Electrode Modification Through Chemical and Electrochemical Deposition of Polytyramine Film for Biosensing Application

Dr. Wajiha Abbasi
Department of Chemistry,
University of Leeds
Leeds, LS2 9JT, United Kingdom

Prof. Dr. Long Lin
Professor of Colour & Polymer Science
University of Leeds
Leeds, LS2 9JT, United Kingdom

***Abstract*— Developing modified electrodes through electrodepositing conductive polymer layer has been continuous studied in areas such as technology, electronics, medicine, biology and many others. Modifying layers allow scientists to build electrodes with required properties such as for example: selectivity, stability, precision, durability, range of potentials etc. The aim of the studies was to modify surface of gold electrode with the layer of polytyramine synthesized through chemical and electrochemical methods. The electrodeposition was carried out in 0.025M tyramine solution in 0.3 M NaOH in methanol at the voltage ranging from 0V to 1.6 V at the scan rate of 100 mVs^{-1}. Similarly a new approach was used to chemically polymerize tyramine in an alkaline media using $FeCl_3$ as an oxidant. The resulting particles were deposited onto gold electrode through drop casting method. The modified electrodes exhibited a good sensitivity, reproducibility, and stability. The resulting polytyramine film and particles were characterized through scanning electron microscopy, FTIR, cyclic voltammetry and impedance spectroscopy. The voltammetric and impedance studies showed that polytyramine layer could be employed to determine biologically active substances. Electrodes modified with polytyramine can be also fundamental to construct biosensors by enzymes immobilization at its surface.**

Keywords*— Surface modification; polytyramine; impedance; biosensor; tyramine; cyclic voltammetry.

I. INTRODUCTION

Polymer-modified electrodes, built up by deposition of electroactive polymeric films on conductive substrates, have been a major area of research for more than two decades [1]. The polymer film coated on the transducer surface for the immobilization of biomaterial must be able to efficiently spread the electrical potential produced by the biochemical reaction to the transducer, to ensure reproducibility and to amplify the signal. Modified electrodes are useful because they can be applied to several electrochemical systems without modification of their characteristic or efficiency, thus enabling their reuse. Electrodes coated with polymeric films containing reactive groups can be connected to a cell so that they function as working electrodes, leading to the desired organic substrate transformations [2].The use of nanoscale polymer films permits better selectivity and faster measurements and has stimulated the development of new polymer films of varied chemical natures. Electropolymerisation of conducting polymers, such as polypyrrole (PPy), polyaniline, polyacetylene, polyindole, polythionine and polythiophene, has been studied extensively for the development of biosensors [3]. This is because these polymers have a high conductivity and stability in both air and aqueous solution. Also, the thickness of the electropolymerised film and the amount of immobilized enzyme can be controlled easily during electropolymerisation.

After conducting polymers, non-conducting polymers are emerging as a novel support matrix for the immobilization of biomolecules because they offer impressive advantages, including excellent perm selectivity and high reproducibility, in addition to most of the reported merits of conducting polymers. The biosensors based on immobilizing enzymes in non-conducting films have some advantages over conducting films: First, the film thickness of the non-conducting polymer is self-controlled during electropolymerisation, and a very thin and uniform film can be obtained. Biosensors prepared in this way generally have the advantages of fast response and high sensitivity because of relatively high enzyme loading. Second, the non-conducting polymer films are generally found to be more effective in both preventing the biosensor from fouling and eliminating the interference from electroactive species, such as ascorbic acid and uric acid [4]. Furthermore, non-conducting films are well suited for capacitance measurements where the electrode surface needs to be properly insulated [5]. An example of a non-conducting polymer is the thin electropolymerised film of poly[1,3-diaminobenzene (DAB)], which can be used to eliminate electrochemical interference from ascorbate, urea, acetaminophen and other oxidizable species [6]. Previous studies indicated that monomers containing aromatic groups that are directly bonded to oxygen are easier to polymerize [7]. The synthesis of such polymers is reproducible, producing films with good mechanical resistance, which allows higher stability to the modified electrode. The use of non-conducting polymers of phenol and its derivatives for the development of biosensors has been reported [8]. The routes of polymerization of tyramine [4-(2-aminoethyl) phenol] have been extensively studied for application as support for biosensors [9].

Tyramine (Tyr) was chosen as monomer in this study due to its pendant amine group. This polymer presents one primary aliphatic amine per tyramine moiety which can be used to attach to organic molecules or to biomolecules of interest. For instance [10], the amino group can covalently bond through a carboxamide or a phosphoramidate bond and anchor to the transducer. Thus, poly-Tyr has been employed for enzyme [11], antibody immobilization, oligonucleotide (ODN) [12] and antigen detection at very low concentrations.The electrochemical polymerization of phenol derivatives such as tyramine (Ty) has received a considerable attention for its applications in bio- and immuno-sensors due to the presence of a free amine group which facilitates immobilization of bio-recognition molecular species. Moreover, the phenol moiety is known to be preferably oxidized to initiate the polymerization reaction. Conductive properties can also be improved depending on the experimental conditions under which the electrodes are modified. The use of acidic aqueous medium to produce poly-[4-(2-aminoethyl) phenol], poly-Tyr, enables the formation of thicker films with improved conductive properties than those obtained in neutral or alkaline solutions.

II. MATERIAL AND METHODS

A. Materials

The solutions were prepared from analytical grade reagents; Tyramine, methanol, sodium hyrdroxide (NaOH) and potassium ferrocyanide were purchased from Aldrich. Ferric chloride ($FeCl_3$) purchased from Sigma was used to initiate the polymerization. HCl (hydrochloric acid) (2N) was supplied by Fluka. The reagents were of analytical grade and de-ionized water was used throughout the experiments. A monomer solution 0.2 M tyramine solution was prepared in 1M NaOH solution. 0.5M $FeCl_3$ was used as an oxidant. The chemical polymerization was performed at room temperature. Once polymerization was completed, thick brown precipitates were collected filtered and washed several times with dH2O to remove any impurities.

Electropolymerization of tyramine was carried out in 0.025M tyramine solution in 0.3 M NaOH in methanol by using GPES software on an AUTOLAB type III electrochemical. The solution was allowed to stand for couple of minutes to allow un-dissolved NaOH to settle down. Once settled, a small amount of the solution was taken into the beaker and polymerized at the voltage ranging from 0V to 1.6 V at the scan rate of 100 mVs^{-1}. Total two scans were carried out and a uniform polytyramine film was electrodeposited on the gold electrode. Following electropolymerisation, polytyramine electrodes were rinsed with dH_2O and blow-dried gently in a stream of argon.

B. Electrochemistry experiments

All electrochemistry experiments were based on a three electrode system in an electrochemical cell. The experiments were carried out by using transducers that integrated all three onto one sensing chip. The working and counter electrode used were gold, where as the reference was Ag/AgCl. The three electrode system was controlled by an EcoChemie µAutolab Type III frequency response analyser (FRA-2) (Windsor Scientific Limited, Slough, Berkshire, UK) potentiostat. This was used to apply a current to the electrochemical cell through the counter electrode. The voltage difference between the working electrode and the reference electrode was then measured. The Autolab measured the difference between the cell voltage and the desired voltage completing a feedback loop, causing an amplifier to drive current into the cell to maintain the voltages. Autolab software was used to acquire electrochemical measurements and for further data analysis. All electrochemical interrogation was done at room temperature. The obtained polytyramine film was characterised using two electrochemical techniques: cyclic voltammetry and electrochemical impedance spectroscopy (EIS). Cyclic voltammograms (CVs) were recorded with various scan rates up to 100 mV/s. EIS measurements were carried out over a range of frequencies ranging from 0.1 Hz to 10,000Hz, using a modulation voltage of 10 mV at an applied voltage of 0.4 V. Both impedance and CV experiments were mainly performed in an electrolyte solution of 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1 ratio) in 100 mM PBS, pH 7.0. All tests were performed at room temperature (25^0C).

C. Scanning electron microscopy (SEM)

Various polymers were prepared through chemical polymerisation and the morphologies and size of these particles were studied with field emission scanning electron microscopy (JOEL JSM-6610LV). All SEM pictures were taken after drop casting polymer suspension onto chip and coated with gold by sputtering for 10 s. Photographs were obtained at 5 or 15 kV and at a working distance 1µm-100µm.

D. FT-IR analysis

Polytyramine films casted on electrodes surfaces through drop casting and electrodeposition were characterised by attenuated total reflectance (ATR) spectra recorded by Alpha FT-IR Spectrometer.

III. RESULTS AND DISCUSSIONS

A. Chemical polymerisation of tyramine

A significant amount of studies have been done on polyaniline and this constitutes a large family of polymers which are formed by oxidative either electrochemically or chemically of aniline or its derivatives. Aminophenols are interesting electrochemical materials since, unlike aniline and other substituted anilines, they have two groups ($-NH_2$ and–OH) which can be oxidised. Therefore, they can show electrochemical behaviour resembling anilines and phenols. The extensive literature survey shows that some work has been carried out in aminophenol and their application in different fields specially polytyramine in biosensors. Previously no attempt was made to synthesise polytyramine through chemical oxidation method. In this research work tyramine was successfully polymerised for the first time in NaOH solution. As the aim of this study was to develop printed medical device therefore an attempt had been made to chemically polymerise tyramine through oxidative polymerisation which could be formulated into conductive ink for the fabrication of a biosensor. The schematic presentation of polymerisation of tyramine is given in Figure 1.

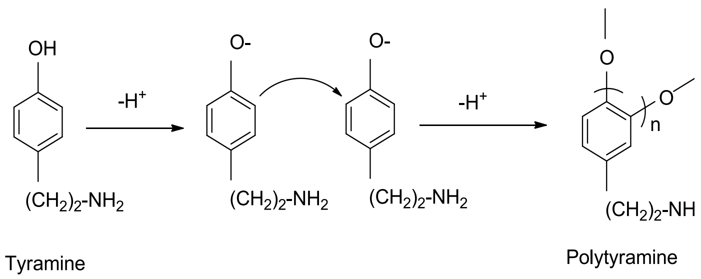


Figure 1 Schematic presentation of the tyramine polymerization

The resulting polytyramine particles were characterized through SEM. SEM pictures of polytyramine are given in Figure 2. It showed that most of polytyramine particles were cubical shaped and dispersed evenly in the solution. There was also some coagulation apparently visible on higher resolution.

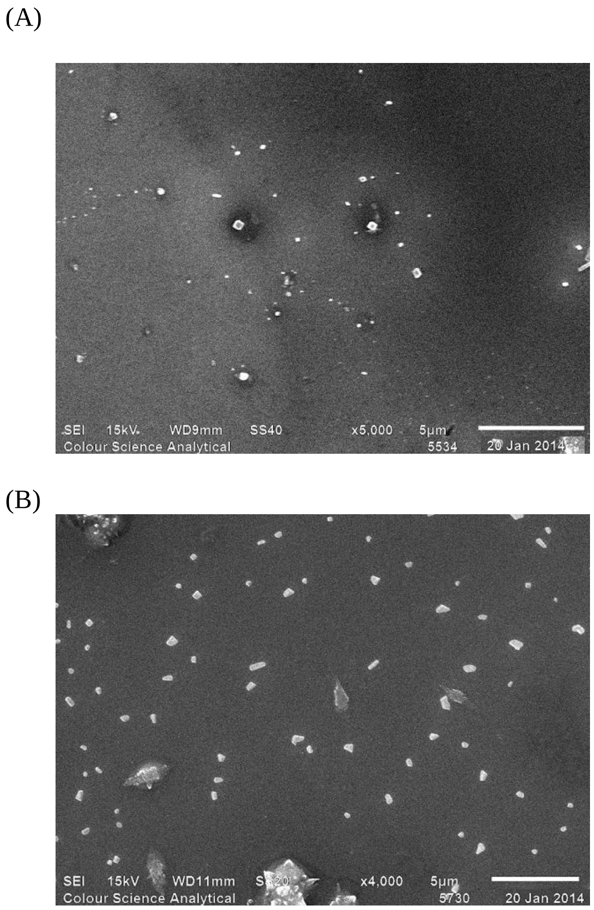


Figure 2 SEM pictures of polytyramine at (A) 4,000 and (B) 10,000 magnifications

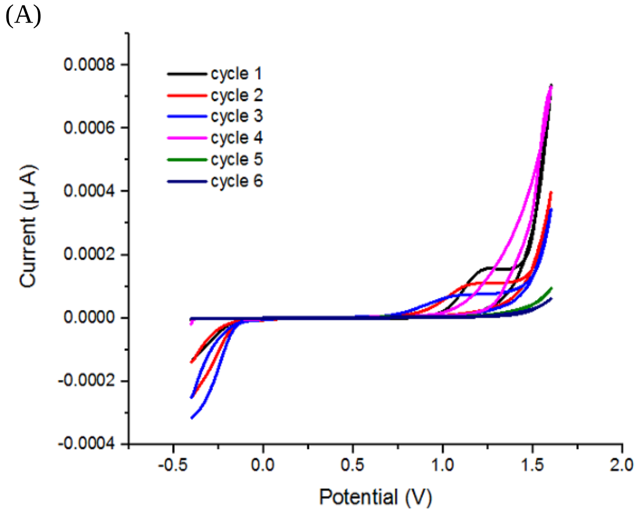
B. *Electropolymerisation*

Electrochemical methods for obtaining polymers are widely used. A polymer can be acquired by applying potentiostatic or galvanostatic conditions or by using the cyclic voltammetry. Electrochemically synthesized layers, unlike the one obtained during chemical synthesis, are free of residual oxidant. Another advantage of the electrochemical synthesis is the ability to control the polymerization process (for example, the polymerization rate or the thickness of the resulting polymer) by changing the current, potential, the scanning rate potential or the number of voltammetric cycles [13].

Figure 3 (A) shows typical cyclic voltammogram obtained from electropolymerisation of polytyramine consisting total six scans resulting into uniform thin layer onto the gold electrode.

Figure 3 (B) showing first two scans generating a thin film on the electrode surface showing oxidation and reduction peak. The first cycle a gradual decrease in the peak current was observed during continuous potential cycling, but oxidation of the monomer continued after second scan. It was obvious that as the number of cycles was increased the poor conductivity of the polytyramine films resulted in passivation of the electrode and hence electrodeposition current was decreased [14]. After several cycles, the electrode was sufficiently blocked such that only very small oxidation currents were observed. As with the electroploymerization of other phenols, linking occurs through the ortho position of the phenol group leaving the amine available for covalent attachment of enzyme [15].

The electrochemical trend demonstrated the reversible reaction (protonation/deprotonation) associated with the transfer of one electron and one proton between the monomer and its cationic phenoxy radical [16]. This initiation step was followed by dimerization reaction (one electron + one proton) of the phenoxy radical with Tyr molecule (linkage via ortho position) to form a dimmer (oligomerization) followed by nucleation of oligomers and subsequent deposition of the polymer on the electrode surface as depicted in literature [17].



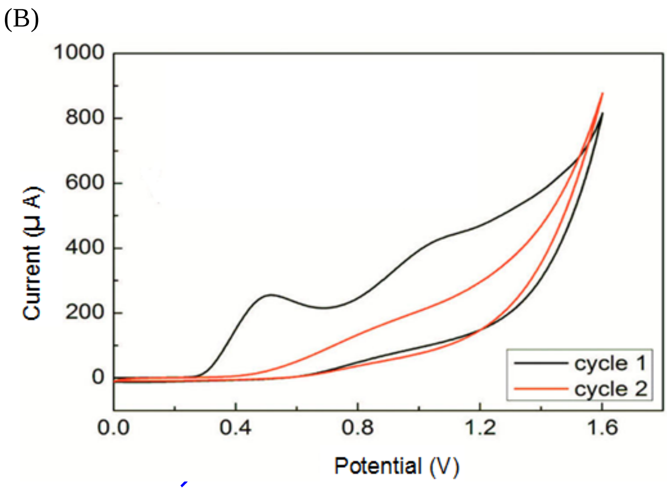


Figure 3 Cyclic voltammogram of polytyramine

C. *FTIR analysis*

The FT-IR spectrum of a PTyr film formed through chemical polymerization is shown in Figure 4 and compared to the spectrum of the monomer. The main bands are reported in Table 1. For the monomer, the (C = C_{ar}) stretch appears at 1623 cm^{-1}. For the polymer, this vibration appears at 1642 cm^{-1}. The band at 1665 cm^{-1} could be attributed to the (C = C_{ar}) stretch of the oxidized part of the polymer chain. The δ(N–H) of primary amines is present at 1517 cm^{-1} for the monomer and at 1571 cm^{-1} for the polymer. The absence of bands from secondary amines indicates that the amino group remains free after electropolymerisation.

The C–O–C stretch vibration is present at 1222 cm−1 for the polymer. The out-of-plane C–H deformation band (of two adjacent aromatic hydrogen's) is present on the monomer at 826 cm−1. This band, which is also present on the polymer, at 826 cm−1, is weaker, and partially replaced by a band at 940 cm^{-1}, which is due to C–H def. of 1 isolated aromatic H. Finally, the band at 772 cm^{-1} on the polymer spectrum could be attributed to the inter-ring C–O–C deformation. It should be noted that no inter-ring C–C bonds are detected [18],[19].

The characteristic peaks of monomer and polymers are demonstrated in Table 1.

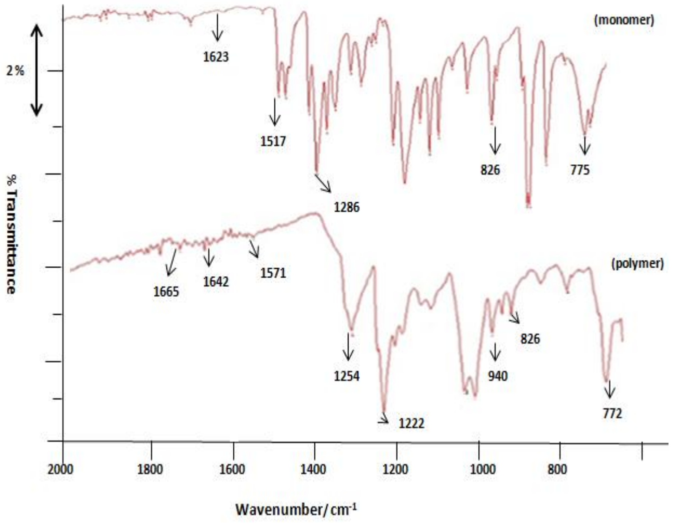


Table 1 FT-IR absorption peaks for polytyramine

Group contribution	Wavenumber (cm^{-1}) tyramine	Wavenumber (cm^{-1}) polytyramine
$(C=C)_{ar}$ strectching Oxidized Reduced	- 1623	1665 1642
Angular deformation of N-H	1517	1571
Angular deformation of terminal O-H	-	1254
C-O-C stretching	1286	1222
C_{ar}-H out of plane deformation (two adjacent hydrogen's)	-	940
C_{ar}-H out of plane deformation (isolated hydrogen)	826	826
C_{ar}-O-C_{ar} deformations	775	772

D. *SEM analysis of polytyramine modified surfaces*

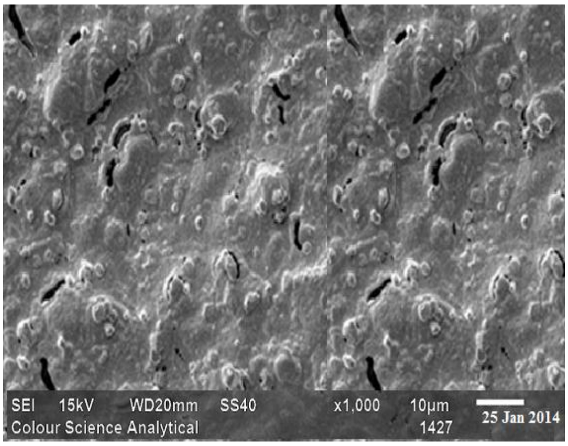
SEM images shown in Figure 5 were obtained before and after polytyramine deposition on gold electrodes. Two different types of electrodes were prepared. Keeping in mind of feasibility of printing sensors, drop casting was adapted as alternative method to electrodeposition.

The resulting polymer surfaces onto gold electrode were analyzed under SEM and compared with bare gold electrode. It was revealed that blank gold drop sensor had rough surface with crevices as shown in Figure 5 (A). Apparently there are fissures and cracks are apparent which made electrode surface quite uneven.

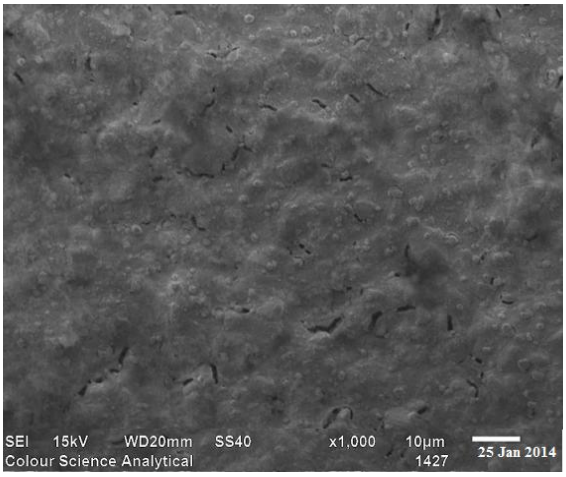
Once polytyramine has been electrodeposited onto the electrode through cyclic voltammetry as given in Figure 5 (B), it covered and filled the surface crevices. There are few cracks quite visible but they appear quite small as compared to bare electrode.

In the third set of electrode modification, polytyramine suspension synthesized through oxidative chemical polymerization was drop casted onto the electrode surface and let it dried away for 5-10 min before washing with distilled water. The SEM of resulting modified electrode is given in Figure 5(C) which apparently confirms a thin and uniform polymeric film covering the electrode surface.

(A) Bare gold electrode



(B) Polytyramine film through electrodeposition



(C) Polytyramine film through drop casting method

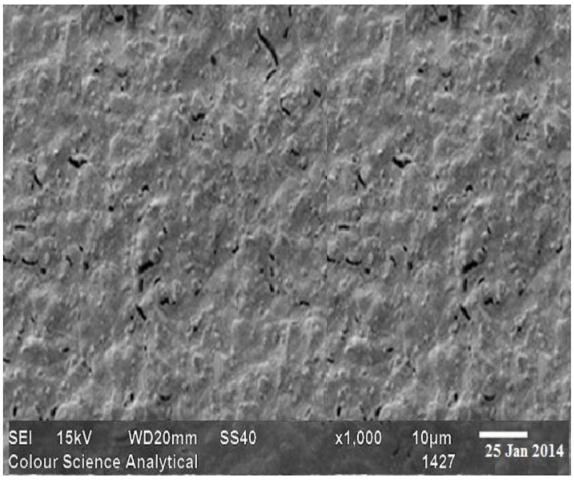


Figure 4 SEM analysis of polytyramine film on electrode surface

E. *Electrochemical impedance spectroscopic studies of modified electrode*

Impedance spectroscopy is a very powerful tool for the analysis of interfacial properties such as changes of modified electrodes upon bio-recognition events occurring at the modified surfaces. Formation of the complex on a conductive or semi-conductive surface alters the capacitance and the resistance at the surface electrolyte interface. Furthermore, the build-up of the sensing biomaterial film on the conductive or semi-conductive support alters the capacitance and resistance properties of the solid support-electrolyte interface. Impedance measurements provide detailed information on capacitance/resistance changes occurring at conductive or semi-conductive surfaces. Impedance spectroscopy for biosensor technology has the same working principle as other electrochemical measuring techniques.

EIS is composed of electrical circuits based on AC current, which is generally used for impedimetric experiments. The complex impedance can be presented as the sum of the real, (Z’) and imaginary (Z”), components originating from resistance and capacitance of the cell, respectively. In the Nyquist format, the imaginary impedance component (Z”) is plotted against the real impedance component (Z’) at each excitation frequency giving information about the electrified interface and the electron transfer reaction.

A typical form of Faradaic impedance scan is presented in the form of Nyquist plot which includes a semicircle region laying on the real component ($Z^{/}$) followed by a straight line. The semicircle observed at higher frequencies corresponds to the electron transfer process, also known as charge transfer resistance, R_{ct}. whereas the linear part of the spectrum represents diffusion limited process at lower frequency range. The experimental data of impedance scan was fitted with computer stimulated spectra using an electronic circuit based on the Randles and Ershler theoretical model as shown in Figure 6 (A) [20],[21].

The Randles comprises the uncompensated resistance of the electrolyte (R_s), in series with the capacitance of the dielectric layer (C_{dl}) also known as double-layer capacitance, the charge-transfer resistance (R_{ct}) and the Warburg impedance (Z_w) resulting from the diffusion of the redox-probe. In the Nyquist plot shown in Figure 6 (B), a typical shape of a Nyquist plot includes a semicircle region lying on the real axis followed by a straight line.

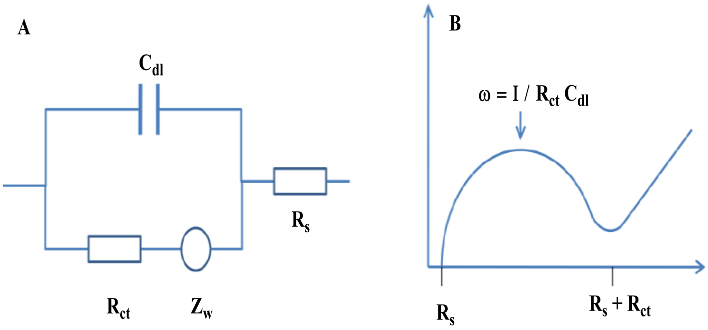


Figure 5 A) the typical Nyquist diagram for the AC impedance measurements; (B) the Randle equivalent circuit

Figure 7 shows the impedimetric data collected, presented in the form of a Nyquist plot depicting the real (Z’) and imaginary (Z”) components of the ac impedance analysis of blank gold electrode shown in 7 (A) following by impedance scan of polytyramine coated gold electrode shown in Figure 7 (B). From Figure 7 (C), both electrodes impedance scans are presented together, it is apparent that the curve for gold electrode is smaller as compared to polytyramine electrode.

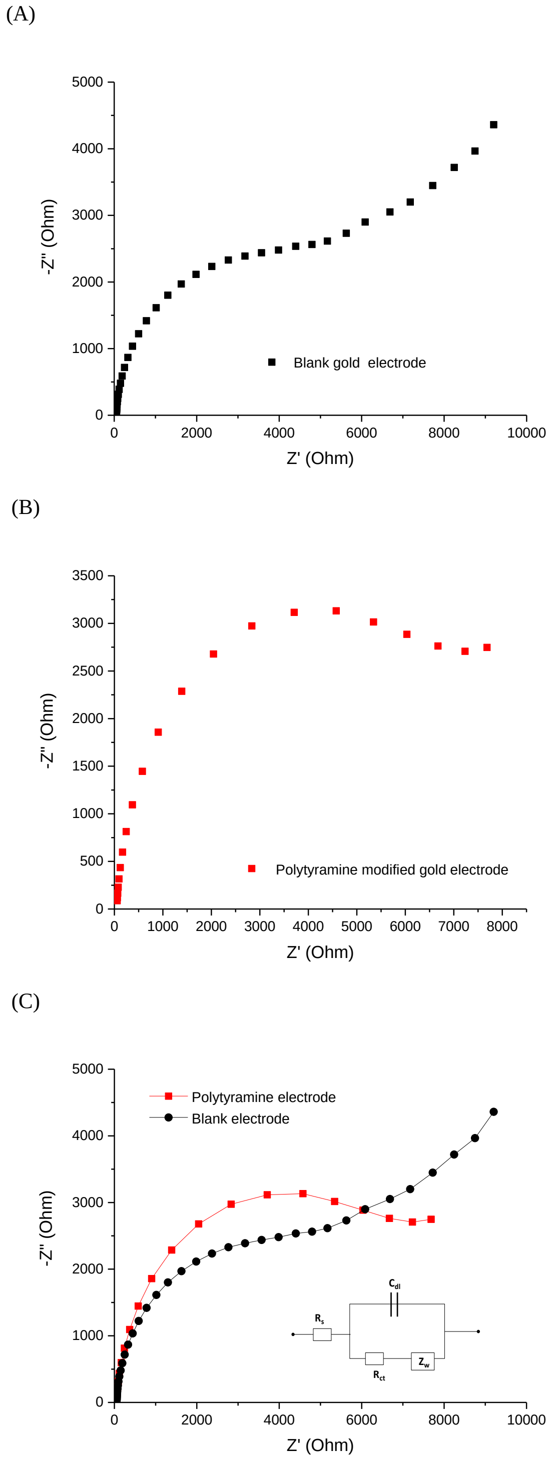


Figure 6 Nyquist plot for gold electrode and polytyramine modified electrode

The increase in the semicircle of polytyramine film *coated* electrode in the high frequency of the Nyquist diagrams is mainly due to the increase in R_{ct}. The semicircle of bare gold electrode shown in black is smaller showing high capacitance as compared to polytyramine coated electrode shown in red. It reflects the increase in multilayer thickness and decrease of ion content and mobility, which are associated with film electrical conductivity. The film resistance becomes detectable immediately after the second or third layers.

IV. CONCLUSION

The study of modified electrodes remains a field of high activity. Many new types of surface structures are being prepared, and electrochemical studies are leading to better insights into the way charge is transported through surface layers and how charge is exchanged between surface species and molecules in solution. In this research work a gold electrode surface was modified through depositing polytyramine film and impedance studies were carried out to study the change in capacitance after polymer deposition. The studies with very promising results showed that the resulting polytyramine modified gold electrode is conductive enough to be a prospective surface for immobilization of antibodies or proteins.

V. REFERENCES

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