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# Fabrication of ultra sensitive Nano biosensors based semi conductive Nano wire integrated Nano electrodes and application for ultrasensitive detection of proteins and individual virus particles

Hamida MB Darwish
Department of Physics, Faculty of Sciences, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia

**Abstract:** The main objective of this project is to develop a biosensor based on freezing elements receptors on a Nano wire containing electrode to detect Nano-virus. Viruses are very small micro-organisms that could lead to damage and disease between simple to very serious disease and be the cause affecting the health of humans, animals and even plants. This may include, for example flu or cold that affects life and more life-threatening humans, for example, HIV / AIDS. The virus particles are a hundred times smaller than a single cell of bacteria. A bacterial cell alone is much smaller at about ten times that of a human cell that is ten times smaller than the diameter of a human hair. It is gravity that viruses may also infect bacterial cells and controls the immune organs by knowing the private life of the bacterial cell and then grow and multiply and then infected cell produces viral particles rather than perform its normal function. Thus, the viruses are among the most important causes of serious diseases that infect humans and there is a growing interest detects viruses and selective disclosure of sensitive sensors work for viruses. This is essential for the implementation of an effective response to a viral infection, such as medication or through quarantine. There are some ways set up to analyze the viral assays include immune assays panel.

**Keywords:** Biosensor, HIV, AIDS, Nano wire containing electrode, Nano-Virus

## 1. Introduction

Viruses are among the most important causes of human disease and an increasing concern as agents for biological contamination. Rapid, selective, and sensitive detection of viruses is central to implementing an effective response to viral infection, such as through medication or quarantine. There are some established methods for viral analysis includes plaque assays, immunological assays, transmission electron microscopy, and PCR-based testing of viral nucleic acids. These methods have not achieved rapid detection at a single virus level and often require a relatively high level of sample manipulation that is inconvenient for infectious materials. Yet, the ability to detect rapidly, directly, and selectively individual virus particles has the potential to significantly impact health care, since it could enable diagnosis at the earliest stages of replication within a host's system.

One promising approach for the direct electrical detection of biological macromolecules uses semiconducting Nano wires or carbon Nano tubes configured as field-effect transistors, which change conductance upon binding of charged macromolecules to receptors linked to the device surfaces. These Nano scale devices, stochastic sensing offers important scientific advantages, including the following: selective detection is not limited by the affinity of the receptor as in previous equilibrium measurements; analysis of single particle on off times provides direct information about binding kinetics crucial to understanding, for example, virus–receptor interactions and single particle sensitivity could enable simple charge-based detection of macromolecules.

The main goal of this project is developing of a biosensor based on immobilization of receptor elements on conducting Nano wire containing Nano electrode for detection of viruses. Nano electrodes will be prepared by a special lithographic technique and it will modify with different types of Nano wire. Electrode will be then modified with receptor proteins which has specific for binding of specific virus. By this method viruses will be strongly bound on to Nano electrode which will allow us to identify samples whether viruses found or not. Also this will give us a chance early diagnosis of certain disease in order to prevent the damaged which can causes by the virus.

## 2. Background of Statement of the Problem:

The interface between Nano-systems and bio-systems is emerging as one of the broadest and most dynamic areas of science and technology, bringing together physics, biology, chemistry, biotechnology, medicine, and many areas of engineering. The combination of these diverse areas of research promises to yield

revolutionary advances in healthcare, medicine, and the life sciences through the creation of new and powerful tools that enable direct, sensitive, and rapid analysis of biological and chemical species. Devices based on Nano wires have emerged as one of the most powerful and general platforms for ultrasensitive, direct electrical detection of biological and chemical species and for building functional interfaces to biological systems, including neurons

Various biosensors based on new materials have been developed that improve the resolution of detection, cost efficiency, and portability for diagnosis of diseases [1]

To achieve label free and highly sensitive detection, Nano materials such as Nano wires, nanoparticles, and carbon Nano-tubes are very appealing due to their fast transport of electrons in one-dimensional structure [2]. In this regard, Nano wire-based biosensors have been widely investigated because of the advantages of Nano-wires such as high sensitivity as a result of the high surface to volume ratio, low power consumption, and the potential for miniaturization of application devices [3]. To date, Nano-wire-based sensors have demonstrated good reproducibility, portability, and label-free detection [4]. In order to utilize Nano wires for biosensors, functionalization of the Nano wire is one of the essential steps in satisfying requirements such as high resolution and good specificity of target detection. Several biomoleculars functionalization methods have been developed including entrapment of bio-receptors, conjugation of biotin–streptavidin, and surface immobilization of monoclonal antibodies (MABS) [5]. Among these functionalization methods, the surface immobilization method is easy and efficient as many biomoleculars can be covalently coupled on the surface area of Nano-wires. Furthermore, many Nano-biosensors have been developed by employing inorganic Nano-wires or carbon Nano-tubes through the chemical bonding of bio-receptors to their surfaces [6]. However, surface immobilization of inorganic Nano-wire often requires multiple steps of chemical reaction due to its low reactivity to biomolecules [7]

In this regard, organic Nano-wires, such as polyaniline (PANI) and Polypyrrole (PPy), are very promising candidate materials for biosensor applications because they can be functionalized relatively easily because of the tunability of the amine group to provide the functional group to biomolecules [8].

## 3. Research Objectives:

**Biosensors and Conductive Polymers**

The development of biosensors requires the achievement of an efficient interface between the biomolecules and the electronic transducers. Conducting polymer interfaces are particularly suitable for localizing biomolecules onto surfaces. The poly-conjugated conducting polymers were recently proposed for bio-sensing applications because of number of favorable characteristics such as direct and easy deposition on the electrode surface, control of thickness, and redox conductivity of and polyelectrolyte characteristics of polymer useful for sensor application. Polypyrrole fulfills the above requirements together with having the characteristics of easy oxidation, high chemical stability, and low cost of monomer.

Conducting polymers such as PANI and PPy have been fabricated into thin films, powders, nanoparticles, or Nano-wires and utilized for chemical or biosensors taking advantage of their wide range of conductivity, ease of synthesis, environmental stability, and biocompatibility [9]. Although various biosensors based on conducting polymer Nano wires have been developed, many of them only use bundled Nano-wires or Nano-wire arrays, which limit the development of a single Nano wire application [10]. In this regard, the promising direction is in the use of a single Nano wire or limited number from selected Nano wires in order to achieve high sensitivity and constant performance of biosensors. However, due to the difficulty of handling a single Nano wire, only a few biosensors based on a single Nano wire have been developed[11], and they exhibited poor reproducibility as the single Nano wire in those biosensors is randomly positioned[12] (see Fig,(1) [13&14].

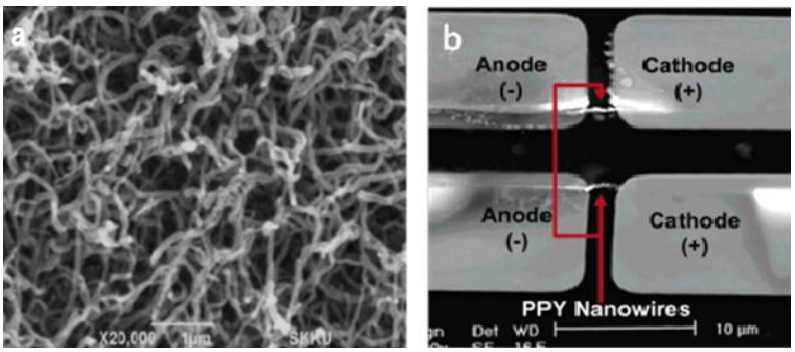



**Figure 1.** SEM image of a) ppy Nano wire[13] & b) and PPY Nano wires deposited on the electrode[14]

### 3.1 Carbon Nano tube in Biosensors

In applications of highly sensitive biosensors, carbon Nano tubes (CNTs) are recommended because of their high surface-to-volume ratio, excellent electrical conductivity, and mechanical strength[15]. Conversely, biosensors with a flexible substrate, which makes the biosensor more portable and biocompatible, have considerable potential in various applications[16].

One-dimensional (1-D) Nanostructure based field-effect transistor (FET)/chemiresistor, with carbon nanotubes, conducting polymer nanowires, metal oxide, etc. is rapidly gaining recognition as a powerful transducer in labelfree monitoring of antigen and antibody binding [17]. Besides labelfree detection, 1-D nano-FET/chemiresistor advantages include, extremely high sensitivity (potentially down to single molecule), easy of miniaturization, low power requirement and development of high density arrays that will allow simultaneous analysis of a range of different species in extremely small sample volume and reduce false negatives/positives due to massive redundancy.

The resistance/conductance of these devices is extremely sensitive to any surface adsorption /perturbation and is a function of the analyte charge. Because of this charge dependence of the sensor sensitivity, successful demonstrations of 1-D nanostructure-based FET immunosensor have been limited to targets with large charges such as proteins, DNA, RNA, viruses, spores and cells.

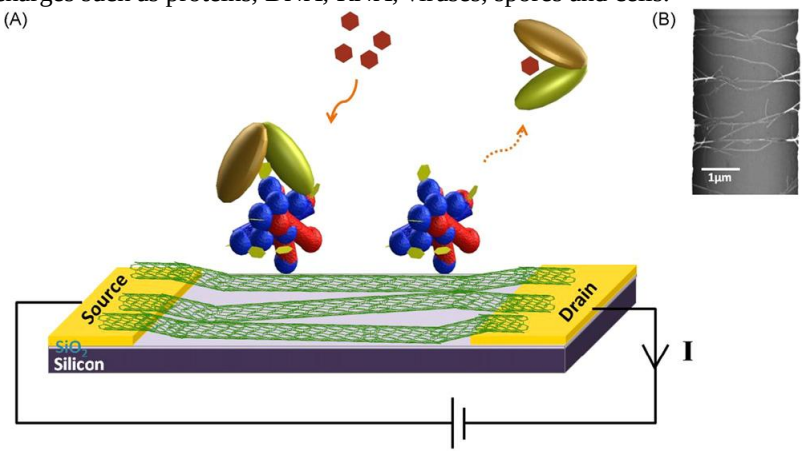



**Figure 2.** (A) Schematic diagram of the SWNT immuno-sensor to detect TNT. Anti-TNP SCAb is leaving from the sensor platform due to displacement by TNT. (B) SEM image of aligned SWNTs between two gold electrodes[18].

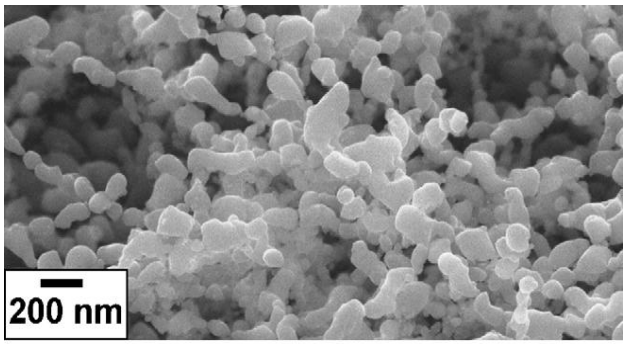
### 3.2 Metal Oxide nanoparticles

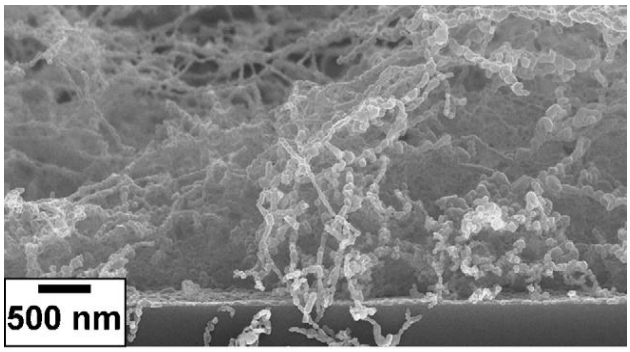
Metal-oxides possess a broad range of electronic, chemical, optical and physical properties that are often stable to vary with the composition of the surrounding gas atmosphere [19]. Because of this advantage, metal oxides like $SnO_2$, $TiO_2$, $WO_3$, ZnO, $Fe_2O_3$ and $In_2O_3$ for gas detection have been investigated for more than five decades and metal oxides gas sensors have been commercially available for more than 30 years. However, traditional sensors based on pristine or doped metal-oxides configured as single crystals, thin and thick films, ceramics, and powders through a variety of detection and transduction principles, typically have to operate at an elevated temperature, which results in large power consumption [20]. The sensitivity and energy consumption of metal-oxide based sensors can be significantly improved by reducing the crystallite size. When the diameter of Nano-wires is close to or less than double thickness of the space-charge layer and, the sensing performances such as sensitivity, response speed, will be increased remarkably. These discoveries likely explain the exploration of the metal-oxide Nano-wires as a platform for chemical sensing is a recently hot [19]. In comparison with their traditional thin- and thick-film counterpart, one-dimensional metal-oxide Nano-wires have two advantages: (i) reduction of their operating temperature and power consumption, and (ii) the possibility of single Nano-wire field-effect transistors (FET) and resistor configuration that allows that could be easily integrated with microelectronics technologies for Nano-scale electronics especially for Nano-sensing technology [19] Trials to form composites of metal oxide Nanoparticles and carbon Nano-tubes with conducting polymer are largely focused for sensing researches [21,22].

Thin films of multi-walled carbon Nano-tubes (MWNTs) and tin oxide composites have been reported to show high response to ethanol or ammonia at either room or moderate temperatures[23] However, while the composites were fabricated by electron beam evaporation of a mixture of CNTs and metal oxide powders, or via spin-coating of a suspension of mixed MWNTs and metal nanoparticles [24], they revealed inherently compact

thin films, thereby showing limited sensitivities. Another common route to synthesize composites of carbon Nano-tubes and metal oxide has been the wet chemical[25].

It requires processing steps including synthesis of CNTs, collection, dispersion, functionalization, mixing with metal oxide solution, filtration, and oxidation, among many others.




## 4. Research Design:

In this project, conducting polymer Nano-wires will be synthesis with two different methods; chemical synthesis and electrochemical synthesis. SWCNT will purchase and it will modify with conducting polymers. Metal oxide (ZnO and TiO) and Au Nanoparticles will be synthesize as describe in the literature. Composite Nano-wires of Nanoparticles with Ppy and PTh will be synthesize chemical methods.

### 4.1 Synthesis of Polypyrrole and Polythiophene Nano wires [27]

Dodecyl-benzene sulfonic acid (DBSA) and Polypyrrole were dissolved in 100ml of distilled water by vigorous stirring to get an emulsion system. The initial molar ratio of DBSA/Py and PTh was always kept at 1/1 and the concentration of them is different in different experiments. $FeCl_3$ pre-dissolved in deionized water was added to the above emulsion at 0 °C. After certain reaction time, large excessive methanol was poured into the solution to terminate the reaction. Then, the resulting Polypyrrole and Polythiophene precipitate was vacuum-filtered and washed copiously with distilled water, methanol and acetone for several times. Finally, it was dried under dynamic vacuum for at least 40 h at room-temperature.

The working electrodes were first cleaned with ethanol and then electrochemically activated in 0.1 M KCl by applying a series of potential pulses from 0 to −2 V vs. Ag/AgCl (3 M KCl) using a CHI750C bipotentiostat. After this, The PPy and PTh NW was electro polymerized on a gold

electrode by chronoamperometry at 0.8 V in 0.15 M polymer solution containing 0.1 M $LiClO_4$ and 0.1 M $Na_2CO_3$. The obtained polymer electrode was rinsed with distilled water and then kept into 10% $HClO_4$ solution for 12 h to remove the carbonate ions[28]. The three-electrode cell included the above described counter and reference electrodes, and the interdigitated electrode with the assembled Nano wires as working electrode.

### 4.2 Synthesis of ZnO, TiO and Au nanoparticles

Initially, 50 mL of 0.1 M $ZnSO_4 \cdot 7H_2O$ and 50 mL of 0.4 M NaOH solutions were prepared and were mixed together in a reaction flask, followed by vigorous stirring for 15 min at ambient temperature. The reaction was further carried out under the influence of microwave radiations by placing the reaction flask in the microwave oven for 2 min. The reaction flask was cooled to room temperature and the product was filtered and washed with deionized water followed by drying at 40 °C in an oven for 4 h[29].

Different weight ratios of Titanium *iso*-propoxide (precursor) and toluene (solvent) are mixed in inert atmosphere. The weight ratios of titanium *iso*-propoxide are varied from 5/100 to 60/100. The mixture is taken in Teflon-lined stainless-steel autoclave and heated for different time durations (4–24 h) with the reaction temperatures varying from 180 to 240 °C. The products have been collected, washed thoroughly and then dried in vacuum at 60 °C for 3 h[30]. The 30-nm-diameter Au nanoparticles were prepared according to the literature[31]. Briefly, 2 ml of 1% (w/w) sodium citrate solution was added into 50 ml of 0.01% $HAuCl_4$ boiling solution. The preparation of 10-nm-diameter Au nanoparticles followed the same protocol as above except that 1 ml of 1% (w/w) sodium citrate solution was added. The mean diameter of the Au nanoparticles was determined using TEM (figures not shown). The resulting Au colloid solutions were stored in a refrigerator in a dark-colored glass bottle before use. Au glassware used in the above procedures was cleaned in a $HCl/HNO_3$ (3/1) solution, thoroughly rinsed with water, and dried prior to use.

**4.3 Synthesis of composite Nano wire**

MWCNT and Metal Nano particle included Nano wires (composite) will be prepared by adding optimum amount to the system while synthesis of nanowires takes place.

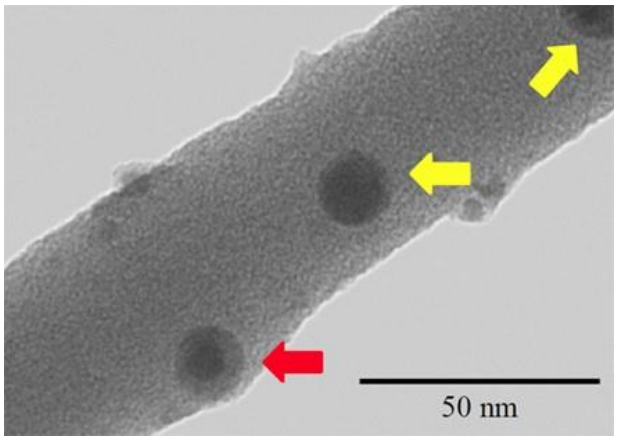



**Figure 4**. Scanning transmission electron microscope image of a polystyrene Nano wire containing iron oxide nanoparticles. The yellow arrow shows the surface of the Nano wire, and the red arrow shows iron oxide nanoparticles which exist in the interior of the Nano wire [32].

**4.4 Nano wire surface modification**

A procedure was used to covalently link antibody receptors to the surfaces of the Nano-wire devices. mAb receptors, anti- hem-agglutinin for influenza A and anti-adenovirus group III were coupled to the aldehyde -terminated Nano wire surfaces by reaction of 10–100 mg/ml antibody in a pH 8, 10-mM phosphate buffer solution containing 4 m M sodium cyanoborohydride. The surface density of antibody was controlled by varying the reaction time from 10 min (low density) to 3 h (high density). Un-reacted aldehyde surface groups were subsequently passivated by reaction with ethanolamine under similar conditions. Device arrays for multiplexed experiments were made in the same way except that distinct antibody solutions were spotted on different regions of the array. The antibody surface density vs. reaction time was quantified by reacting Au-labeled IgG antibodies with aldehyde-terminated Nanowires on a transmission electron microscopy grid, and then imaging the modified nanowire by transmission electron microscopy, which enabled the number of antibodies per unit length of Nanowires to be counted[33].

**4.5 Virus Samples**

Different concentration virus solutions were prepared from stocks by dilution in phosphate buffer (10 MM, pH6.0) containing 10 MM KCl (assay buffer); influenza type A, 109 to 1010 particles per ml and unpurified avian adenovirus group III, influenza A, and avian paramyxovirus virus in allantoic fluid, 1010 to 1011 particles per ml, were used as received after dilution in assay buffer or purified by using a microfiltration device (5,000 rpm, Centricon 30, Millipore). Similar results (sensitivity and selectivity) were obtained with purified and unpurified samples. Viral concentrations were measured by transmission electron microscopy after staining samples with uranyl acetate and by fluorescence microscopy using 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine- labeledviruses

## 5. Electrode Construction

Interdigitated electrodes (IDE) with the spacing between 1and 6 micrometer as shown in Figure 5 will be prepared using DEEP UV photolithography mask aligner and ion beam dry etching system. Magnetron sputtering will be used for platinum electrode deposition. The spacing will be reduced down to 100 nm using Electron Beam Nanolithography system in IKCU labs to increase sensitivity of IDE electrodes

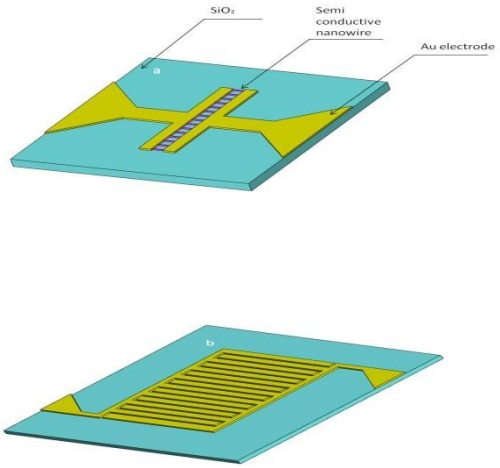



**Figure 5.** a) one terminal b) multiple terminal Au Nano-electrodes.

## 6. Electrical Measurements

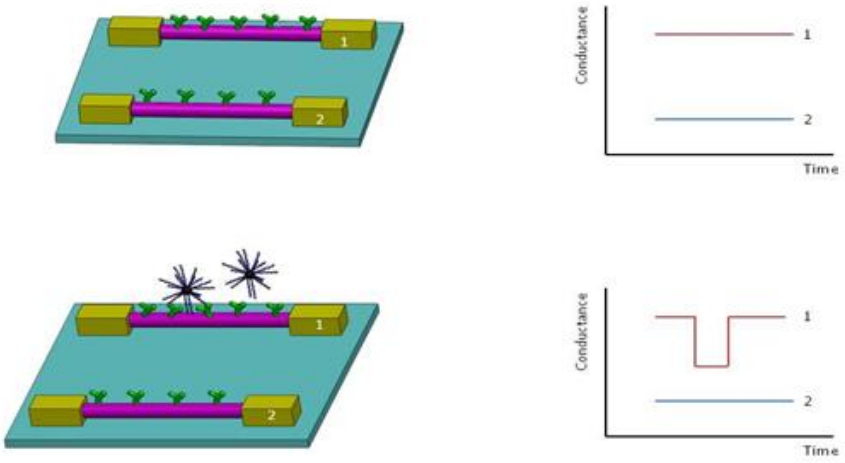



**Figure 6.** Nano-wire-based detection of single viruses.

**Figure 6.** (*Left*) Schematic shows two Nano-wire devices, 1 and 2, where the Nanowires are modified with different antibody receptors. Specific binding of a single virus to the receptors on Nano wire 2 produces a conductance change (*Right*) characteristic of the surface charge of the virus only in Nano-wire 2. When the virus unbinds from the surface the conductance returns to the baseline value

Electrical measurements were made by using lock-in detection with a modulation frequency chosen as a prime number between 17 and 79 Hz, inclusive. Measurements were independent of frequency within this range. The modulation amplitude was 30 mV and the dc source-drain potential was zero to avoid electrochemical reactions. Conductance vs. time data was recorded while buffer solutions, or different virus solutions, flowed through the micro-fluidic channel. Viral sensing experiments were performed in the micro-fluidic channel under a flow rate of 0.15 ml. hr in 10 *m*M phosphate buffer solution containing 10 mM KCl at pH 6.0.

## 7. Utilization:

The fabricated biosensor and electrodes will be used in the detection of the disease and giving doctors a more objective and quantifiable basis for clinical decision-making.

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