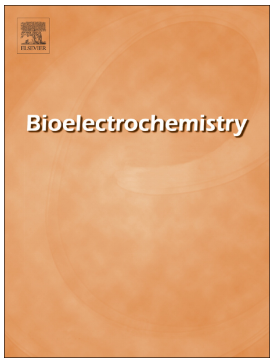

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A new composite consisting of electrosynthesized conducting polymers, graphene sheets and biosynthesized gold nanoparticles for biosensing acute lymphoblastic leukemia

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Abstract:

In this work we report the synthesis of a stable composite with excellent electrical properties, on the surface of a biosensor. Conductive polymers offer both high electrical conductivity and mechanical strength. Many reports have focused on synthesizing conductive polymers with the aid of high-cost enzymes. In the current work we introduce a novel electrochemical, one-step, facile and cost effective procedure for synthesizing poly (catechol), without using expensive enzymes. The poly (catechol) conductivity was enhanced by modification with graphene sheets and biosynthesized gold nanoparticles. Four different robust methods, DPV, EIS, CV and chronoamperometry, were used to monitor the biosensor modifications. The peak currents of the catechol (an electroactive probe) were linearly related to the logarithm of the concentrations of target DNA in the range 100.0 µM to 10.0 pM, with a detection limit of 1.0 pM for the DNA strand. The current work investigates a new, stable composite consisting of conductive polymers and nanoparticles, which was applied to the detection of acute lymphoblastic leukemia.

Keywords: conducting polymer, graphene sheet, biosynthesized, biosensor, acute lymphoblastic leukemia, DNA hybridsation

1. Introduction:

Cancer is among the deadliest human diseases. In general the body forms new cells to replace the old ones, however cancer is uncontrolled cell growth. Such growth can result in cells forming a mass called a tumor, additionally the cells from malignant tumors can break away and spread to other parts of the body, which is called metastatic cancer. After metastasis, the chances of survival are much lower, hence there is an urgent need for clinical diagnostic strategies to detect early stage cancer, before it has had the chance to metastasize [1].

Biosensors, are a new wave of clinical device, which can be designed to detect cancers in the early stages.

A biosensor is an analytical device combining a biological receptor and a physicochemical detector, which is used for the detection of an analyte [2-5].

There are various biosensors available based on different receptor and detector types. Electrochemical DNA biosensors have several advantages, such as ease and speed of measurement, cost effectiveness, ability to analyze complex biological fluids, and high specificity and sensitivity [6, 7].

An ultra-sensitive electrochemical DNA biosensor based on recent advances in conductive polymers and nanomaterials has the potential to be very useful [8].

Conductive polymers are organic polymers that offer high electrical conductivity, in addition to air stability and mechanical strength. The combination of these features leads to the potential to further increase the sensitivity of biosensors.

In recent years, many studies have focused on aromatic polymers because of their extensive applications and their excellent electrical conductivity. Poly (catechol) is a useful polymer with redox behavior that is responsible for many of its features and applications. Many reports have focused on the synthesis of poly (catechol) with the aid of enzymes, such as horseradish peroxidase (HRP). These methods have several disadvantages, including the high cost of enzymes, which may denature over time, and the number of steps that can be involved [9].

In the current work, an electrochemical, one-step, facile and inexpensive procedure was developed for synthesizing poly (catechol) without using enzymes. This poly (catechol) was used for the modification biosensors, resulting in the enhancement of the electrical properties.

Nanomaterials offer interesting properties owing to their large surface area to volume ratio and high electrical conductivity, which can have a significant role in the development of many areas of healthcare, in particular biosensors [10].

A new class of nanomaterial is graphene, which is a thin layer of pure carbon, bonded in a hexagonal lattice structure. Potential benefits of graphene include high electrical conductivity and high mechanical strength [10].

In this work, a combination of graphene and conductive polymer is used to create a surface with high electrical conductivity, which results in a sensitive and selective biosensor.

The most significant challenge in the development of electrochemical biosensors, is the immobilization strategy, which involves the immobilization of biological components on the electrode surface. One of the most successful immobilization strategies in the field of DNA

biosensors, is the high affinity of thiol-modified DNA for gold nanoparticles. To overcome the immobilization challenge, the present work explores the potential application of biosynthesized gold nanoparticles, in electrochemical biosensors. Numerous methods have been used to synthesize gold nanoparticles, with biosynthesis being regarded as the best. Biosynthesis is a facile, bioenzyme-catalyzed process, which converts the substrate into a modified compound. There are many benefits to green synthesis of nanoparticles, such as physiological relevance, robustness, low environmental impact, biocompatibility and lack of starting material toxicity, as well as uniformity of the nanoparticles [12].Cancer is not one disease, there are more than 100 kinds of cancer such as lung, breast, prostate, ovarian, and leukemia.

Acute lymphoblastic leukemia (ALL), also known as acute lymphocytic leukemia, is a cancer that originates in lymphoid cells.

A particular challenge of ALL is that the leukemia cells can spread to other parts of the body without traveling in the blood. There is therefore an urgent need for a sensitive and selective biosensor to detect early stage ALL [13–16].

In this work, a DNA electrochemical biosensor for the early detection of ALL is presented, based on a composite, consisting of electrosynthesized conductive polymer, graphene sheets and biosynthesized gold nanoparticles. Based on our findings, we anticipate that this biosensor has potential applications in simple, rapid and quantitative detection of many kinds of cancer.

2. Experimental

2.1. Apparatus and reagents

Tris (2-carboxyethyl)phosphine hydrochloride (TCEP), 6-mercapto-1-hexanol (MCH), graphite powder, catechol, trisodium citrate, hydrochloric acid, hydrogen peroxide, sulfuric acid, sodium hydroxide, phosphoric acid, disodium hydrogen phosphate, potassium ferrocyanide

($K_4[Fe(CN)_6]$), potassium ferricyanide ($K_3[Fe(CN)_6]$), ethanol, $Na[AuCl_4]$ and bovine serum albumin were obtained from Sigma-Aldrich ((USA, http://www.sigmaaldrich.com/). All other analytes and solutions were analytical grade and used without further purification.

Saccharomyces cerevisiae yeast was grown in the microbial collection lab of Yazd University, Iran.

All of the functionalization steps involved in the assembly of the biosensor were performed using 2.5 μL of the appropriate solution deposited on the glassy carbon working electrode surface.

The 24-mer DNA sequence was designed based on the BCR/ABL fusion gene (the Philadelphia chromosome (Ph1)), an important prognostic indicator in childhood ALL, which is a minute exchange chromosome between chromosomes 9 and 22 (t (9:22)(q34;q11)). This mutation consists of a fusion between the 5′ part of the BCR gene, normally located on chromosome 22, and the 3' part of the ABL gene on chromosome 9, resulting in a BCR/ABL fusion gene [15]). Sequences were obtained from the ABCOM company (http://www. ABCOM.com).

The sequences used were as follows:

Immobilized probe HS-ssDNA:

5'-Thiol-TTT TTT AGA GTT CAA AAG CCC TTC-3'

Target ssDNA (complementary sequence):

5'-GAA GGG CTT TTG AAC TCT-3'

Non-complementary sequence:

5'-ACG TGG TCC CCA GCT CTC-3'

The stock solutions of the oligonucleotides (100.0 μM) were prepared in 0.1 M pH 7.4 phosphate buffered saline (PBS) and kept frozen at −20°C. The double distilled water and buffers were sterilized using an autoclave.

The electrochemical measurements were performed using an Autolab potentiostat/galvanostat (PGSTAT-302 N, Eco Chemie, Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) and Frequency Response Analyzer (FRA) software.

For the biosensing studies a Ag/AgCl/KCl (3.0 M) reference electrode was used. A platinum wire and a modified glassy carbon electrode (GCE) (Bio AuNP/Pol/Gr/GCE) were used as the auxiliary and working electrodes, respectively.

Another three-electrode system, consisting of a Pt counter electrode, a graphite working electrode and a Ag/AgCl/KCl (3.0 M) reference electrode, was used to synthesize the conductive polymers.

A Metrohm 691 pH/ion Meter was used for pH determination. The yeast growth was accelerated using a GFL1101 shaker.

3. Preparation of modified electrodes

3.1. Electrochemical synthesis of conductive polymer

Electrochemical synthesis of poly (catechol) was achieved using a simple one-step procedure. This is the first report of water-soluble poly (catechol) synthesized without the use of enzymes, to our knowledge.

In detail, a traditional three-electrode system was used: a Pt electrode as the counter electrode, a graphite electrode as the working electrode, and a calomel electrode was used as the reference.

The procedure for poly (catechol) synthesis was as follows: 0.026 g of catechol was added to 60 mL of 0.01 M PBS (pH 6), the reaction proceeded for ~1 h at a potential of 0.5 V until the stock

solution turned reddish-brown. The solution was then dialyzed for 24 h to remove unreacted monomers.

3.2. Preparation of graphene sheets

Graphene oxide (GO) was generated by oxidizing graphite. A combination of concentrated H_2SO_4/H_3PO_4 (360:40 mL) was mixed with graphite/$KMnO_4$ (3:18 g) at 50°C for 12 h with stirring. The reaction was cooled to room temperature and then cooled on ice. The resulting product was collected by centrifugation followed by filtration, and then washed with water, 30% HCl, and 200 mL of ethanol. Graphene was synthesized from GO using hydrazine in ammonia solution. A colloidal suspension of GO (150 mg) in distilled water (50 mL) was prepared after sonication for 3 h. The GO suspension was added to 50 µL of hydrazine solution (98%) in 200 µL of ammonia solution (30% in water). The reaction mixture was then refluxed at 90°C for 12 h. The resulting solution was centrifuged, and the sediment was washed with deionized water and then dried under vacuum at 60°C for 24 h [11, 12].

3.3. Environmentally friendly biosynthesis of gold nanoparticles

For environmentally friendly biosynthesis of gold nanoparticles using *Saccharomyces cerevisiae* yeast, the strains were grown in Yeast Nitrogen Base Agar with Glucose (YNBG) broth liquid medium at 27°C with shaking at 80 rpm for 72 h. The *Saccharomyces cerevisiae* biomass was then separated from the YNBG broth by centrifuging (5000 rpm, for 10 min). 0.6 mM $HAuCl_4$ solution was added to the supernatant (0.15 g) and the mixture was placed in a shaker at 27°C (80 rpm) for 48 h. To isolate the gold nanoparticles, the aqueous solution was centrifuged (10,000 rpm, for 10 min) [12, 17-21].

3.4. Immobilization and hybridization of DNA

The electrode was modified as follows: first, the GCE was cleaned by polishing with 0.30 μm alumina powder for 2 min, followed by sonicating in ethanol solution for 5 min. 2.5 μL of 0.001 M graphene sheet suspension (Gr) was then dropped onto the surface of the GCE. 2.5 μL of 0.01 g/mL poly (catechol) (Pol) solution (pH 7.4) was then added to the Gr/GCE and allowed to dry at room temperature to give Pol/Gr/GCE. Then 2.5 μL of 0.004 M biosynthesized gold nanoparticles was dropped onto the surface of the Pol/Gr/GCE. 2.5 μL of 6 μM 5′-SH probe DNA was then added to Bio AuNP/Pol/Gr/GCE. This assembly method takes advantage of the strong affinity of thiol-modified DNA for Au nanoparticles, which can increase the DNA immobilization.

The ssDNA/Bio AuNP/Pol/Gr/GCE assembly was rinsed with Tris-EDTA buffer solution to remove the non-specifically bound probe DNA. 2.5 μL of 1 mM MCH solution was then dropped onto the surface of the modified electrode for an optimized period of 1 h to avoid nonspecific adsorption of oligonucleotides. In the last step, to achieve hybridization, 6 μL of target DNA solution was incubated on the ssDNA/Bio AuNP/Pol/Gr/GCE assembly, and the reaction was allowed to proceed for an optimum hybridization time. The electrode was then rinsed with Tris-EDTA buffer solution to remove unhybridized target DNA (Schematic 1).

3.5. Electrochemical measurements

To study the assembly of the DNA biosensor, four different electrochemical methods (DPV, EIS, CV and chronoamperometry) were used.

EIS measurements were recorded at a bias potential of 200 mV with an alternating voltage of 10 mV at frequencies ranging from 0.1 to 105.0 Hz.

DPV analyses were recorded in the potential range from −0.5 to −0.1 V at modulation amplitude of 70 mV and potential step of 5 mV.

Chronoamperometric measurements were carried out by setting the working electrode potential at 500 mV.

4 Results and discussion

4.1. Scanning electron microscopy (SEM) and NMR, FT-IR and UV-Visible spectroscopy characterization.

The morphology of the graphene sheets and biosynthesized gold nanoparticles was characterized by SEM. Representative SEM images of graphene sheets and biosynthesized gold nanoparticles are shown in Fig. 1 and 2, respectively. The morphology of the graphene sheets was thin with a wrinkled, paper-like appearance. The biosynthesized gold nanoparticles had uniform mono-disperse spherical structures [10, 17].

FT-IR spectroscopy of poly (catechol), Fig. 3, shows a series of peaks at 3437, 3000, 1500, 1210 and 1120 cm^{-1}, which belong to the Ar-OH-H bond, Ar-H stretch, aromatic C-C stretch, overlapping peaks of asymmetric vibration of C–O–C linkage and C–OH vibrations, respectively [35]. NMR spectroscopy of poly (catechol), confirmed the FT-IR spectroscopy results (Fig. 4).

UV-Visible spectroscopy of biosynthesized gold nanoparticles, showed a peak at 550 nm, which is due to electron transitions between gold nanoparticles.

UV-Visible spectroscopy of graphene sheets, showed a peak at 260 nm. The absorption peak at 260 nm indicates the presence of a π-plasmon from the graphitic structure and the absorption peak at 550 nm indicates the formation of biosynthesized gold nanoparticles (Fig. 5).

4.2. Optimization of experimental parameters

To investigate the preparation of the DNA biosensor, parameters such as DNA probe concentration, optimum incubation time of the DNA probe and hybridization time, were monitored.

In this work, four electrochemical methods (DPV, EIS, CV and chronoamperometry) were applied to a redox probe solution to determine the features of the DNA biosensor. A comparison of the oxidation peak current of Bio AuNP/Pol/Gr/GCEs for different probe solutions, suggested that 2.0 mM catechol in PBS, was the best of the probe solutions tested. The results indicated a considerable increase in the oxidation peak current with a shift to more negative values in the oxidation peak potential for the catechol in PBS, in comparison with the other probe solutions; Fig. S1.

The concentration of the probe DNA strand, has a significant effect on the sensitivity of the DNA biosensor signals. Different concentrations of probe DNA strand solution (10^{-11} – 10^{-7} M) were therefore added to the surface of the Bio AuNP/Pol/Gr/GCE assembly to optimize the probe concentration. The experimental results showed that the optimum DNA probe concentration was 10^{-9} M (Fig. S2.). Because of the saturation of the active sites, there was no considerable signal improvement in concentrations above 10^{-9} M.

4.3. Electrochemical characterization and hybridization detection

To investigate the electrode modification, immobilization and hybridization in each step, the redox reaction was probed using four different electrochemical methods (DPV, EIS, CV and chronoamperometry).

The CV investigations were carried out in 2.0 mM catechol solution, with a scan rate of 100 mV/s.Compared with the bare GCE, modification with 2.5 μL of 0.01 g/mL conductive polymer caused a clear increase in the oxidation peak currents due to the high electrical conductivity of

poly (catechol). This unique property of the synthesized conductive polymer, has the potential to significantly improve biosensor sensitivity and selectivity. In the subsequent step, after surface modification with 2.5 μL of 0.001 M graphene sheet, the oxidation peak current was further enhanced, indicating further potential improvements in biosensor sensitivity, owing to the electrical properties of the graphene sheets.

After modification with biosynthesized gold nanoparticles to give Bio AuNP/Pol/Gr/GCE, the oxidation peak current increased further. In addition a slight shift of the potential peak towards less positive values was observed, which is due to the high surface area to volume ratio of the gold nanoparticles (Fig .6).

The Randles–Sevcik equation describes the effect of scan rate on the peak current, I_p. For simple redox events such as the ferrocene/ferrocenium couple, the active surface area of the modified electrode can be estimated based on the following equation [20, 21].

$$I_{peak} = 2.69\times10^{5} n^{3/2} A D^{1/2} C_{bulk} \nu^{1/2} \quad (1)$$

where I_{peak} is the anodic peak current, A is the surface area of the electrode, n electron transfer number, D is the diffusion coefficient, C_{bulk} is the concentration of $[Fe(CN)_6]^{3-/4-}$ and ν is the scan rate. For 1.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ in 1.0 mol L^{-1} KCl electrolyte the slope of the plot of I_{peak} versus $\nu^{1/2}$, gave a microscopic area of 1.67 cm^2 for the Bio AuNP/Gr/Pol/GCE (Fig. S3).

Cyclic voltammetry studies of DNA immobilization and hybridization were carried out in 2.0 mM catechol solution. The results showed that after immobilization of the probe DNA strand on the surface of the Bio AuNP/Pol/Gr/GCE assembly, the redox peak current decreased, in addition there was an obvious peak potential shift to more positive values, which is associated with the covalent coupling of the thiol-modified probe DNA with the biosynthesized nanoparticles. After hybridization between the probe DNA and target DNA, peak current decreased further, Fig. 7.

DPV investigations were carried out, to support the CV conclusions. As can be seen, after modification of the GCE with conductive polymer, the peak current increased because the poly(catechol) showed high conductivity. After Pol/Gr/GCE modification, the peak current increased again owing to the conductive nature of the Pol/Gr composite. In addition there was a slight peak potential shift to more positive values, which we believe is due to the non-homogeneous distribution of graphene layers at the electrode surface. After Bio AuNP/Pol/Gr/GCE modification, the peak current was at a maximum and the peak potential was shifted to less positive values, because of the conductive nature of the Bio AuNP/Pol/Gr/GCE composite.

Immobilizing the probe (ssDNA) on the surface of the Bio AuNP/Pol/Gr/GCE assembly, caused the peak current to decrease. In addition the peak potential separations (ΔE_p) increased significantly owing to the formation of insulating layers of DNA blocking the electrode surface, which considerably affects the interfacial electron transfer and prevents the electrocatalytic oxidation of catechol. On the one hand, the introduction of complementary DNA increases the blocking layers, increasing repulsion of redox species. On the other hand, it has been shown that DNA immobilization and hybridization could have an effect on the current and peak potential separations of the fabricated electrode surface. (Fig. S4).

EIS is a perturbative characterization of the dynamics of an electrochemical process; the linear part describes the diffusion-limited process and the semicircle portion is associated with the electron transfer-limited process.

Fig. 8. shows EIS investigations of electrode modification, immobilization and hybridization in each step (the $[Fe(CN)_6]^{3-/4-}$ couple was applied as a probe solution).

As can be seen, after dropping Pol on the GCE surface, the R_{ct} slightly increased, which indicates the successful formation of a polymer film, which makes an electron transfer resistant at the GCE surface. When the GCE electrode surface was modified with Pol/Gr, R_{ct} decreased owing to the excellent electrical conductivity of the Pol/Gr composite. When the GCE electrode surface was modified with Bio AuNP/Pol/Gr, R_{ct} decreased again, due to the elevated electrical conductivity of the modified electrode.

Subsequently, when the thiol-modified ssDNA was covalently bonded to the modified electrode, R_{ct} increased, due to the resistance to electrical current, which confirms the immobilization of the probe DNA. The EIS results confirmed the CV and DPV findings.

Chronoamperometry through electrochemical methods, is a powerful method for quantitative analysis, and allows reproducible signals owing to the modification with Pol, Pol/ Gr/, Bio AuNP/ Pol/Gr, ssDNA and target DNA strand to be registered. The chronoamperometry results, confirmed the CV, DPV and EIS findings. (Fig. S5).

CV investigations were used to optimize the immobilization and hybridization times. As can be seen, the optimum times for DNA immobilization and hybridization were 1 and 2 h, respectively. (Fig. S6).

4.4. Repeatability, reproducibility and storage stability study

The repeatability was studied using DPV analysis in 2.0 mM catechol solution (pH 7.4). The hybridization was carried out with three parallel ssDNA/Bio AuNP/Pol/Gr/GCE assemblies which were made individually on 3 different days, before hybridization with the target DNA [22-25]. The relative standard deviation, which provided an indication of the variability, showed an acceptable result of 0.009 (Fig. S7).

The reproducibility of the biosensors was studied by CV analysis. No considerable changes in I_{peak} values were observed for 5 CV cycles.

To study the stability to storage of the biosensor, three modified electrodes were kept in 0.1 M Tris-EDTA buffer solution (pH 7.4) and stored at 4°C for 7 days. The results of tests carried out after storage were very similar to the original.

4.5. Evaluating the selectivity of the DNA biosensor

To investigate the selectivity of the biosensor, complementary target DNA sequences and a random DNA sequence (non-complementary DNA sequence) that contained almost no similar pairs of bases were tested; in addition to testing a target DNA sequence with four mismatches. The ssDNA/Bio AuNP/Pol/Gr/GCE biosensor was subjected to the same hybridization reaction. The results showed that the hybridization step for non-complementary DNA sequences and the four mismatched DNA strands resulted in no significant decrease in peak current, which suggests that no hybridization reaction took place. The small decrease of peak current that was observed could be due to non-specific adsorption (Fig. S8) [26].

4.6. Sensitivity of the electrochemical DNA biosensor

The sensitivity of the DNA sensor was established by hybridizing various concentrations of the complementary DNA strand with the probe DNA strand. The results show that the DPV signals of 2 mM catechol solution (pH 7.4) on the DNA biosensors decreased with increasing probe DNA strand concentration, for each DNA biosensor [27-30].

It could be seen that, the peak current of 2.0 mM catechol on the DNA sensor is linearly related to the logarithm of the concentrations of target DNA in the range 100.0 µM – 10.0 pM, with detection limits of 1.0 pM for the DNA strand (Fig. S9).

4.7. Detection of PCR-amplified clinical samples

The DNA sequence was extracted from 500 μL blood samples using a DNA extraction kit. Polymerase chain reaction (PCR) amplifications of the BCR/ABL fusion gene, were performed with the aid of one pair of primers (forward primer: TTCAGAAGCTTCTCCCTGACAT; nucleotide position: 196-217 and Reverse primer: TGTTGACTGGCGTGATGTAGTTGCTTGG; nucleotide position: 655-628; with a product (amplicon) length of 460 bp). The amplification of 600 fragments of bases pairs of the BCR/ABL fusion gene was carried out using these primers. To do this, 0.5 μL of each primer (10 pmol), 0.8 μL of $MgCl_2$ (50 mM), 0.5 μL of dNTP (10 mM), 2.5 μL of 10X PCR and 1.5 Units of Taq DNA polymerase gave a total volume of 25 μL, containing 100 ng total DNA as a template (and the thermal cycle program was 94°C for 1 min, 94°C for 1 min 64°C for 1 min, 72°C for 1 min and 72°C for 10 min).

To investigate the hybridization reaction with the PCR amplified real-life samples of ALL, the samples were denatured by heating at 95°C for 7 min and then cooled down in an ice bath for 3 min. Subsequently, 1 μL of the target DNA real-life sample (which was extracted from human blood) was diluted with 9 μL of hybridization buffer [31, 32]. 2.5 μL of the described real-life sample was then dropped onto the surface of the ssDNA /Bio AuNP/Pol/Gr/GCE assembly. The hybridization reaction was allowed to proceed for 2 h, and then the biosensor was washed with Tris-EDTA buffer solution. DPV analysis (in 2.0 mM of catechol solution) was used to study the hybridization process. The results showed that there is a decrease in the current signal after hybridization of the probe DNA with the complementary PCR amplified real-life sample. This suggests that hybridization took place between the ssDNA and the complementary real-life sample DNA. No hybridization took place between the ssDNA and the noncomplementary real-

life DNA sample, and the small decrease in the peak current could be due to non-specific adsorption (Fig. S10).

5. Conclusion

Electrochemical biosensors have many advantages, such as facile use, specificity, cost effectiveness and the ability to perform continuous monitoring; yet their standout quality is the potential for rapid results. These features make electrochemical biosensors helpful tools in DNA mutation and cancer marker detection in clinical samples such as blood or urine. Early diagnosis of cancer can remarkably increase the chance of successful treatment.

In the current work, a DNA electrochemical biosensor for the detection of acute lymphoblastic leukemia was demonstrated, based on a stable, uniform composite, consisting of electrosynthesized poly(catechol), graphene sheets and biosynthesized gold nanoparticles. Conductive polymers offer high electrical conductivity and mechanical strength. In this work the conductivity of poly(catechol) was enhanced through modification with graphene sheets and biosynthesized gold nanoparticles.

The peak current of the catechol (as an electroactive probe) is linearly related to the logarithm of the concentration of the target DNA in the range 100.0 μM–10.0 pM, with detection limits of 1.0 pM for the DNA strand. This level of sensitivity compares favorably with reported values (Table 1) [33–40].

Sensitivity, selectivity, reproducibility and real sample detection were tested using DPV, EIS, CV and chronoamperometry methods, showing positive results.

It is anticipated that this system has potential applications in the simple, rapid and quantitative detection of many kinds of cancers.

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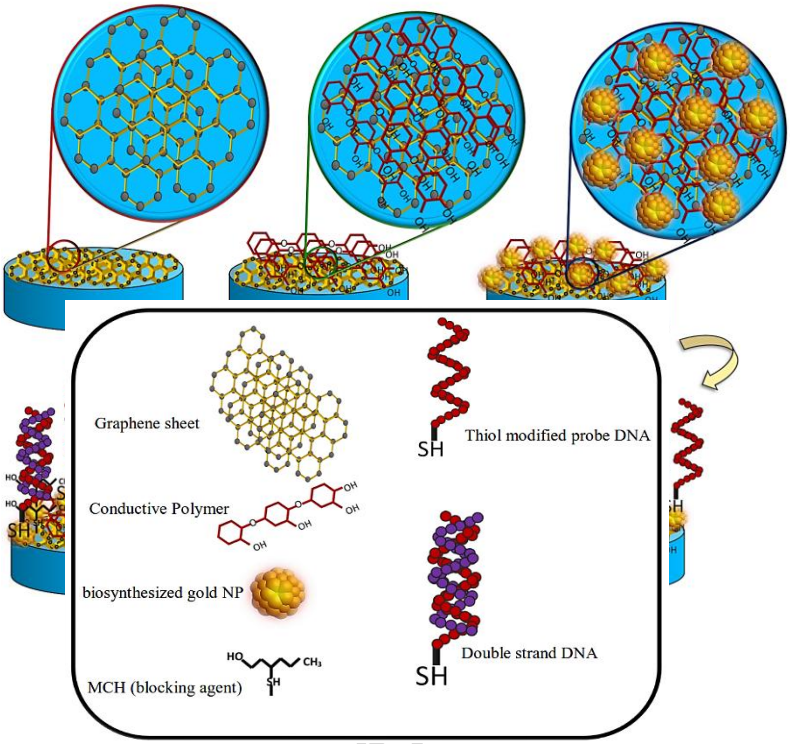
Table.1. Comparison of analytical parameters of the DNA biosensors for the Leukemia cancer detection.

Sensor platform	Detection technique	Detection time	Detection limit (M)	Reference
Gold surface/DNA probe (thiolatedhairpin locked nucleic acids)	CV, EIS and DPV	60 min	1.20×10^{-10}	33
Gold surface/AuNPs/DNA probe (thiolated hairpin locked nucleic acids)	CV, EIS and DPV	60 min	1×10^{-10}	34
Glassy carbon surface/aminated DNA probe	DPV	35 min	5.90×10^{-8}	35
Glassy carbon surface/aminated DNA probe	CV and DPV	30 min	6.70×10^{-9}	36
Glassy carbon surface/aminated DNA probe	CV and DPV	30 min	$6.70 \times 10_{-9}$	37
Gold surface/thiolated DNA probe	SPRi (Surface plasmon resonance imaging)	20 min	1.03×10^{-8}	38
Cadmium-telluride quantum dots functionalized with carboxylic groups/aminated DNA probe	FRET (Fluorescence resonance energy transfer)	20 min	1.50×10^{-10}	39
Conducting polymers (poly (cathechol), graphene sheets and	CV, EIS, Chronoamperometry and DPV	120 min	1.02×10^{-12}	Current work

gold nanoparticles



Schematic 1

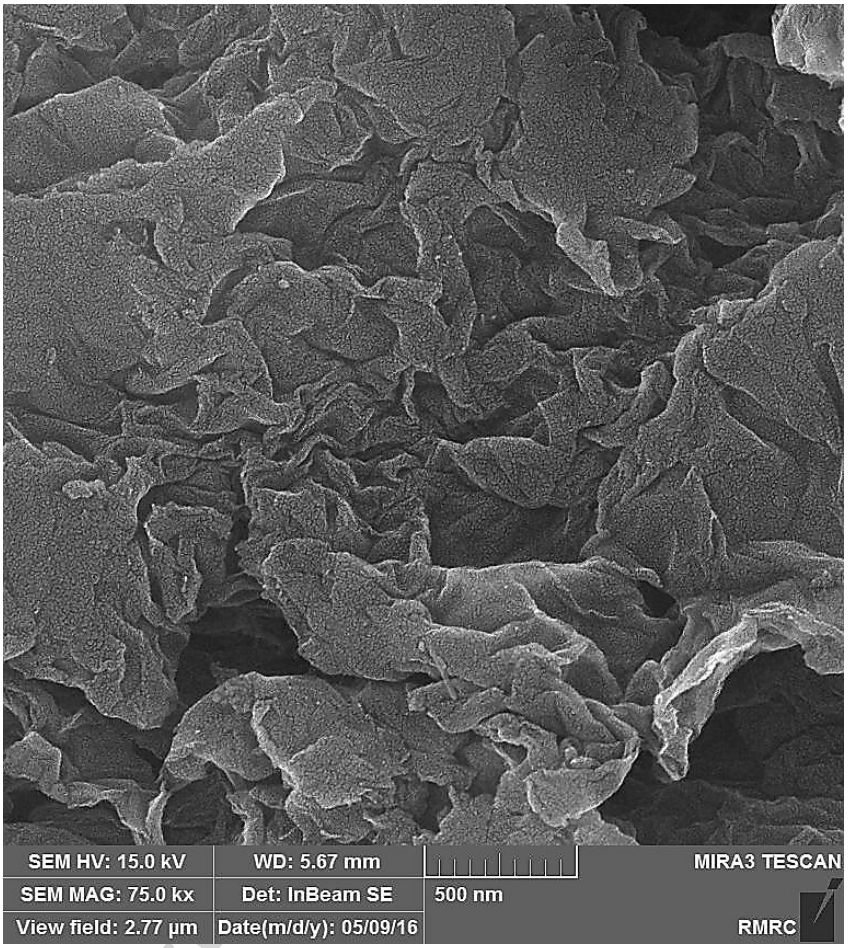


Fig.1. A SEM micrograph of the grapheme sheet

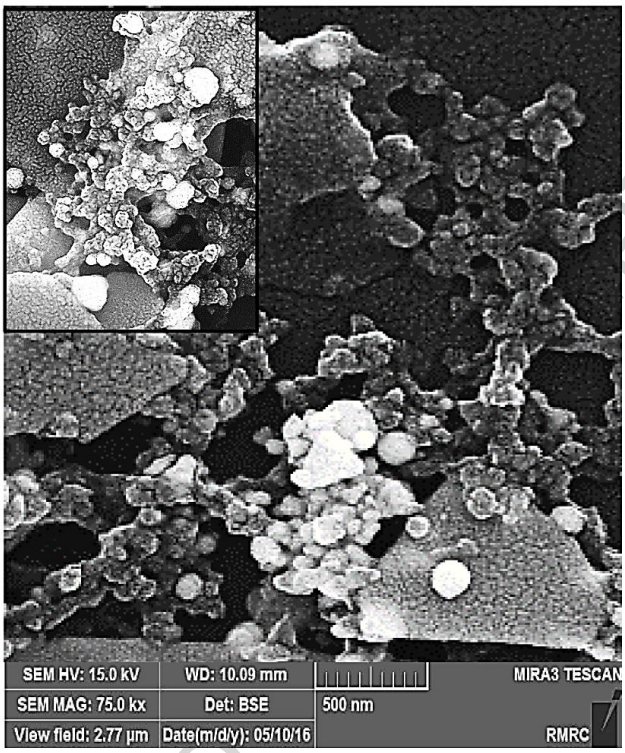


Fig.2. A SEM micrograph of the biosynthesized gold nanoparticles

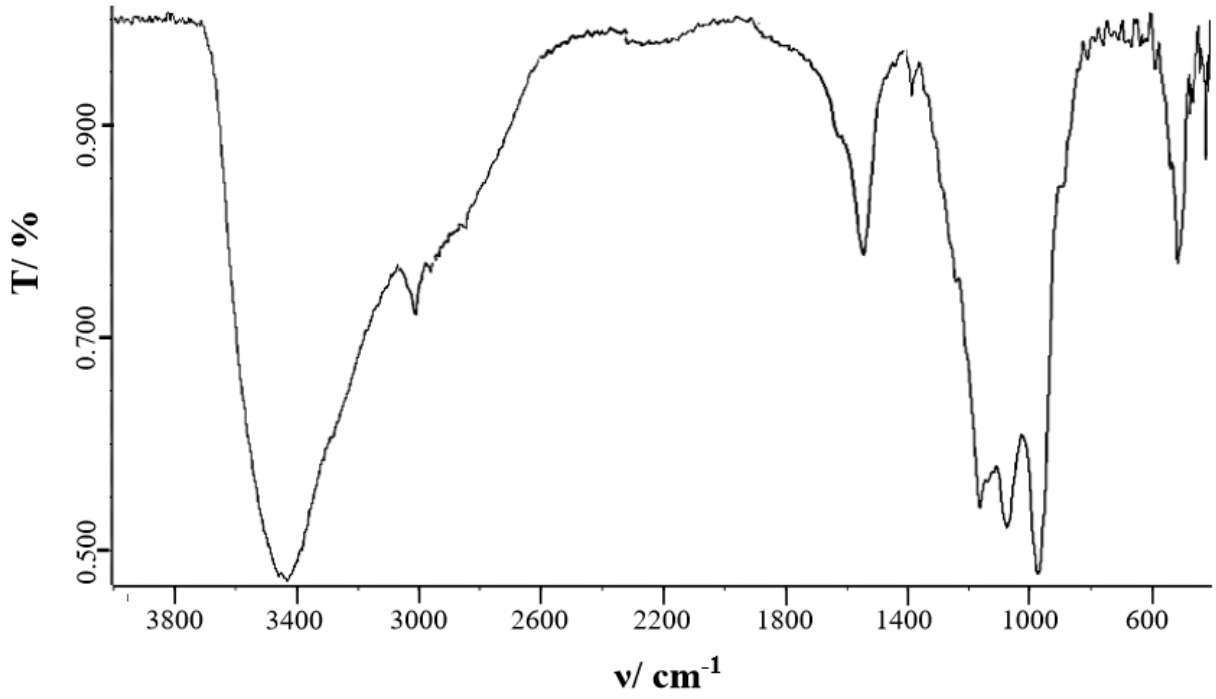


Fig.3. FT-IR spectroscopy of poly (catechol)

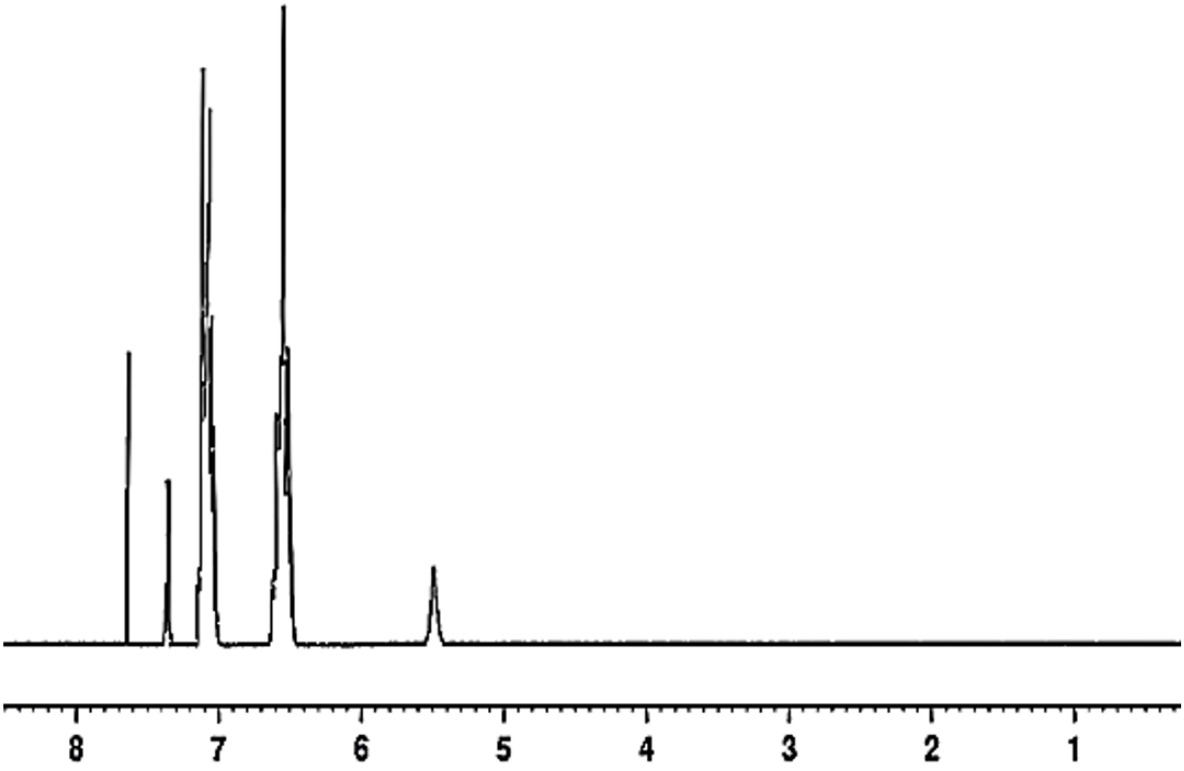


Fig.4. NMR spectroscopy of poly (catechol)

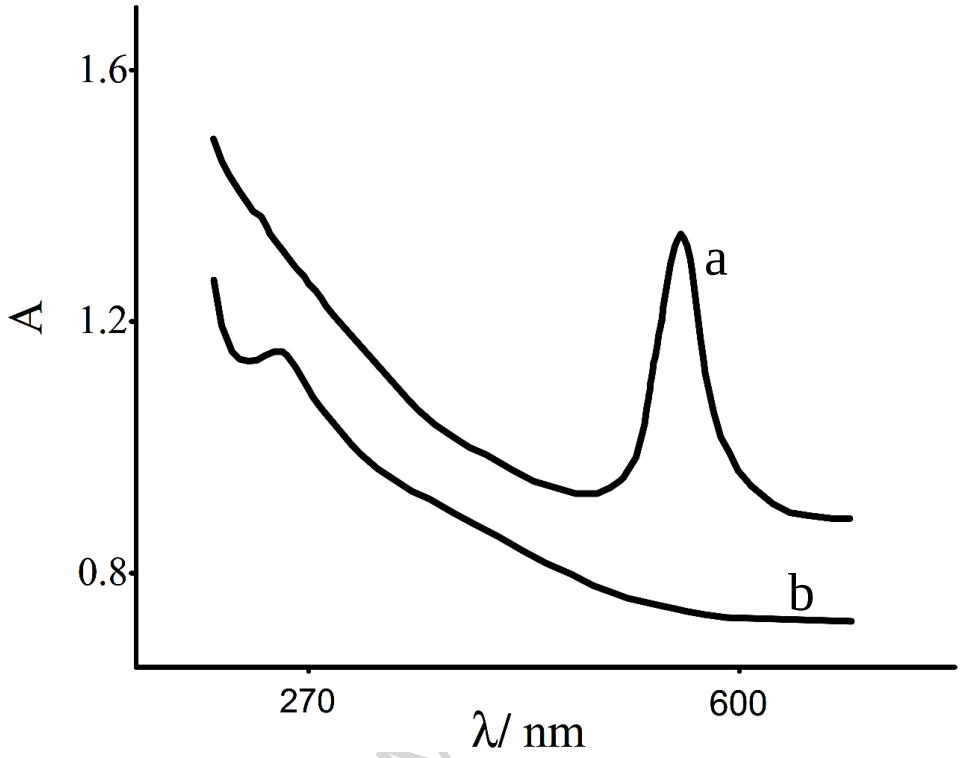


Fig.5. UV-Visible spectroscopy of biosynthesized gold nanoparticles (a) and graphene sheets (b)

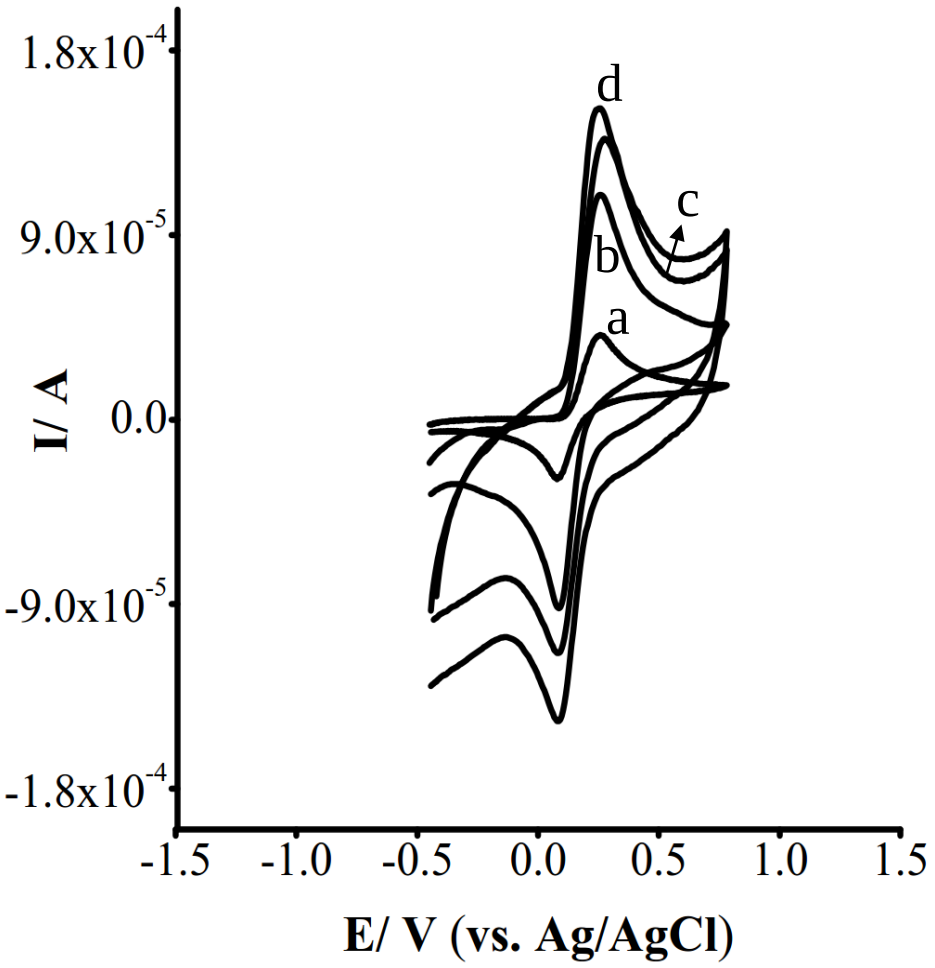


Fig.6. Detecting of the GCE (a), Pol/GCE (b), Pol/Gr/GCE (c) and Bio AuNP/Pol/Gr/GCE (d) in 2 mM of catechol in PBS.

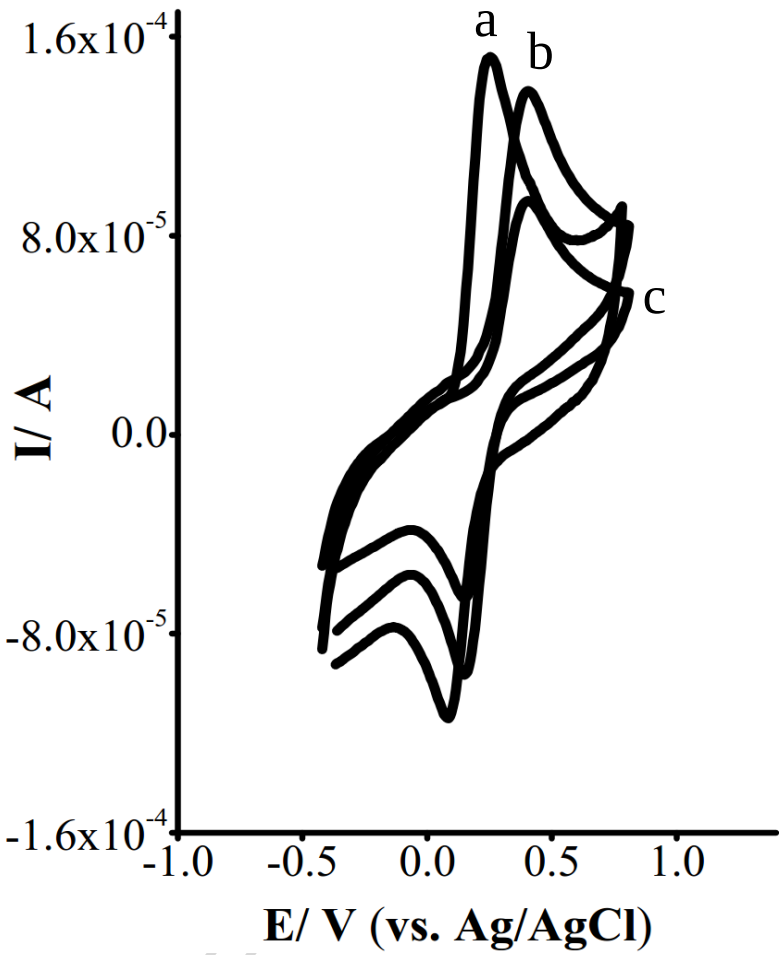


Fig.7. Detecting of the immobilization and hybridization process at Bio AuNP/Pol/Gr/GCE (a) and ss DNA/ Bio AuNP/Pol/Gr/GCE (b), respectively, in 2 mM of catechol in PBS, the (c) is ds DNA/ Bio AuNP/Pol/Gr /GCE

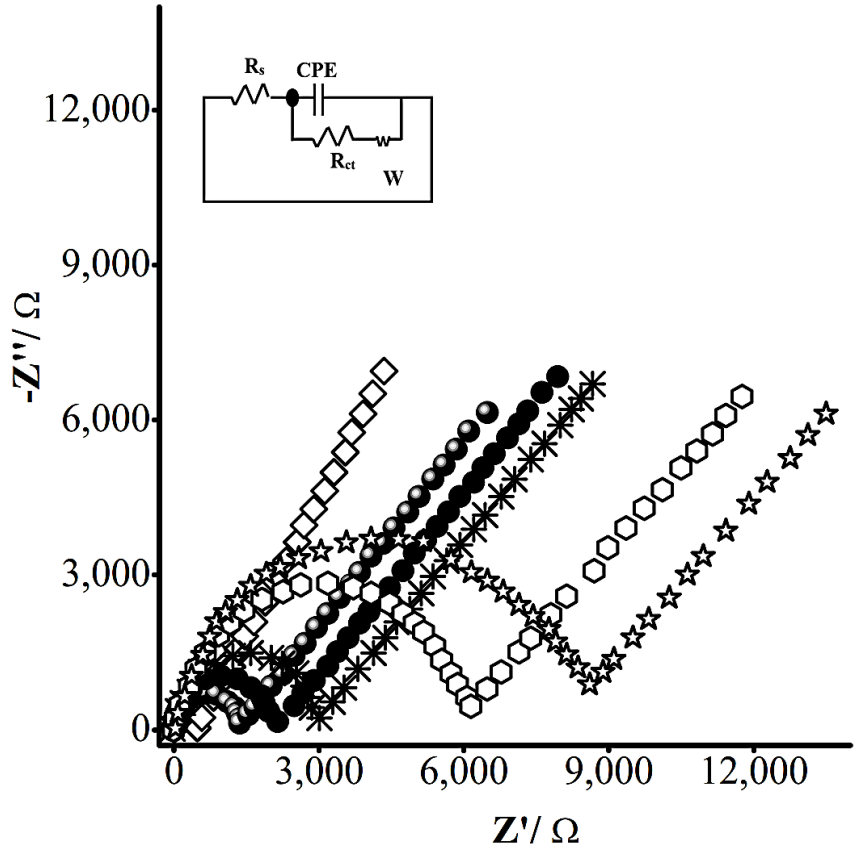
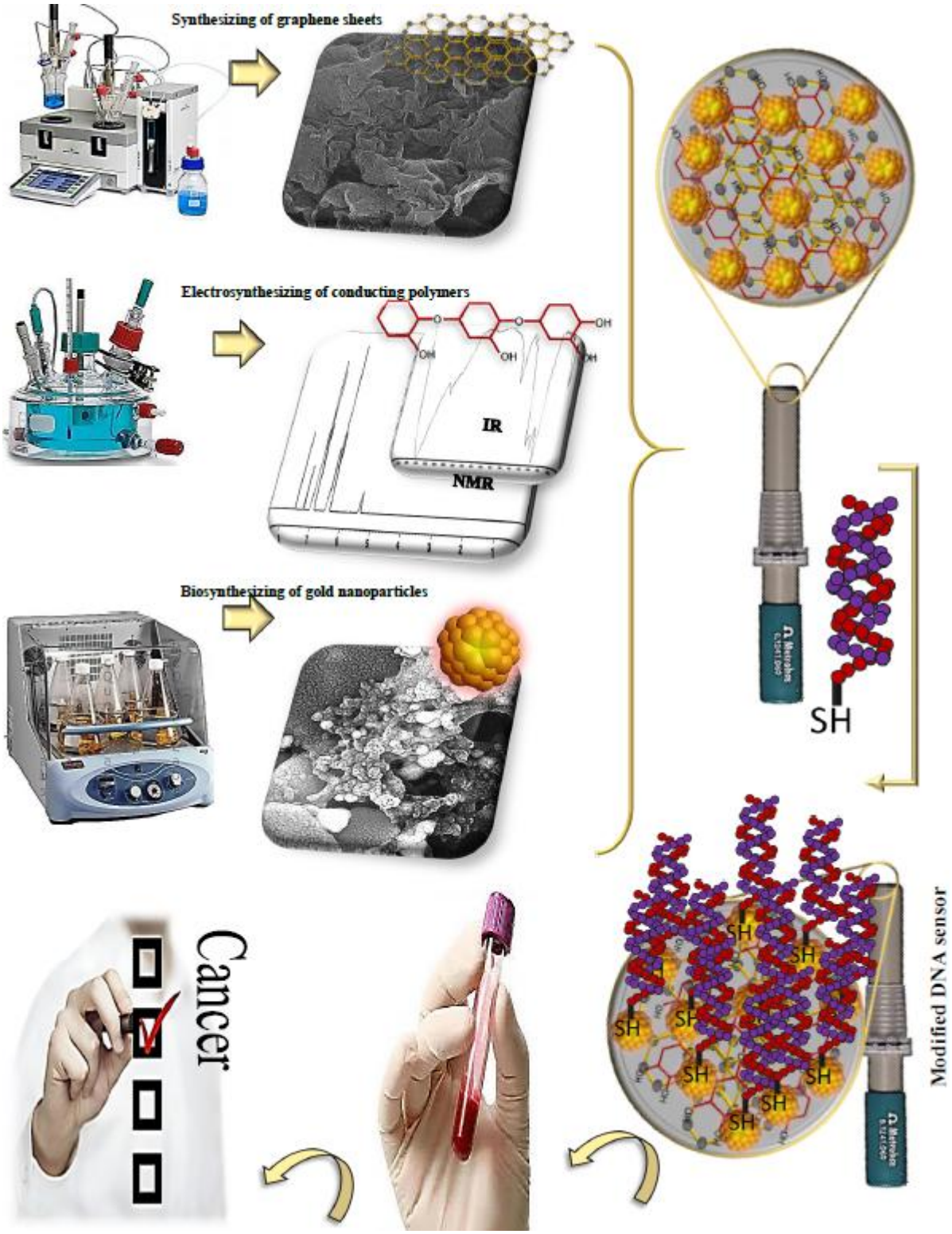


Fig.8. Nyquist plots of GCE (a), Pol/GCE (b), Pol/Gr/GCE (c), Bio AuNP/Pol/Gr/GCE (d), ssDNA/ Bio AuNP/Pol/Gr/GCE (e) and dsDNA/ Bio AuNP/Pol/Gr/GCE (f) in 1 mM of $[Fe(CN)_6]^{3-/4-}$ in PBS. Inset: Equivalent circuit for fitting the plots.

Graphical abstract



Highlights

- Bio AuNP/ Gr/ Pol as composite was employed for attachment of ss DNA onto the GCE.
- Cathechol was used as the redox probe.
- The DNA sensor showed high selectivity for the detection of ALL cancer.