity towards heat treatment so construction of sensing platform is difficult for it (specially the last stage of hybridization; where denaturation of genomic DNA is carried out). A wide number of tests have been reported showing toxicological as well as immunological aspects for heat stable enterotoxin [Leitner et al., 2006: Son et al., 1900]. Genetic information for *St* and *Lt* is plasmid mediated. PCR serves as an important tool in identification of ETEC from other E coli strains, which is very sensitive and rapid. Conventional microbiological detection of *E. coli* includes plate counting, membrane filter technique, multiple-tube fermentation and turbidimetry [Joanna et al., 2008; Pachaury et al., 2013;Loukiadis et al 2006; Ram et al., 2008; Guiti et al., 2011]. These methods are reliable but require long incubation time (1–2 days) and complexity in operation. Effective method of bacterial species detection is the need of the hour. Moreover sensitive detection requires rapid, simple handling operation and low cost for E coli quantification. In these areas, requirement of rapid, sensitive, simple and low cost detection methods of *E. coli* is also recommended from health viewpoint. In recent years DNA/RNA biosensors showing high sensitivity and excellent selectivity have been developed and used for detection of pathogen.

Table 4.1 Shows comparative study for detection of *E. coli* over past decade.

S.No.	Immobilization matrix	Detection Limit	Detection Technique	Reference
1	Fe_2O_3@Au core/shell nanoparticle	pM DNA 500 cfu/mL,	Amperometric	Li (2011)
2	Nanoporous alumina	10^2 CFU/mL to 10^5 CFU/mL, 10^2 CFU/mL	Impedance	Tian (2016)
3	Au aggregates	4 aM to 24 fM, Impedance	Impedance	Singh (2015)
4	Alginic acid coated cobalt magnetic beads	1.0 × 102 to 2.0 × 10 voltammetry 3 cells/mL with a detection limit of 50 cells/mL	Cyclic voltammetry	Geng (2011)
5	Platinum nanoparticle	10^{-12} M	impedance	Kashish (2016)
6	Lectin functionalized SAM	1.0 × 10^2 to 1.0 ×10^5 cells/mL	Impedance	Yang (2016)
7	Nanoparticle/SWCNT	10^2–10^5 CFU/mL	Change in current vs concentration	Yamada (2016)
8	Ferrocene antimicrobial peptide	10^3 cfu/mL	Impedance	Li (2014)
9	Real-Time PCR with Molecular Beacon	4CFU/mL. efficiency greater than 500 times than regular PCR	Real time PCR	Ram (2008)
10	Ruthenium complex-labelled Concanavalin A	5.0 × 10^2 to 5.0 ×10^5 cells/mL. detection limit was 127 cells/mL	Fluorescence	Yang (2012)
11	Self-assembly of mercapto-hexanoic acid	Low limit of detection (2 CFU/mL): linear range (3×10^3-10^4 CFU/mL).	Impedance	Santos (2013)
12	Silica nanospheres with fluorescein	4 - 4.0 X 108 cfu/mL, LOD of 3 cfu/mL	Fluorescent immunoassay	Hu (2016)

DNA biosensors based on various detection techniques such as optical, electrochemical, chemiluminescence, fluorescence, impedance and quartz crystal microbalance have been

developed for *E. coli* [Li et al., 2010, 2014; Guo et al., 2012; Geng et al., 2011]. The limit of detection for as mentioned E coli sensors points at 10^3 -10^7 cfu/ml with ferrocene based matrix, carbohydrate based matrix LOD peaks at 10^2-10^3 cfu/ml, gold nanoaggregates based matrix via Differential Pulse Voltammetry LOD calculated as 10^{-10} M [Guo et al., 2012]. Supplementing in this regard, we have proposed a sensitive and efficient impedimetric DNA biosensor based on stable and selective ssDNA/PtNp@CS probe for *E. coli* detection. Novelty of the sensing platform lies in its simplicity and limit of detection is of order 10^{-14} M for such prototype sensor lies in order of nanoscale (10^{-12} M).Nanomaterials mainly carbon based materials and metals are significantly enhancing stability, surface chemistry, porosity and surface area of sensing platforms [Chan et al., 2013; Yamada et al., 2016; Tan et al., 2011; Yang et al., 2012]. Based on our previous work we found that cationic polymers, like chitosan (depending on pH), have potential for DNA complexation and an excellent matrix for immobilization of biomolecules and development of low cost probes [Tanveeer et al., 2015; Chandra et al., 2011. More recently, platinum nanoparticles decorated with chitosan attracted attention for DNA sensing [Chan et al., 2013]. Moreover, it is a biocompatible, biodegradable and non-toxic for sensor applications [Nigam et al., 2009; Srivastava et al., 2014]. In the present work we have developed an electrochemical sensor based on glassy carbon electrode (GCE) and conducting carbon ink screen printed electrode (SPE) decorated with platinum nanoparticles and chitosan (PtNPs@CS) and subsequently immobilized with a specific single-stranded (ss-DNA) oligonucleotides selective for *E. coli* DNA. Such selective probe is developed based on *St* gene (gene coding for heat stable toxin in Enterotoxigenic *E. coli* physically immobilized over PtNPs@CS matrix. The hybridization event of the *E. coli* DNA with ssDNA probe was monitored based on impedance spectroscopy (change of R_{CT})

and relative change of R_{CT} is used as sensor parameter for detection. The schematic diagram of thus developed *E. coli* biosensor

based on EIS method is shown in following Figure 4.1

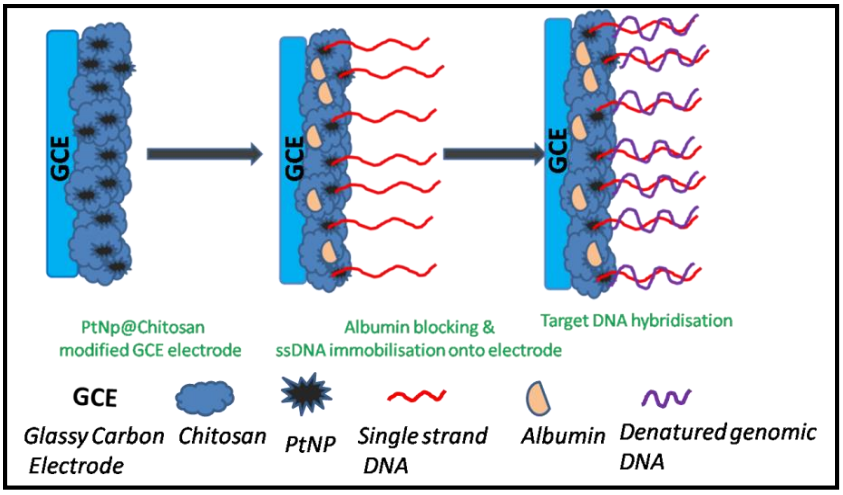

Figure 4.1 Schematic illustration for fabrication of the biosensor electrode (PtNPs@CS assembly for DNA detection.

4.2 Experimental

4.2.1 Chemicals

The ssDNA oligomers of *Escherichia coli* MTCC723 was purchased from Sigma, India. The ssDNA (*St*-1 gene) primer was assembled onto the modified electrode. The sequence for the complementary oligomer was taken as target (PCR product of 145 bp sequence). The target was amplified using specific primer pair and run onto agarose gel. The band obtained was cut and extracted from the gel.

The DNA products were dissolved in deionized water (18.3 M Ω cm) and desired concentrations were prepared prior to use. Phosphate buffer solutions (PBS) of specific pH

values were prepared using 0.1M NaH_2PO_4 and 0.1M Na_2HPO_4. High grade deionized water (18.3 M Ω cm) was used and all the chemicals were used of analytical grade. All the experiments were done at ambient temperature (25 ± 2 °C). Poly-D glucosamine (chitosan) of lower molecular wt. (<5400 g/mol) with 84.5% of deacetylation was purchased from Sigma Aldrich, USA. Chitosan mixture (due to poor solubility) was vigorously stirred in 1% Glacial acetic acid and kept for nearly 18 h for a homogenous transparent solution. $NaBH_4$ is obtained from SRL, India and BSA was from Sigma-Aldrich A4503. Freshly prepared aqua-regia solution (HCl: HNO_3= 3:1) bath is used for cleaning glass wares and finally rinsed with deionized water.

4.2.2 Bacteria isolate and DNA isolation: *Selection of enterotoxin gene*

ETEC colonies were grown on EMB (Eosin Methylene Blue) Agar. This agar is a selective microbilogical medium for gram negative bacteria as shown in Figure 4.2

PCR amplification of *St*-1 gene was carried out using primers with sequence

5’- GCACAGGCAGGATTACAAC-3’ (Forward primer) and

5’- CAACTGAATCACTTGACTCTTC-3’ (Reverse primer). The final volume of reaction mixture was 0.2 mM dNTP (50 µL), 10×reaction buffer (5 µL), 1.5 mM $MgCl_2$, Taq DNA polymerase (1.5 units), DNA template (5 µL) and primers (0.4 µM, each) [Courtesy: Anurag Jyoti, Amity University, Gwalior].The PCR program was shown in Table 4.2. Amplification condition are as follows: PCR program was carried out at for initial denaturation 95˚C for 3 min, 35 cycles of Denaturation at 95˚ C for 20 sec , annealing at 55.5 ˚ C for 30 sec and a final extension step at 72˚ C for 30 sec.

Electrophoresis of PCR products (25 µl) were performed along with 50 bp DNA ladder at 100 V/cm for 1-2 h (0.5 µg ml^{-1} of EtBr). Amplicons were seen under UV light and documented by Gel Doc Bioimaging system [Pachaury et al., 2013].

4.2.3 Isolation of *St* gene

The standard MTCC 723 produced amplicons of expected sizes 145 bp for *St* genes is shown in Figure. 4.3 [Courtesy lab of Anurag Jyoti, Amity University).

4.2.4 DNA isolation

NCBI GenBank database was used to retrieve the coding sequences for the *St1* gene (V00612, M25607 and M58746) and alignments were matched to determine conserved sequences of the *St*1 gene (ClustalW software used). A set of primer (*St*1F: 5'-GCACAGGCAGGATTACAAC-3', *St*1R: 5'-CAACTGAATCACTTGACTCTTC-3', Product size: 145 bp) were generated against sequences that were conserved for the target gene (pathogenic gene). Product size shows divergence from the analogous region of the cholera toxin gene (*Vibrio cholerae)* using Beacon Designer 5.0. PCR product of 145 bp: >NC_017633 *Escherichia coli* ETEC H10407 plasmid p666 - nucleotides 57063-57207 (145).

GCACAGGCAGGATTACAACAAAGTTCACAGCAGTAAAATGTGTTGTTCATATTTT
CTGATTTTTTTTCACTGTTGTTTTTTACAACATCACACTTTTTAGTCTCTAATGTAA
TTTTCTCTTTTGAAGAGTCAAGTGATTCAGTTG (underlined portion showing complementary region to reverse primer *St*1R).

S. No.	Temperature	Time
1.	Initial denaturation (95º C)	3 min
2.	Denaturation (95 º C)	20 min
3.	Annealing (55.8 º C)	30 sec
4.	Extension (72 º C)	30 sec

35 cycles

Table 4.2 Showing PCR Program for amplification of *St*1 Gene.

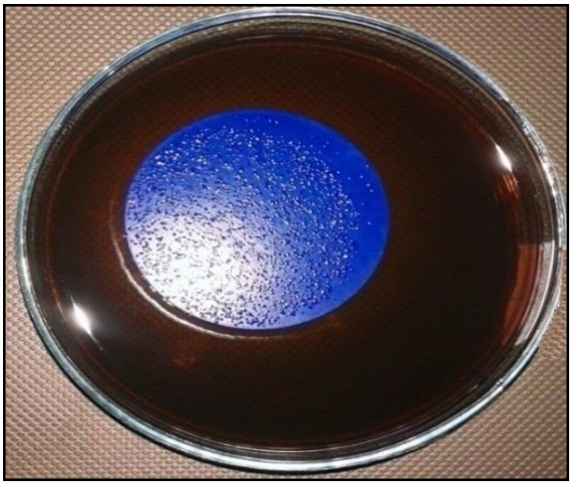

Figure 4.2 ETEC colonies grown on EMB Agar (blue colour) [Courtesy: Amity university, Gwalior; Dr. Anurag Jyoti Lab].

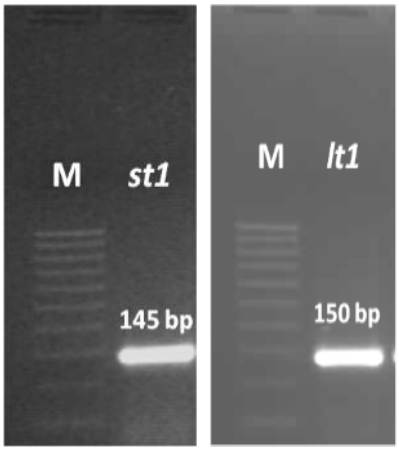

Figure 4.3 Amplification of *St*1Gene from ETEC. [M: denotes the DNA ladder of known base pair and presence of 145 bp band confirms the presence of *S* t1 gene.

4.2.5 Instrumentation:

UV–vis spectrum was taken (UV-visible spectrophotometer, Lambda-25, Perkin Elmer, Germany) in the wavelength range 200-800 nm and FT-IR (8400S Shimadzu, Japan) from 500 to 3750 cm^{-1}. Electrochemical studies were performed using electrochemical analyzer (CH 17041C instruments Inc., USA). FRA module on Autolab PGSTAT 20 installed with its associated software (Nova 2.1) was used for all the electrochemical impedimetric measurements and the numerical analysis. Ferri/ferrocyanide (5 mM) prepared in phosphate buffer (0.1 M) is used for observing the electro-activity of the PtNp@CS modified electrode. Impedance spectra at open circuit potential vs. Ag/AgCl using modulation amplitude of 10 mV is recorded in the frequency range from 0.10 Hz to 100 KHz. Impedance spectra were analyzed using a circuit consisting of RC connected in parallel and a solution resistance connected in series which appropriately corresponds to the experimental data.

4.2.6 Synthesis of Platinum nanomaterials decorated with Chitosan (PtNPs@CS)

For the synthesis of platinum nanoparticles, 2.5% chitosan stock solution was prepared by dissolving 1.8 mg of chitosan in 1.0% glacial acetic acid solution. 4 mM of H_2PtCl_6 solution was mixed well with 4 ml of chitosan solution in acetic acid. Later the solution was stirred for 2hrs. 250 µl of 30 mM aq. Sodium borohydride was then added in drop wise manner to the stirring precursor solution. Thus obtained solution was centrifuged twice at 4000 rpm for 6 min and colloidal solution of nanoplatinum impregnated with chitosan was obtained. Resultant product was washed thrice with deionized water (18.3 M Ω cm). Washing removes all unreacted H_2PtCl_6, Cl^- ions and any undesired impurity. Obtained solution can be redispersed in required amount of water for development of sensor electrodes. Chitosan has wide number of highly reactive functional groups such as hydroxyl and charged amino group, due to which it shows affinity towards transition metals viz., platinum and forms metal complex [Kashish et al., 2015]. Efficient binding occurs between chitosan NH^{3+} to $PtCl_6^{2-}$. This further leads to sufficient nucleation and growth of platinum nanoparticles in chitosan matrix during reduction process.

4.2.7 Sensor probe development

Sensing is performed employing the CS/PtNP as working electrode, platinum foil as a counter electrode and Ag/AgCl as reference electrode. Working electrodes were polished with 0.05 mm alumina and rinsed thoroughly with double distilled water and subsequently ultra sonicated formerly in water and finally with acetone for 5-10 min.

Biosensor has been prepared in accordance with scheme as mentioned. Platinum nanoparticles decorated with chitosan is dropcasted onto the glassy carbon and dried in controlled conditions. Later 5µl of 100nM of ssDNA solution was drop casted onto the

platinum nanoparticle decorated chitosan modified electrode (100 nM over 2 mm^2 disc area). Electrode was left for incubation at 4 ºC for 12 h to make sensing probe with GCE/PtNP/ssDNA configuration. Immobilization of reverse primer is via electrostatic interactions between PO_4^{3-} of DNA and NH^{3+} of Chitosan. For increasing the specificity we make use of blocking agents albumin (1mg/ml) prepared in PBS buffer solvent for 6 h. It was washed with buffer prior to carrying out it Impedance spectroscopy. Hybridization is performed with different consecutive decreasing concentration of target DNA under optimized conditions. This process was carried out by immersion of modified electrode into a glass vial containing denatured genomic DNA. Heat treatment was given by providing conditions, 92-94ºC for 3-5 min and finally cooled for denaturation. At temperature > 75 ºC the) immobilized electrode was dipped into the glass vial containing target DNA and when temperature reached 40-42ºC hybridization occurs. Optimum time of hybridization is 15 min.

The hybridized GCE/PtNPs/ssDNA/albumin/denatured DNA electrode was then rinsed with buffer before recording its EIS. Using the same platform EIS was recorded for successive decreasing concentrations of DNA (soil sample) [Sirhan et al., 2013; Richardson et al., 1999].

4.2.8 Impedance analysis

Impedance spectroscopy acts as model technique onto which a sensor system is designed for specific detection of hybridization events on probe for hybridized non-labeled target DNA based on the change of surface properties. Redox mediator free electrochemical DNA detection was made possible directly through the measurement of electrical surface properties such as conductance, resistance and capacitance [Leypon et al., 2015; Lisdat et al., 2008; Bonnani et al., 2010; Kafka et al., 2008;tiwari et al., 2015; Rao et al., 2006;

Choosakoonkriang et al., 2001]. The electrical resistance and capacitance was used as indicators for changes in surface properties in response to DNA hybridization event over the modified electrode. Impedance spectra (Nyquist plot) were mathematically analyzed using an equivalent circuit (Randles circuit) including solution resistance, *Rs* and the double layer capacitance, C_{dl} (electrode/solution interface) and charge transfer resistance R_{CT}. Significant differences in the impedance spectra are observed with increasing concentrations of DNA (target). A typical impedance spectra displays a semicircle portion in which at higher frequencies corresponds to the electron-transfer limited process may be trailed by a linear part detonating the lower frequency at which a diffusion limited electron transfer process occurs.

4.3 Results and discussion:

4.3.1. Fourier-transform infrared spectroscopy (FT-IR) characterization

The interaction between PtNPs with the amine group of chitosan was confirmed by Fourier-transform infrared spectroscopy analysis. Fourier-transform infrared spectroscopy (Figure. 4.3) was used to characterize the PtNPs@CS, and after immobilization of ssDNA as shown in the spectra of region of 390–3500 cm^{-1} [Kashish et al., 2015]. Bands observed in the spectrum were further compared with the literature and values were assigned from the literature [Mao et al., 2001; Lee et al., 2012; Rioux et al., 2006]. Furthermore, FT-IR spectra of the PtNP@CS/DNA was recorded over the range of 390–3500 cm^{-1}. Absorbance bands of base pairs in DNA are seen at 1603 cm^{-1} (adenine), 1680 cm^{-1} (thymine), and 1487 cm^{-1} (cytosine). Phosphate ester is seen at 1232 cm^{-1} and the band at 973 cm^{-1} is of 2'- endo deoxribose conformation. FTIR spectra of PtNPs@CS /DNA as shown in curve d of spectra

displayed the appearance of new absorbance bands at 871 cm^{-1} and 1571 cm^{-1} corresponding to C-0 bond stretching due to pyranose ring and vibrations from carbonyl group.

Peaks showed shift to lower wavenumbers for bases guanine as 1643 cm^{-1}. This shifting is due to the hydrophilic binding with guanine N7 with NH_2 group of chitosan. We also observed disappearance for the CH_3OH and CH_3 in amide in Figure 4.4.

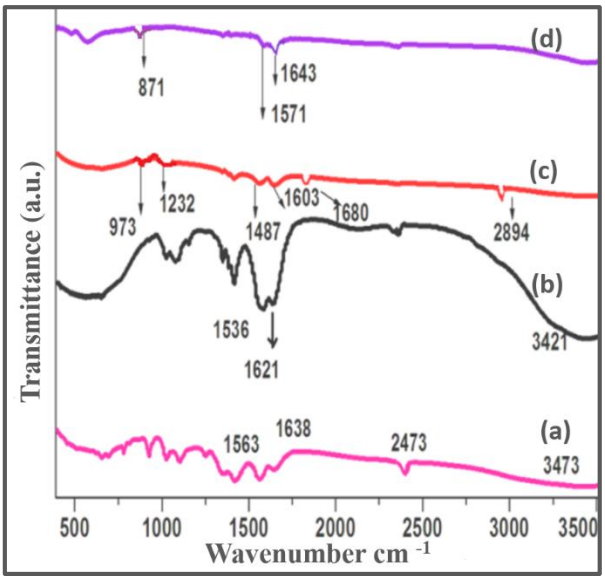

Figure 4.4 FT-IR Spectra of (a) Chitosan and (b) CS-PtNPs (c) DNA (d) CS-PtNPs/DNA.

4.3.2 Electrochemical characterization

Electrochemical activity of PtNP in ferricyanide containing phosphate buffer at pH 6.8 is studied using cyclic voltammometry (CV) for successive increasing scan rates as shown in Figure 4.5 over glassy carbon electrode. The inset of Figur 4.5 shows that the redox peaks current increase with the square root of scan rates in range of 20–400 mV/s in a linear fashion.It indicates excellent electroactivity of the PtNP modified electrode

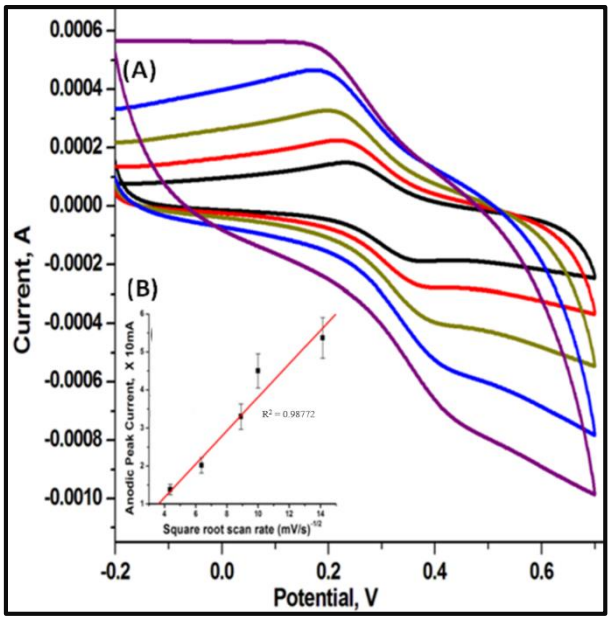

Figure 4.5 Cyclic voltammogram of PtNP/GCE (at different scan rates: 20, 80, 100, 200, 400 mV/s) in 0.1M Phosphate buffer solution containing 0.1mM $K_3[Fe(CN)_6]$. (B) Inset showing the fitted anodic current versus square root of the scan rates.

4.3.3 TEM Characterization

The morphology of platinum nanoparticles was observed under TEM. TEM images showed that the platinum nanoparticles are uniform, nearly mono-dispersed and were spherical as shown in Figure 4.6A.

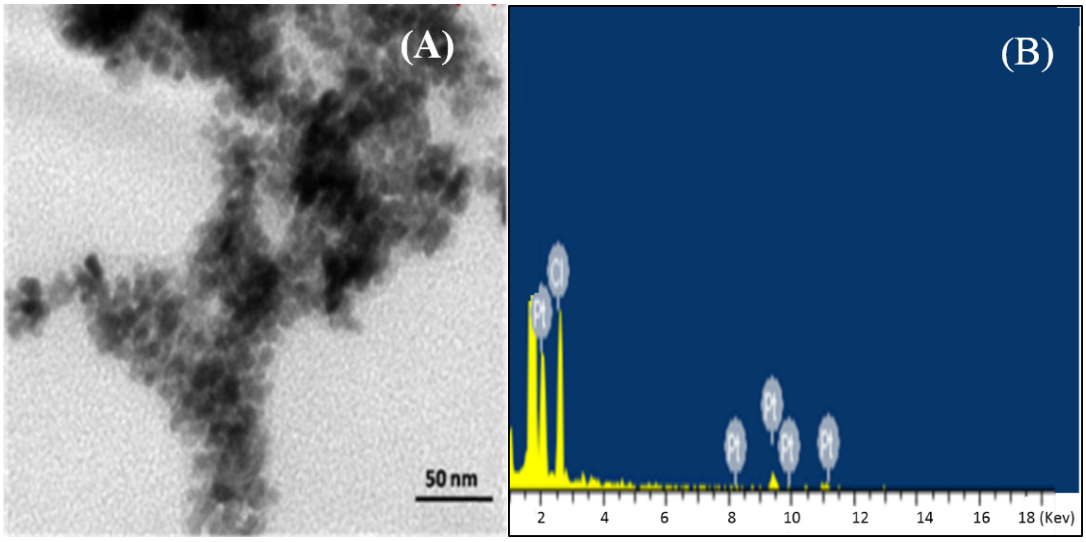

Figure 4.6 TEM images of (A) PtNP and (B) EDAX of PtNP nanoparticles.

The particle diameter was estimated using Image J software. It is observed that chitosan is also responsible for its uniform structural morphology and control size due to capping effect. EDAX data were collected to further confirm the presence of Pt nanoparticles in capped form. Figure 4.5B showed the EDAX spectrum of chitosan capped Pt nanoparticles with approximate composition 30% indicating that Pt nanoparticles were successfully obtained as also discussed earlier [Gupta et al., 2014; Liu et al., 2005 Yadav et al., 2014].

Herein, the positively charged PtNPs nanoparticles contributed both in providing biocompatible environment for binding with negatively charged DNA molecules, and also in suffice electron movement between DNA and the electrode.

4.3.5 Impedance analysis

Electrochemical impedance study was performed over modified electrode and data (Z_{img} vs. Z_{real}) was recorded to study the hybridization event. The modified electrode was formed and electrode surface was blocked by blocking agent albumin to prevent non-specific

DNA binding [Raoofa et al., 2013]. Later modified electrodes were washed with phosphate buffer and stored in freeze at 4 ˚C. All the experiments are carried out in the electrochemical cell containing 3 electrodes set up for impedance measurements. The modified electrode was kept in a stirred solution 50 mM PBS buffer (pH 6.8) and impedance was recorded followed by addition of target DNA (either oligomer or full genomic DNA) for hybridization process.

The hybridization event was observed by recording impedance spectra after each addition of known concentrations of the target ssDNA (complementary145 mer) as shown in Figure 4.7. The impedance spectra for bare GCE and modified with CS was recorded each time (not shown in the Figure). The impedance of ssDNA immobilized probe (curve a) was recorded as blank before addition of target DNA as shown in the Figure 4.7A. Impedance was recorded after each addition of target ssDNA for wide concentration range. A significant increase in the R_{CT} was observed after each addition of target ssDNA due to hybridization over the electrode. Accumulation of negative charge from the DNA backbone after hybridization attributed to the R_{CT} increase. This confirmed that a barrier for interfacial electron transfer and thus an increase in the electron transfer resistance [Hayat et al., 2014]. Corresponding increase in R_{CT} for successive reading of increased concentration of target DNA was recorded for plotting calibration plot as shown in Figure 4.7B.

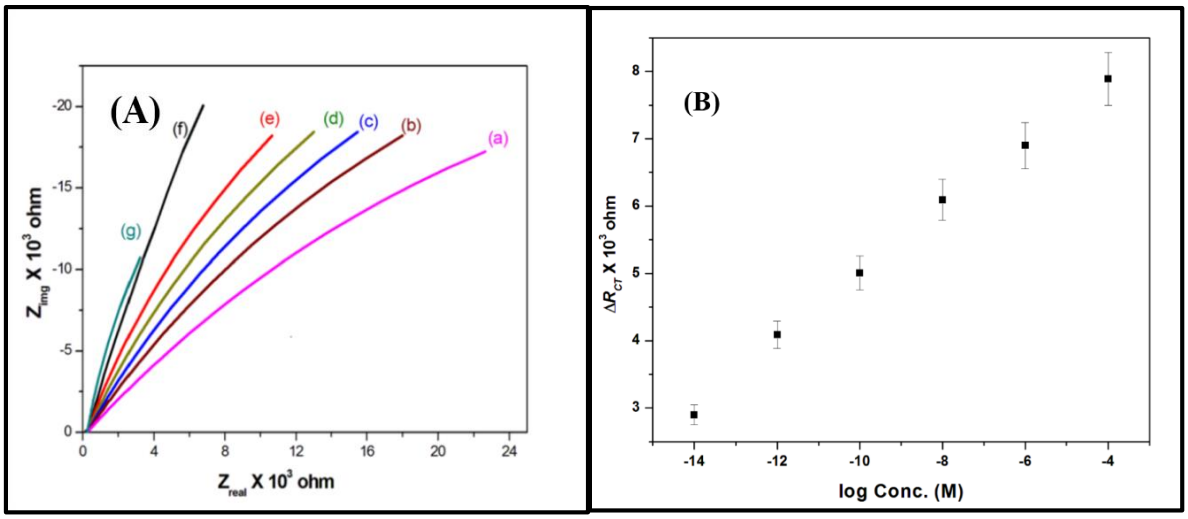

Figure 4.7 (A) Nyquist plot for (a) GCE/CS-PtNPs/ssDNA and after hybridization with complementary target ssDNA of 145 mer of concentration range from (b) $1x10^{-14}$M (c) $1x10^{12}$M (d) $1x10^{-10}$M (e) $1x10^{-8}$ M (f) $1x10^{-6}$M (g) 1 $X10^{-4}$M. **(B)** Corresponding calibration plot for complementary oligomer in form of R_{CT} vs. log concentration (M).

Similarly, hybridization event was also studied for full genome after denaturation. Nyquist plot is obtained as shown in Figure 4.8A for GCE/CS-PtNPs (a) and after hybridization of denatured dsDNA real sample (full genome) for various concentrations. Sensing platform is sensitive since the probe sequence used and immobilized was specific for *St* gene.The calibration plot as shown in Figure 4.8B is showing linearity for wide range of concentration of denatured dsDNA with detection limit of $1x10^{-12}$ M. The calibration plot was obtained similar to 145mer target ssDNA with relatively lower detection limit.

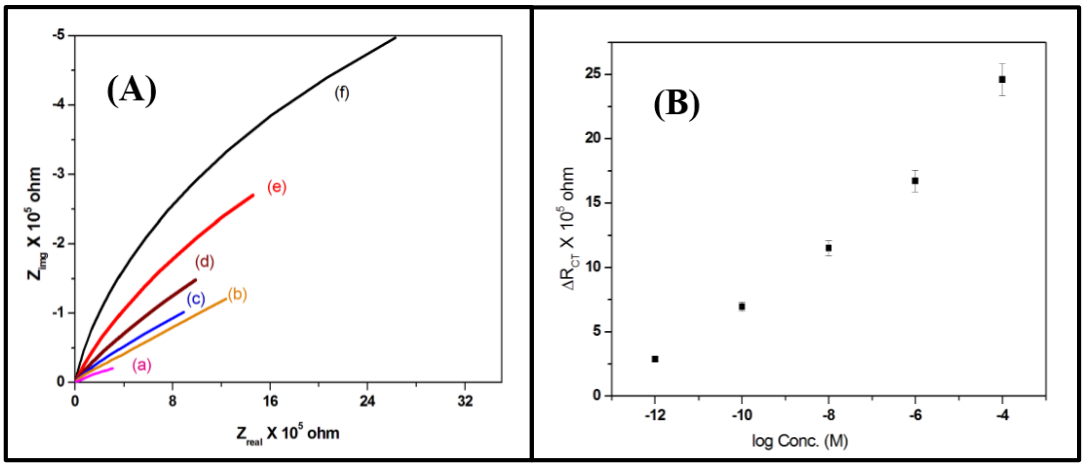

Figure 4.8 (A) Nyquist plot for (a) GCE/CS-PtNPs/denatured ds DNA and after hybridization with real sample (genomic DNA) of concentration range from (b) $1x10^{-12}$M (c) $1x10^{-10}$M (d) $1x10^{-8}$M (e) $1x10^{-6}$ M (f) $1x10^{-4}$M. (B) Corresponding calibration plot for corresponding complementary bounded target DNA in form of R_{CT} vs. log concentration (M).

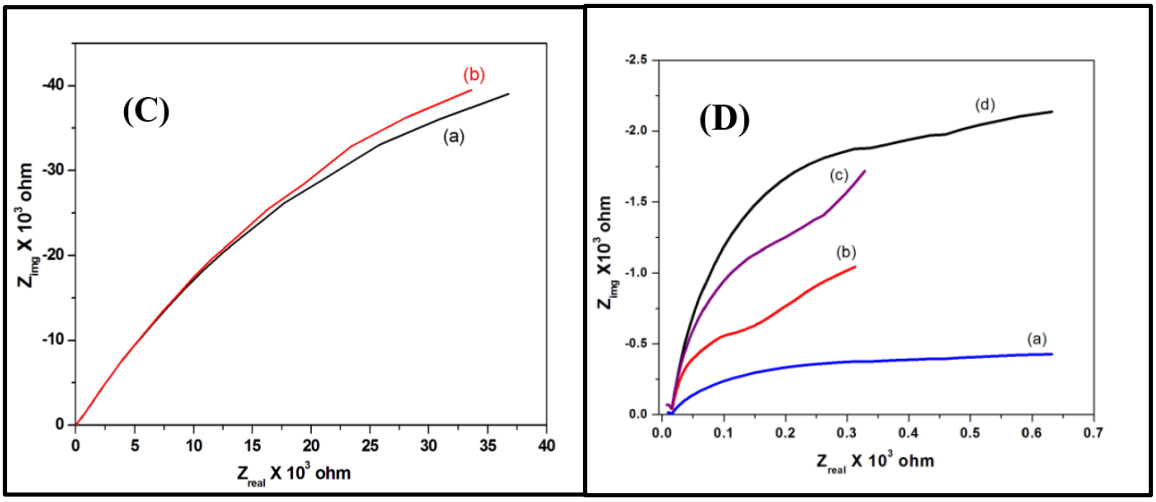

Figure 4.8 (C) Nyquist plot (a) PtNP with probe *St* gene of E.coli after hybridisation event with target DNA (1 X 10^{-8}M) (b) After heat treatment and dip wash (showing the stability). (D) Nyquist plot showing optimisation of hybridisation time (a) Response after 5 min (b) after 10 min (c) after 15 min (d) after 2 min.

In addition to that Nyquist plot for *Lt* gene was also taken.

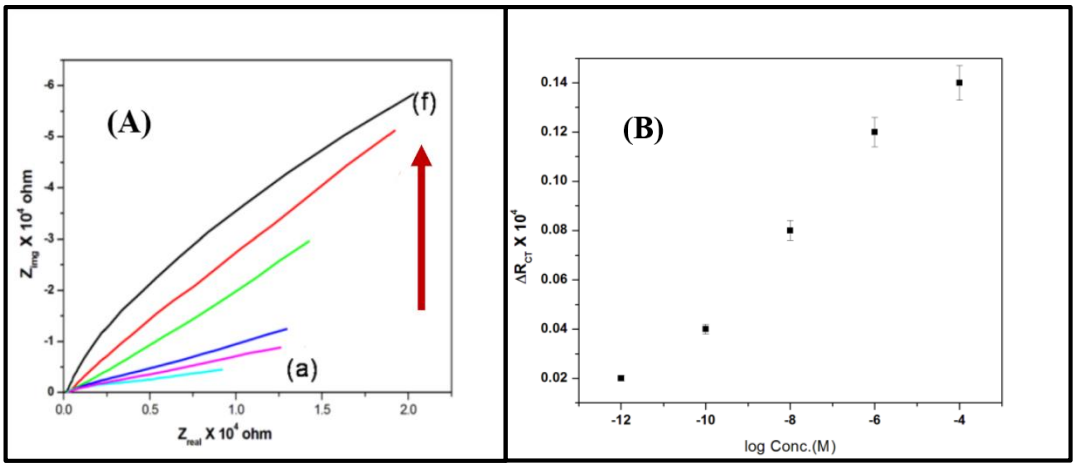

Figure 4.9 Nyquist plot for (A) GCE/CS-PtNPs/ssDNA and after hybridization with complementary target ssDNA of 150 mer of concentration range from (B) $1x10^{-12}$M (c) $1x10^{-10}$M (d) $1x10^{-8}$M (e) $1x10^{-6}$ M (f) $1x10^{-4}$M.

In addition to that of St gene impedance analysis of *Lt* gene is also carried out. Since *Lt1* gene is a heat labile gene so it shows lower R_{CT} difference as compared with *St*1 gene. However the limit of detection is in picomole.

The stability of the modified probe was studied by heat treatment during dip washing using impedance analysis. A fixed concentration of target ssDNA (1 X 10^{-8}M) was used for hybridization event over the modified probe and impedance was recorded after the hybridization event as shown in Figure 4.8C curve (a). The hybridized probe was dip washed in buffer above 70^{o}C and impedance was again recorded as shown in Figure 4.8C curve (b). It is observed that both the curves were almost similar and negligible difference in R_{CT} (corresponds to a difference of 500 Ω) was observed before and after the heat treatment of the hybridized probe. It is clearly indicating the stability of the sensing probe and capability of the sensor working in real environment. Figure 4.9D clearly explains that the curve c and

d show overlap in curves (R_{CT} value) showing the saturation limit of hybridization, in other terms, it depicts the optimization value of the hybridization time.

Further, experiments were performed with screen printed carbon electrodes (SPCE) using all the parameters similar as used for the GCEs. Modified SPCEs were used for the study of hybridization of three different concentrations of full genomes to explore the possibility of development of low cost and portable sensors [Gupta et al., 2015; Amin et al., 2014; Liu et al., 2013; Hayat et al., 2014]. A Fig 4.10 depicts the sensing of target DNA on a miniaturized platform based on SPCE modified with biocompatible PtNP. Further we have incorporated the DNA probes onto it, for sensing the complementary target DNA. On the basis of any drop in the impedance value (Resistance in charge transfer, R_{CT}) due to the hybridization process. It clearly demonstrates the enhanced conductivity of dsDNA configuration formed after hybridization on the SPCE as compared with the single stranded DNA. Our modified probes showed potential for sensitive, low cost and portable sensors for detection of *Escherichia coli St* Gene in real samples and real environment.

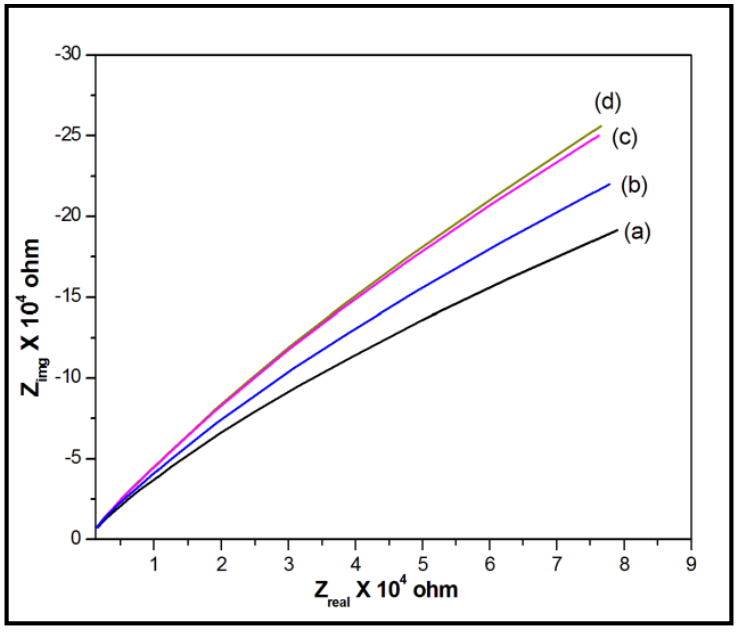

Figure 4.10 Nyquist plot for (a) SPCE\CS-PtNPs\ssDNA and after hybridization with real sample (genomic DNA) of concentration (b) $1x10^{-12}$M (c) $1x10^{-10}$M (d) $1x10^{-8}$M.

4.4 Conclusion

Novel probe developed based on platinum nanoparticles @ chitosan (PtNPs@CS) and immobilized with a specific oligonucleotides (ssDNA) for detection of *Escherichia coli St* Gene. Sensor probes were developed based on conventional glassy carbon and conducting carbon ink screen printed electrodes. Electrochemical impedance spectroscopy (EIS) technique is used for detection of hybridization even and the change in charge transfer resistance (R_{CT}) after *E. coli* DNA hybridization with ssDNA immobilized probe was used as detection tool. The experimental conditions are optimized and the change in charge transfer resistance (ΔR_{CT}) is calculated for varying concentrations of *E. coli* DNA. The combination of our stable ssDNA probe with EIS provides a label-free, specific and user-friendly biosensing system. Further it showed potential for development of low cost sensing probes based on carbon ink screen printed electrodes for portable sensing device devices for environmental monitoring, food industry and clinical laboratories. Issues regarding structural changes of the immobilized DNA films need to be addressed as it may affect the sensitivity and reproducibility of measurements. Furthermore research is focused towards development of prototype for real samples detection.