2.1 Introduction

Food-borne pathogens considered as serious health hazards include Salmonella typhimurium, *Listeria monocytogenes, Staphylococcus aureus, Campylobacter jejuni, Clostridium perfringens, Yersinia enterocolitica, Escherichia coli,* etc. *Listeria monocytogenes* has recently been reported as the most virulent food-borne pathogen with high mortality (30%) and hospitalization (91%) [Mook et al., 2011; Tolba et al., 2012]. Listeria is ubiquitous in the environment as it tolerates extreme pH, temperature and salt concentration levels. As of now, listeriosis caused by *L. monocytogenes* is the major cause of abortion, stillbirth, septicemia, meningitis and meningoencephalitis in pregnant women, neonates, elderly or immunocompromised humans [Kyoui et al., 2014; Soni et al., 2014]. The detection of *L. monocytogenes* is difficult because of its low copy number and dominance of other background pathogenic bacteria [Garrec et al., 2003; Vivant et al., 2013]. The present work focused on the development of a method for rapid and sensitive detection of L. monocytogenes in diseased samples.

Detection and identification of Listeria by conventional methods based on morphological and biochemical tests require up to 5–7 days for the confirmation of L. monocytogenes in the samples [Chen et al., 2004; Yang et al., 2005; Jadhav et al., 2012; Datta et al., 2013]. Subsequent techniques viz., Enzyme-linked immunosorbent assay (ELISA), flow cytometry and matrix-assisted laser desorption/ionization (MALDI) have also been developed for Listeria detection [Low et al., 1992; Barbuddhe et al., 2008; Ikeda et al., 2009]. However, due to several limitations (long duration, labor-intensive protocol, necessity of pure sample and costly reagent), there is a need to develop simple to use, rapid and sensitive afford-able method for the detection of pathogen in environmental and clinical

samples.

Biosensors using surface plasmon resonance (SPR), fiber optic techniques, fluorescence resonance energy transfer (FRET), and piezoelectric devices with the detection range 10^2–10^6 cfu mL^{-1} are reported for the detection of *L. monocytogenes* [Bhattacharya et al., 2008; Poltronieri et al., 2009; Ohk et al., 2010; Sharma et al., 2013]. But these techniques have certain specific limitations such as SPR biosensor restricted in respect to penetration depth of evanescent field, refractive index change and size of bacteria [Banik et al., 2008]. Accuracy of fiber optic biosensors depend on nature of antigen, quality of antibody, fiber variation and nonspecific binding to non-target analytes which can affect the overall signal [Steemers et al., 2000; Ohk et al., 2010]. FRET based biosensors need higher fluorescence resolution to improve specificity. Additionally, as more FRET based technologies extend to medical diagnostics; the affordability of FRET reagents becomes a significant concern. Similarly, piezoelectric biosensors are having main drawback of immobilization of antibody through the binding of amine groups (likely to their Fab sites) which ultimately inhibits antigen binding.

Electrochemical DNA biosensors have attracted the attention of several researches because of their reliability for sequence specific information combined with the advantages of their simple fabrication process, rapid response time, and better sensitivity [Mohan et al., 2010; Tolba et al., 2012]. The fast method to detect virulent L. monocytogenes is based on the virulence factors i.e., listeriolysin O (LLO). It is an extracellular, pore-forming, thiol-activated toxin essential for *L. monocytogenes* virulence. The LLO protein (a 58 kDa hemolysin protein encoded by hly gene) is reported as the main virulence factor and pathogenic marker for the detection of *L. monocytogenes* [Soni et al., 2014]. Polymerase

chain reaction (PCR) followed by hybridization of the target gene with a single-stranded oligonucleotide probe has been observed as the effective method of sequence-specific DNA detection [Ligaj et al., 2003; Farabullini et al., 2007; Gao et al., 2010]. Lately, a few DNA hybridization assays are reported for the detection of Listeria, nevertheless, a more sensitive, faster, cost-effective and easy-to-use techniques is yet to be developed. Some of the recent studies focused on the development of polymers based sensors to detect the target DNA based on hybridization event over the transducers. Electrochemical detectors have been developed using conducting polymers immobilized enzymes/antibodies/ssDNA as the recognition layer for sensors [Shimidzu, 1987; Li et al., 2009; Nie et al., 2009]. Conducting polymers (e.g., poly-5-carboxy indole) are also suitable transducers for biomolecule recognition, and can be used to enhance the stability and sensitivity of biosensors [Li et al., 2008; Mohan et al., 2010]. Electrochemical detection of DNA employing the labels and DNA markers has the ideal detection limit (detection level which is required to know the preliminary stage of the disease). Rapid hybridization event has been recently reported for the detection of hlyA gene employing electrochemically active intercalator (label) applying amperometric technique (square wave voltammetry) [Ligaj et al., 2003]. Similarly, hlyA gene has also been detected using gold electrode modified with mercaptan activated by N-hydroxysulfosuccinimide and N-(3-dimethylamino) propyl-E^{t}hyl-carbodiimide hydrochloride and [Co (phen)$_3$] $(ClO_4)_3$ as the indicator (label) by amperometric technique using cyclic voltammetry [Wu et al., 2010].

However, in view of the several limitations of label-based sensing and amperometric methods, use of electrochemically active conducting polymers as the transducer for label-free measurement of DNA hybridization have been proposed [Peng et al., 2005; Zhang et al.,

2009; Mohan et al., 2010]. The incorporation of the oligonucleotide/ssDNA into a conducting polymer and the hybridization of the incorporated ssDNA is expected to perturb the electrochemical response of the polymer [Korri-Youssoufi and Yassar, 2001; Peng et al., 2009]. The change in electrochemical responses on the interaction of ssDNA or the hybridization events may be used as the signal for detection. This entire process requires a suitable transduction technique. Among the various transduction techniques, electrochemical impedance spectroscopy (EIS) is effective to probe the interfacial properties of modified electrode (in terms of basic parameters viz. charge transfer and capacitance). Even the minimal change in parameters can be measured instantly compared to other tedious electrochemical techniques [Mohan et al., 2010; Tolba et al., 2012].

This work focuses on the electrochemical impedance study for determination of DNA hybridization (without any label) that enables label-free detection of target DNA [Gheorghe and Guiseppi-Elie, 2003; Thompson et al., 2003; Mejri et al., 2010]. Recent researches have shown a wide acceptance of electro-chemical impedance technique for detection and quantification of pathogenic bacteria [Thompson et al., 2003; Yang and Bashir, 2008]. With the foregoing analysis in mind, in this work, we describe a sensitive and label-free electrochemical impedance based biosensor for the detection of L. monocytogenes based on the conducting polymer probe. The morphological and electrochemical characterization of the chemically immobilized ssDNA probe was done prior to sensing. The detection of DNA hybridization with the target DNA was executed by impedance spectroscopy. Moreover the effect of pH, hybridization time, temperature, reproducibility and stability of the modified electrodes are standardized and further demonstrated for sensing of Listeria based on EIS technique and also using screen printed electrodes.

2.2 Experimental

2.2.1 Apparatus

All the impedimetric measurements were performed on electrochemical workstation (Palm Sens3 Instrument, The Netherlands and CH instruments Inc., USA) with screen printed electrodes and a three-electrode system consisting of a bare gold or DNA modified gold working electrode, aqueous Ag/AgCl reference electrode and a platinum wire auxiliary electrode. Surface morphologies were observed through scanning electron microscope (SEM; FESEM SUPRA 40, CARL ZIESS, Germany) and optical microscope (Metallux-3, Leica Germany). All the electrochemical measurements were performed at room temperature ($25 \pm 1^{\circ}$ C).

2.2.2 Reagents

Monomer 5-carboxyindole, tetrabutyl ammonium perchlorate (99%), 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were obtained from Sigma–Aldrich (India), and acetonitrile (HPLC grade) and HCl from Merck (India). Sodium monophosphate and diphosphate were obtained from SRL Pvt. Ltd., India. Bangalore Genei (India) synthesized the 24-mer synthetic, unmodified oligonucleotides (5‑GCA ACG TAT CCT CCA GAG TGA TCG-3) related to hlyA of *L. monocytogenes* gene and its complementary sequence [Soni and Dubey, 2014]. All stock solutions were prepared in deionized and autoclaved water.

2.2.3 Bacterial isolate

Bacterial isolate L. monocytogenes (Pb2) was used in this study from our laboratory stock collection. Identification of the pathogen was confirmed by biochemical and molecular characterization methods [Soni et al., 2013, 2014]. The 16S rRNA and hlyA gene sequences have been deposited with NCBI Genebank under accession number KJ765622 and KJ504112.

2.2.4 DNA isolation

Chromosomal DNA was extracted from L. monocytogenes isolate (Pb2) grown overnight (37 °C) with shaking (200 oscillations per min) in brain heart infusion broth (BHIB, Difco, USA) using QIAGEN DNeasy® Blood and Tissue kit. Harvested cells (maximum 2×10^9 cells) in a microcentrifuge tube (7500 rpm, 10 min), were re-suspended in 180 L lysis buffer [20 mM Tris–Cl (pH 8.0), 2 mM sodium EDTA, 1.2% Triton® X-100, 20 mg mL^{-1} lysozyme (Sigma)] and incubated (30 min; 37 ° C). Proteinase K (25 L) and 200 L Buffer AL (without ethanol) were added, mixed by vortexing and incubated at 56 °C (30 min). Thereafter, 4 L RNase A (100 mg mL^{-1}) was added and incubated (2 min) at room temperature (25 ± 1 °C). Pure ethanol (200 L; Merck) was added to the sample, and mixed thoroughly by vortexing. DNA was eluted in Buffer Tris–acetate–EDTA buffer (TAE) and the concentration/purity determined using Nano Drop Spectrophotometer (ND 1000, Nano Drop Technologies, Inc, and Wilmington, DE, USA).

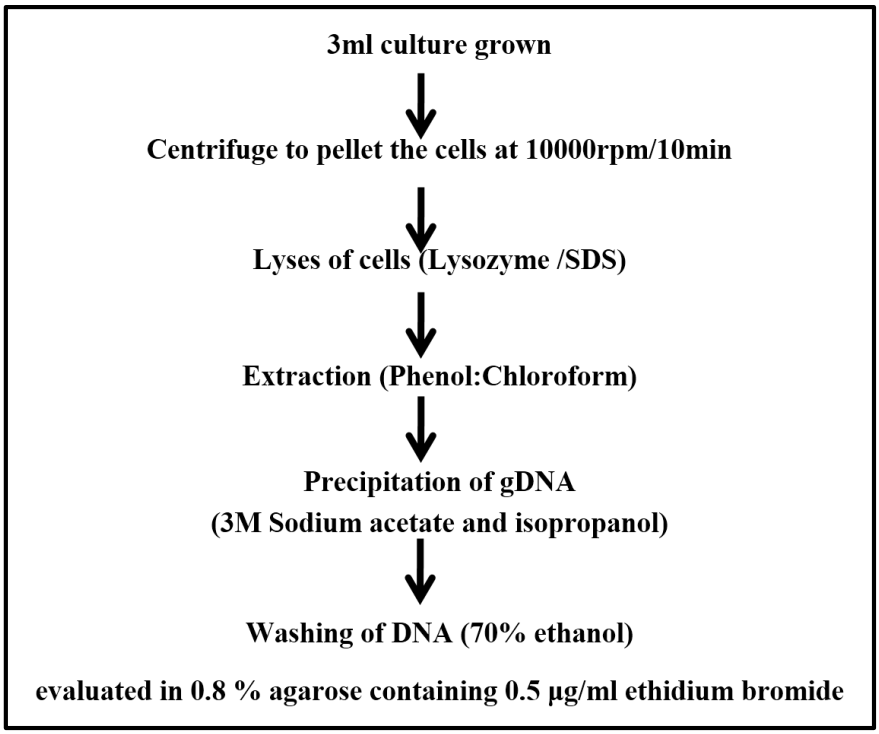

Figure 2.1 Flowchart showing steps for isolation of DNA.

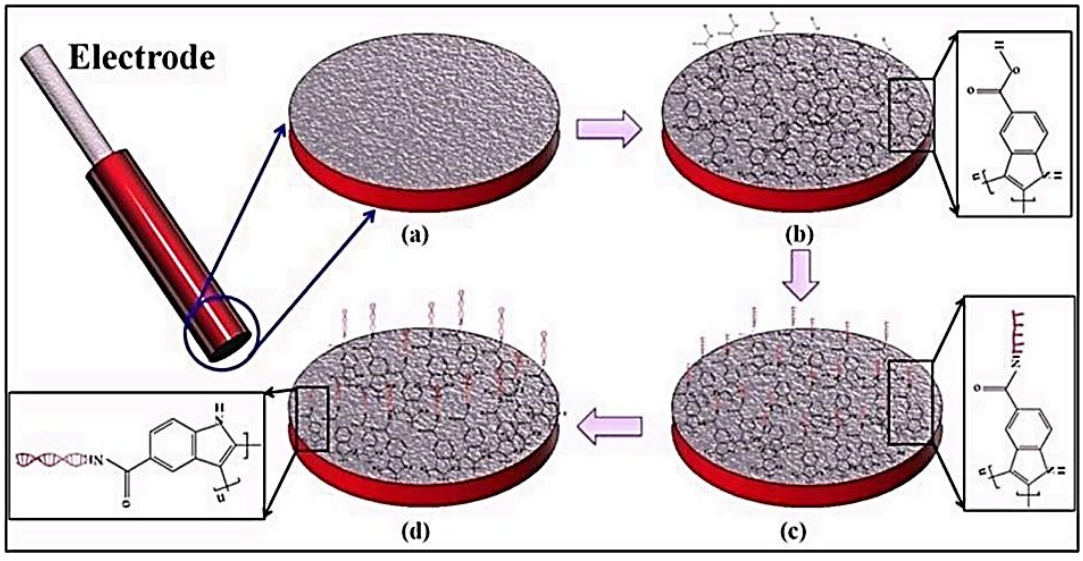

Figure 2.2 Schematic representation (a) Polished gold electrode with alumina slurry. (b) Electropolymerization of 5-carboxyindole. (c) Carbadiimide coupling of probe of hlyA gene of L. monocytogenes over poly-5-carboxyindole. (d) Target DNA hybridization on poly-5-carboxyindole modified electrode.

2.2.5 Electrochemical polymerization

Electrochemical polymerization of monomer was carried out using a three-electrode cell system at a fixed potential of 1.1 V or cycling potential in the range of −0.2 V to 1.0 V vs. Ag reference electrode [Bartlett et al., 1992; Sadki et al., 2000; Mohan et al., 2010]. The gold electrode was polished with alumina slurry before electropolymerization (Figure 2.2). After polishing, the electrode was washed with distilled water and acetone before use. Acetonitrile solution containing 5-carboxyindole monomer (60 mM) and the supporting electrolyte (0.1 M) tetrabutyl ammonium perchlorate (TBAP) with the molar ratio of 1:2 was used for electro-polymerization using cyclic voltammetry for more than 10 cycles at the scan rate of 50 mV s^{-1} (Figure 2.3 B). After polymerization, the 5C Pin film formed on electrode was dip washed in acetonitrile (2–3 times) to remove the unpolymerized monomers. The formation of radical cation is proposed on the oxidation of monomer followed by resonance of the radical cation and formation of the polymer (as shown by equation) [Prakash et al., 2002; Joshi and Prakash, 2013]. The polymer electrode was characterized for its electroactivity based on cyclic voltammetry at various scan rates (Fig. 2.3 B). Redox reaction of 5C Pin is shown by eq1(oxidation) and eq 2 (reduction).

$$(\mathbf{5C\ PIn})_n + \mathbf{mnClO^{4-}} \rightarrow [(\mathbf{5CPIn})^{m+} + \mathbf{mClO^{4-}}] + \mathbf{mne^-} \qquad \mathbf{2.1}$$

$$[(\mathbf{5C\ PIn})^{m+}] + \mathbf{mClO^{4-}} + \mathbf{mne^-} \rightarrow (\mathbf{5C\ PIn})_n + \mathbf{mnClO^{4-}} \qquad \mathbf{2.2}$$

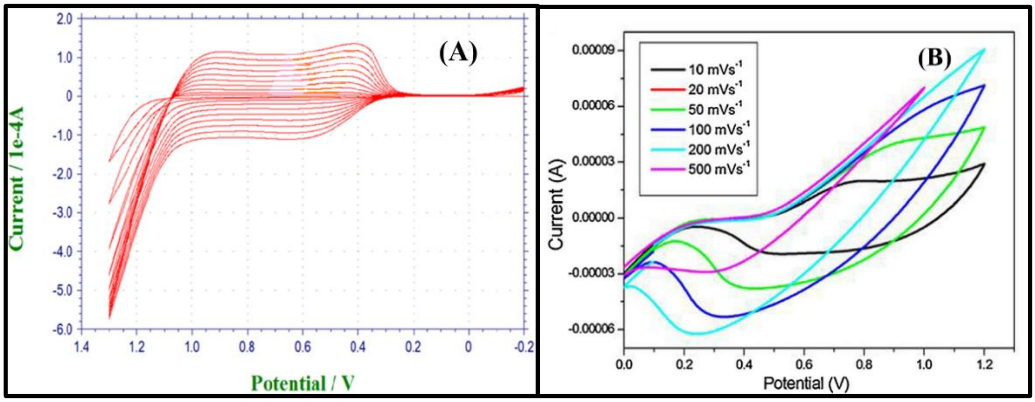

Figure 2.3 (A) Cyclic voltammograms of 5-carboxyindole over gold electrode in acetonitrile and TBAP for 10 cycles at the scan rate 50 mVs^{-1}. (B) Cyclic voltammogram of poly-5-carboxyindole coated over gold electrode in acetonitrile and TBAP (monomer free) solution at various scan rates.

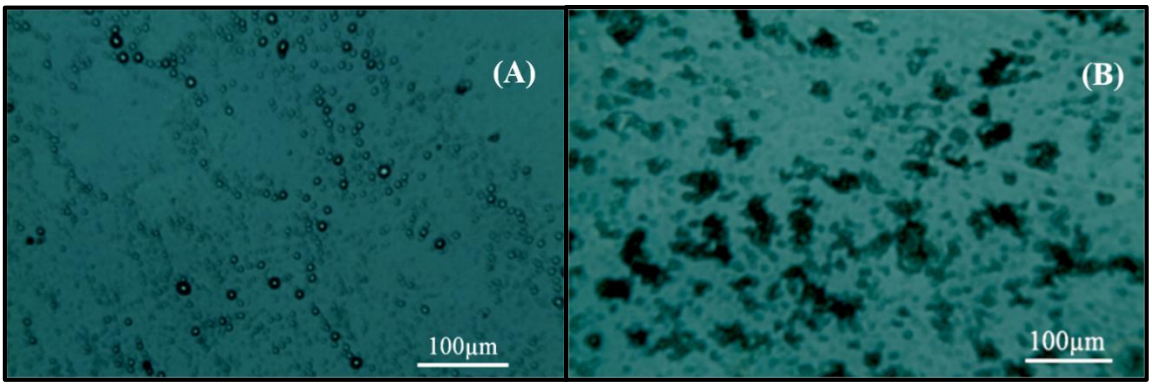

Figure 2.4 Optical images of poly 5-carboxyindole electrode surface (left images) and modified electrode (right images) formed by immobilizing probe of *hlyA* gene of *Listeria monocytogenes*.

2.2.6 Probe immobilization

After polymerization, the 5C Pin coated gold electrode was immersed in the solution of EDC-NHS (coupling agent for amide formation) for 1.5 h at room temperature (25 ± 1 ° C). The EDC-NHS solution was prepared in a 3:1 molar ratio in phosphate buffer saline (PBS, 50 mM), pH 6.8 and stirred for 3 h in a sealed tube. A 15 L of the probe solution was dropped on the EDC-NHS treated electrode surface, and left for 4 h (at room temperature; 25 ± 1°C) followed by an overnight incubation below 8 ± 2°C. After immobilization, the electrodes were washed thoroughly with PBS buffer to remove any unattached DNA. The electrodes were refrigerated (4 ° C) under sealed conditions if not in use.

2.2.7 Hybridization of target DNA (hlyA gene)

Hybridization with the complementary probe was carried out by immersing the modified electrode in PBS buffer (50 mM, pH 6.8) for various time intervals at 42°C. Hybridization was achieved in glass vials taking the target DNA (hlyA gene) followed by denaturation of the target dsDNA. The dsDNA solution was first sonicated for 10 min at the pulse rate of 15 s on, 15 s off, and was then subjected to heat treatment (denaturation; 92–94 °C) for 3 min followed by cooling for hybridization. During cooling step, ssDNA immobilized probe electrode (temperature > 75 ºC) was dipped in the solution containing denatured dsDNA (heated solution to denatured temperature) maintained at 40–42 °C for hybridization. A competitive binding of denatured dsDNA present in the solution occurred with the ssDNA immobilized at the sensor probe as well as in the solution with its complementary DNAs. This competition was depending on concentration of dsDNA present in the solution and detection signal (R_{CT} charge transfer resistance) was calibrated with respect to the concentration of dsDNA. Following hybridization, the probe was washed with

the same buffer to wash out the non-hybridized DNA existing in adsorbed state. The schematic representation of the steps for electrode preparation is shown in Figure 2.5.

2.2. 8 Measurements of electrochemical impedance

Impedance measurement was performed in PBS buffer (50 mM, pH 6.8) at the open circuit potential (+0.35 V vs. Ag reference) using modulation amplitude of 10 mV in the frequency range from 0.1 Hz to 0.1 MHz. Nyquist plot for 5C Pin with hlyA gene as the probe was recorded as the control, and further for hybridization with the denaturated target DNA concentration in the range of 1×10^{-12} to 1×10^{-4} M with the increments of 10^{-2} M. R_{CT} calculated from the Nyquist plot is based on the best fit of the equivalent circuit model, and the calibration plot for the same is presented as changes in R_{CT} vs. log concentration of target DNA (genomic).

2.3 Results and discussion

2.3.1 Enrichment of bacteria *Listeria monocytogenes*

Enrichment is the process in which we provide growth conditions that are favourable for one organism of interest (*Listeria*) and is unfavourable for other competing microorganism (Courtesy of Prof. SK Dubey's lab, Department of Botany, BHU). Two step enrichment procedure is preferred for culturing of *Listeria monocytogenes*

2.3.1.1 Primary Enrichment

Here we have taken selective Liquid enrichment medium with reduced concentration of selective agents (Half Fraser broth, Difco) or growing the bacteria. We take 5 ml of each blood sample and was mixed with 45 ml of Half Fraser broth. Incubated at 30º C for 24 h.

2.3.1.2 Secondary Enrichment

0.1ml of each cultures grown in primary enrichment were transferred into 10 ml of of selective liquid enrichment medium with full concentration (Fraser broth, Difco) and incubated at 37º C for 48 h.

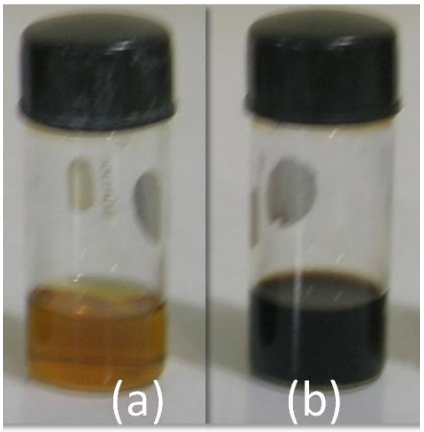

Figure 2.5 Enrichment of Bacteria (a) Absence of *L. monocytogenes* (b) Presence of *L. monocytogenes* in Fraser broth.

2.3.2 Isolation of *L. monocytogenes*

Later to enrichment, we do plating of the cultures obtained in Fraser Broth on the PALCALM Agar, Difco plates with incubation at 37º C for 48 h. Bacterial colonies were considered as positive for *Listeria* that yields grey-greenish appearance surrounded by black precipitate on PALCALM agar plates. Occurrence of black precipitate is due to esculin hydrolysis as shown in Figure 2.6. The presumed colonies of Listeria were further confirmed by molecular analysis.

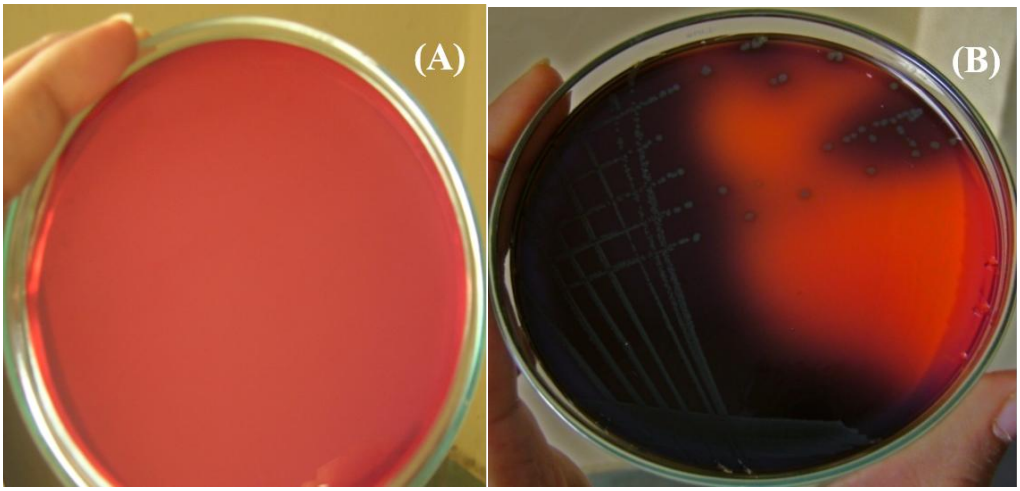

Figure 2.6 (A) PALCALM Agar plate (B) Colonies of *Listeria monocytogenes* on PALCALM Agar.

2.3.3 Characterization of the modified electrode

Morphological changes in the film were observed under optical microscope before and after the immobilization of ssDNA over the modified electrode (Figure 2.4). The changes clearly revealed the reductions in the surface pores with marked surface roughness changing into small globular structures in the polymer matrix following DNA immobilization [Minehan et al., 1994]. Changes in the morphology indicated immobilization of ssDNA over the polymer surface which was confirmed by optical microscope.

2.3.4 Electrochemical behavior of poly-5-carboxyindole/gold electrode

Cyclic voltammetry of 5-carboxyindole monomer over bare gold electrode and polymerization of monomer is shown in Figure. 2.3 A for the scan range −0.2 V to 1.3 V. The polymerization was evident by the couple of redox peaks with increasing peak height (current) and the increasing scan cycles. Cyclic voltammograms with increasing current is the typical signature of conducting polymer over the metal electrodes. Further, polymer used

in this study revealed good electroactivity as evident from the cyclic voltammogram recorded for the monomer free electrolyte as shown in Figure. 2.3 B. Oxidation and reduction peaks showed reversible redox nature of the polymer. Further the polymer was also studied using impedance spectroscopy technique.

2.3.5 Immobilization and hybridization of DNA

Single stranded DNA (15µ L) was drop casted onto the modified electrode and incubated for 1 h at room temperature (25 ± 1 ˚ C) and subsequently kept overnight in freeze. Such a modified electrode is highly stable, and can be used for hybridization with the target DNA. The hybridization reaction mainly depends on time given for hybridization and also temperature apart from DNA composition. Therefore, optimization of these factors is important for accurate sensing. In the present study, the length of base pair and base composition (% G + C) were fixed because only one strain of *L. monocytogenes* was selected for experiments. Moreover, the time and temperature parameters for hybridization reaction on the modified electrode were optimized. The optimum temperature range for hybridization for this system is observed as 40–42 ˚C. Optimum hybridization time was calculated by observing the changes recorded in impedance spectra (R_{CT}) for the sensor probe after every interval of 5 min and it is continued till no change in spectra is seen.

The time after which no significant change in R_{CT} observed, was considered as optimum time for hybridization, which is observed as 15 min as shown in Figure 2.7. Generally R(CRQ(RW)) circuit is used for such studies but in our case R(C(RW)) circuit showed the best fit and matching with the actual value of the solution resistance (R_s). Therefore this slightly modified R(C(RW)) circuit was used for the study.

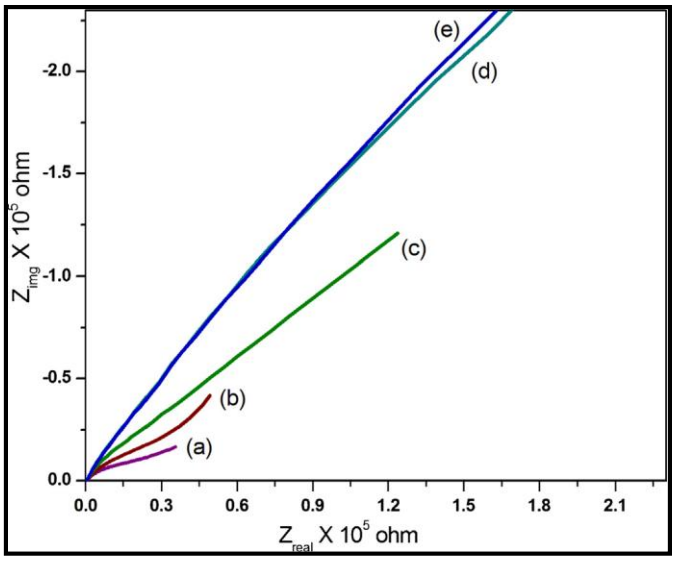

Figure 2.7 Nyquist plots showing effect of hybridization time on change in impedance of poly 5-carboxy indole/ssDNA immobilized electrode for hybridization event with a fixed concentration of 10^{-8} M DNA for various time periods: (a) 0 min (b) 5 min (c) 10 min (d) 15 min and (e) 20 min.

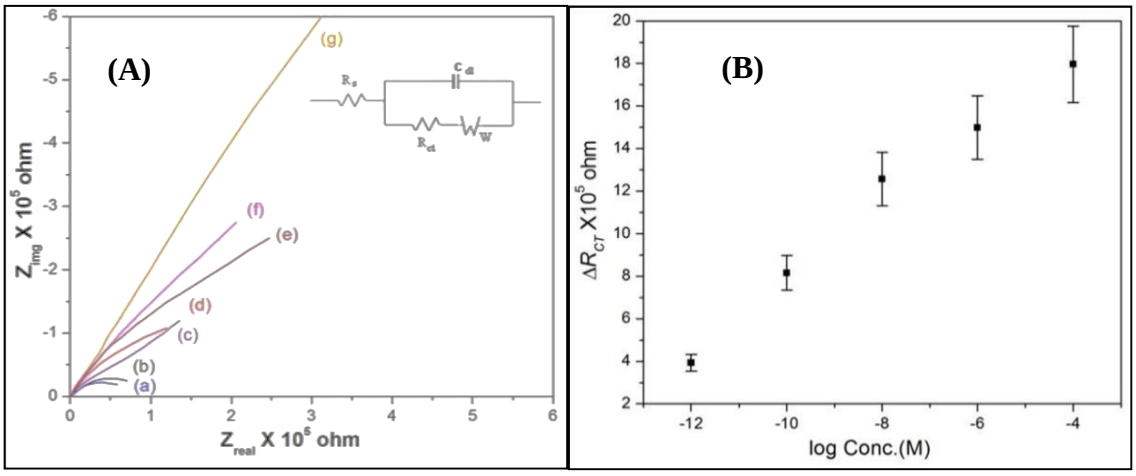

Figure 2.8 (A) Nyquist plots for (a) poly 5-carboxyindole film, (b) poly 5-carboxyindole with probe of *hlyA*, after hybridization with (complementary) target DNA of concentration from (c) $1x10^{-12}$; (d) $1x10^{-10}$ (e) $1x10^{-8}$ (f) $1x10^{-6}$ and (g) $1x10^{-4}$ M. (B) Calibration plot for ΔR_{CT} vs. concentration of target (genomic) DNA sequence.

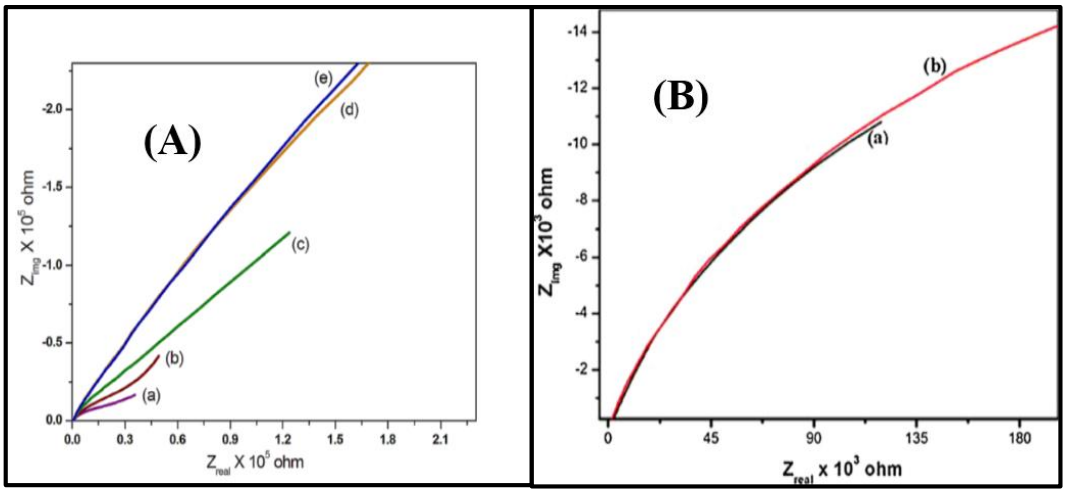

Figure 2.9 Nyquist plots for (A) Poly 5-carboxyindole with probe of *hlyA* after hybridization event with target DNA ($1x10^{-8}$ M), and (B) After heat treatment and dip wash.

2.3.6 Electrochemical detection based on Impedance

Importance of Impedance Technique: Impedance data are normally recorded in a range of frequencies, using alternating current of small amplitude, thus the EIS is often referred to as AC Impedance. Electrochemical DNA biosensors, impedimetric detection relies on the variation in the electrical properties of the DNA modified electrode before and after hybridization. The change of double-layer capacitance, heterogeneous electron transfer resistance, impedance or current is measured quantitatively. DNA sensors based on electrochemical impedance spectroscopy (EIS) detection records the changes in interfacial properties between the electrode and the electrolyte induced by DNA hybridization. These changes have arised due to either DNA conformational changes or the DNA damages to an electrical signal [Palecek et al., 1998]. Unique advantages of impedimetric technique as compared to other electrochemical methods, lies in its high sensitivity, and shows capacity to separate the surface binding events from the solution impedance, easy quantification of signal [Grieshaber et al., 2008]. Other merits lies in AC Impedance being less destructive as

compared to the measured biological interactions since it is carried out over narrow intervals of small potential. To characterize the DNA immobilized on the modified electrode surface using EIS, the change in impedimetric response upon hybridization can be measured either in terms of kinetics of electron transfer process by Faradic impedance measurements or in terms of alterations of capacitance and molecular layer organization, originating from biorecognition events, by non Faradic approach. The polymer is electrochemically switched between its oxidized and reduced states on application of positive and negative voltage (case of imposed AC potential). The oxidation (means creation of positive charge over polymer backbone) is resulting insertion of anions in the polymer matrix from solution, which is also known as doping of the polymer. While during reduction process polymer is going back to its original state (neutral) and anions inserted may move out from the polymer matrix to solution known as dedoping of the polymer. The change in charge transfer resistance (R_{CT}) in the impedance plot is depending on anions (dopants) movement, which is affected by the modification of electrode surface by interaction with DNA [Tang et al., 2004; Mohan et al., 2010]. The impedance plot for the gold electrode is observed as a small semicircle that corresponds to the interfacial charge transfer, followed by a low frequency Warburg line with the slope near the unity that corresponds to the domination of mass diffusion effects over the electron transfer process. In the typical Nyquist plot as already mentioned, the semicircle portions correspond to a combination of the charge-transfer resistance (R_{CT}) with the double layer capacitance (C_{DL}), as supported by the equivalent circuit obtained using Z_{sim} program (Figure 2.8 A). Numerical values of R_{CT} were derived from the impedimetric data obtained by fitting it into the slightly modified Randles equivalent circuit (Figure 2.8 B) as also discussed above. The R_{CT} is the most sensitive and straight forward parameter that can be

used to characterize the events on the surface of modified electrode/probe. Other components such as background solution resistance (R_s) and Warburg impedance (W) represent bulk properties of the electrolyte solution and diffusion features of the redox probe and ions in the solution, are unaffected by chemical modifications at the electrode surface (Li et al., 2008; Moradi et al., 2013). Nyquist plots for 5C Pin film as shown Figure 2.8 shows the change in R_{CT} (ΔR_{CT}) 67 kΩ (with respect to bare electrode). With the immobilization of 24 mer ssDNA probe significant change in R_{CT} was 77 kΩ (with respect to 5C Pin electrode) for a typical experiment. Further hybridization with the target DNA, increased the diameter of semicircle (difference in successive reading approx. 20 kΩ) due to increase in the charge transfer activity with the successive increasing concentrations from 10^{-12} to 10^{-4} M (Figure 2.6 A). The Warburg resistance was observed 3.2×10^{-7} and the R_s was observed 660 Ω in a typical experiment which showed variation with concentration. Experiments repeated several times, to verify the reproducibility of the sensor and showed standard deviation of 12%. The detection limit was observed as 2.34×10^{-13} M (by extrapolating the calibration curve). The increase in the R_{CT} value may be explained based on the increase in the negative charge over the probe-polymer film following hybridization with the target DNA (negatively charged due to PO_4^{2-}). As discussed above the anionic dopants (in this case ClO_4^-) which are acting as charge carrier responsible for change in R_{CT} value is repulsed and hurdled by the negative charges over the DNA (PO_4^{2-}) and caused increase R_{CT}. This repulsion increased with increasing the concentration of the DNA over the probe. Therefore, with increasing concentrations of target DNA, the hybridization also increased with the concomitant increase in R_{CT}.

In order to check the stability of the probe during cyclic heating and cooling

(hybridization event), the impedance spectroscopy was performed for the probe (ssDNA immobilized over 5C Pin) at room temperature (25 ± 1 °C) (Figure 2.7 A). Figure 2.9 shows Nyquist plot for (a) poly-5-carboxyindole with probe of hlyA after hybridization event with target DNA (1×10^{-8} M), and (b) after heat treatment and dip wash. It is showing stability of the modified electrode for various cycles of heating and cooling (as the typical plot is for 5 runs) which was the condition of the denaturation and hybridization during the sensing. There was no significant change in the R_{CT} and this ascertained that the probe was stable against cyclic heating and cooling and any change in R_{CT} was only due to hybridization of target DNA.

2.4 Conclusion

A label-free genosensor for the explicit detection of *L. monocytogenes* was developed based on the hlyA gene related 24 mer ssDNA probe. A significant rise of R_{CT} was observed following hybridization with increasing concentrations of the target DNA. The limit of detection with target DNA was 10^{-13} M. The method used in the present study is rapid, simple and free from the complex chemistry of redox indicator and markers for DNA hybridization detection. The proposed impedimetric approach may be considered for early diagnosis of listeriosis in clinical and environmental samples.