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An Overview of a Recent Development in Electrochemical nCovid-19 Detection Biosensor

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Abstract:

The outbreak of novel Corona virus, Covid-19 pandemic has uprooted the entire world. A highly contagious infection related to the respiratory system, also designated as SARS-CoV-2, is spreading rapidly across the globe. The World health organization has reported that about 215 countries are affected around the world, with 3.5 million (still growing) cases is confirmed and 0.2 million deaths are reported so far. The recovery rate is found to be very slow. Although, various organizations globally are racing against time for developing vaccines, treatment, medicine for this catastrophic virus, yet there is a long battle left. Presently, early detection of Covid-19 is considered as the best way to contain its spread by following the quarantine method of the patient. However, so far, rapid detection is challenging. This article focuses on a very recently developed electrochemical detection possible point of care sensor for novel corona virus.

Keywords: electrochemical, biosensing, covid-19 detection, point-of-care

1. Introduction

The novel Corona virus is a major concern today globally. First reported in China, in December 2019, the virus has spread across the globe to about 215 countries. Approximately, 3.5 million confirmed cases, 0.2 million deaths occurred worldwide according to the world health organization bulletin [1]. So far, there is no medicine or vaccine to fight against this deadly virus. Many industries, laboratories, academicians are working day-night for the possible treatment, yet it appears too far. The only way globally adapted is the early detection of the virus in the patient and quarantine of them. Unfortunately, this virus is expected to stay in dormant state inside a person upto a period of 14 days. Some clinical reports have also suggested that, certain patients were asymptomatic, i.e; they showed no signs of infection like cold, cough, fever or difficulty in breathing [2]. Such type of carriers is way hazardous in transmission of the virus. Although, fortunately, it is a contact based disease, social distancing is playing a great role in arresting the spread. Nevertheless, the early detection of virus in a patient is extremely important in the present circumstances. The test for Corona virus is being conducted in specialized laboratories only. It takes at least approximately 12 hours for the confirmation test to show results. Another major concern is the cost. Especially in the developing countries, Covid-19 test is being carried only for the patients who are suspected to carry the disease based on symptoms. Therefore, there is a possibility, that, large group of people might be carrying the virus without symptoms. Thus, in such scenarios, it is highly required, that affordable and rapid test kits must be developed. In comparison to the existing conventional method, one exquisite root could be electrochemistry based chemically modified electrode approach.

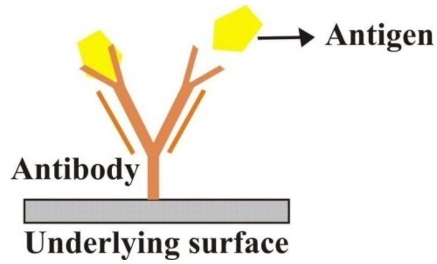
Electrochemistry deals with the relation of electrical and chemical properties. Basically, it is the analysis of chemical changes occurring upon passage of an electric current and the production of electrical energy by chemical reactions [3]. Basically, a chemically modified surface or underlying electrode (CME) is fabricated that works as an electrochemical sensor. In the past few decades, there has been a constant rising interest amidst researchers for fabricating biosensors/electrochemical sensors due to the growing demand of the miniaturized gadgets in the healthcare sector [4]. Such type of sensors are foremost as they are commercially viable in various fields like medicinal, healthcare, clinical, industrial, environmental and agricultural studies. The idea of CMEs was first established meticulously in the early 1970s. Since then, this technique has acquired a leading position in present day to day routine. It has become a prominent area of attention in recent years as they are considered to be one the best and quick approaches to inspect the electron transfer behavior in biological and non-biological system [5]. CMEs are nothing but a type of electrochemical sensors that have established an extensive array of uses for the detection of chemicals, biochemicals, real time physiological fluids, cells etc. They consist of an electro-active species (redox mediators) and a signal transducer that helps to create an electrical signal. The redox mediators used can be a chemical or biological substance. These electro-active redox mediators are responsible for the selective detection of the target analyte whereas the transducer transforms a chemical reaction into suitable current signal which with /without amplification can be used to quantify the analyte concentration in an unknown sample [6,7]. Electrochemical sensors are broadly classified into two types: (1) Chemical sensor (2) Biosensor.

2. Chemical sensors

These categories of electrochemical sensors are those in which the electro-active redox mediator used for modifying the underlying surface are any desired chemical/metallic complex/nano particle etc. of interest. Mostly, the chemical used has functional groups or electro-active sites which are responsible for its electrochemical feature of sensing specific analyte at a fixed applied potential. Generally, biochemicals like Ascorbic acid (AA), Uric Acid (UA), Cysteine (Cys), Dopamine (DP), sulfide, hydrogen peroxide (H_2O_2) etc. For instance, Vishnu et al. reported multiwalled Carbon nanotube modified gold electrode for polymerization of aniline (Au–MWCNT@PANI pH7) and its application for AA sensing [8], Barathi et al. in 2013, described protocol for immobilization of polyhydrocarbons as surface confined quinones and studied its electrochemical oxidation [9]. Azo-dye based CME was reported in 2015 which was used for sensing AA [10]. Very recently, a molecular imprinted polymer based electrochemical sensor for selective detection of paraben was developed by Yucebas et al., in 2020[11]. Wang et al., reported a metal–organic framework and conducting polymer based electrochemical sensor for high performance cadmium ion detection [12]. These sensors continue to have a noteworthy influence in day to day life and there has been a robust entreaty to develop such kind of highly selective, sensitive and economic chemical sensors.

3. Biosensors

In recent years, researchers in the electrochemistry are extensively focusing on developing biosensors. Several imperative technical developments have been done to ensemble the equipment, methodologies and resources required to build efficient biosensors [13]. In the year 1999, the IUPAC designated biosensor as sovereign, incorporated receptor transducer tool that can give highly sensitive and specific quantitative information [14]. Basically, it is an electro- analytical sensor in which a biological component is used as a redox mediator (immobilized over the substrate) to fabricate CME that can sense either biological or chemical analytes [15]. The transducer present has the ability to convert the biological signal to electrical current signal. The main focus of this device is to deliver rapid, simple and accurate information about the analyte being sensed. Since the first biosensor was invented (i.e.; Clark oxygen electrode) [16] till today, massive advances in terms of the selectivity, sensitivity and accuracy of the biosensors has taken place. Biosensors are presently anticipated to show a substantial role in several diverse fields of biochemical, environmental, medicinal, pharmaceutical, diagnostic and industrial research [13- 27].



3.1 Antibody Based Biosensor (Immunosensor)

An antibody based biosensor was first invented in early 1950s. Meanwhile, extensive research is being done to prepare immunosensors as a diagnostic tool [28]. An antibody, basically, Immunoglobin (Ig) is used over the substrate as a bio-receptor. Ig is made up of two light and two heavy chains. Each chain of these antibodies has a constant and a variable portion. It is this variable part which selectively binds with the antigen [28-29]. Therefore, an immunosensor exploits the aptitude of the antibody to bind with the analogous antigen in an extreme selective manner. In contemporary approach, there are two types of detection techniques used in an immunosensor: (1) Optical (2) Electrochemical. Both of these techniques have gained mutual importance [30-32]. Certain current investigations demonstrate that the immunosensor is being broadly used in the detection of cancer cells and tumors. Due to their high sensitivity, they have become a popular tool in early diagnosis of certain types of cancers. Usually, antibody is Y shaped and has special binding sites for antigen. Scheme 1 projects the mechanism of antibody-antigen interaction.

Scheme 1: Illustration of an Antibody-Antigen Based Biosensor

In comparison to the conventional available detection method for Covid-19 which takes a lot of time, antigen-antibody approach comes as a savior. Development of immunosensor, that has, Covid-19 antibody anchored could help in capturing the Covid-19 antigen instantly as it comes in contact with the infected sample. Since, the antibody will be highly specific in nature, it binds selectively with the specified Covid-19 antigen in swab sample from mucous or throat (saliva). Upon the formation of antigen-antibody complex, instantly changes in the electrochemical signals will occur that can be amplified to comprehend the presence of virus. Such type of method is not only time saving but also rapid and affordable due to which large number of tests can be conducted.

4. nCovid-19 Electrochemical Detection

Based on the similar approach of antigen-antibody complex formation, Mahari et. al., very recently, developed a highly sensitive, in-house built, electrochemical detection tool for Covid-19 spike protein [33]. This article reviews mainly the electrochemical approach adopted by the aforementioned group for the development of rapid nCovid detection. Scheme 2 briefly states the approach of Covid-19 electrochemical sensor by Mahari et. al.

Scheme 2: Illustration of stepwise process for electrochemical Covid-19 sensor preparation reported by Mahari et. al., 2020.

4.1 Chemical and reagents

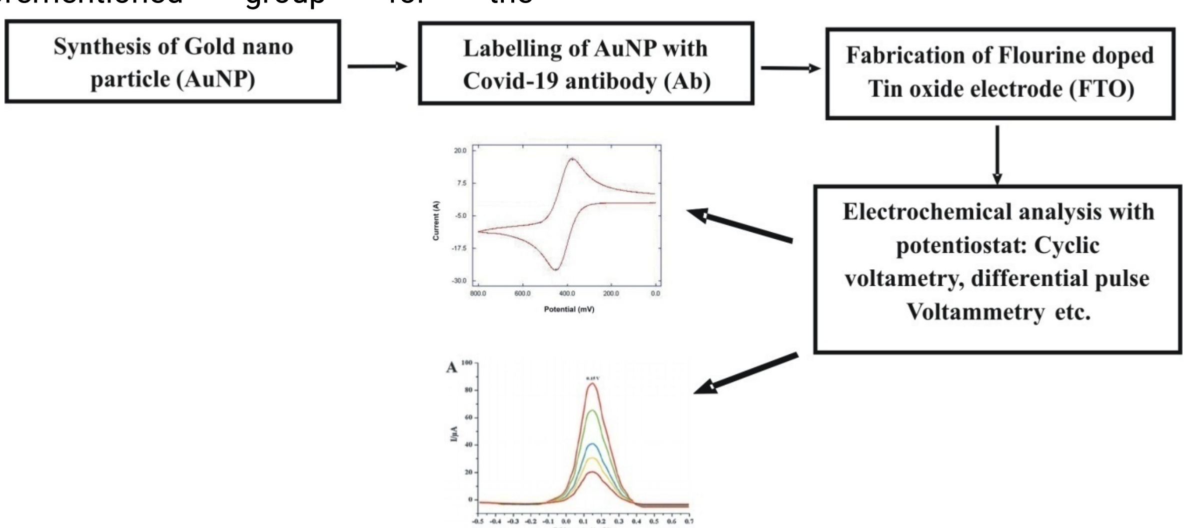
The authors have procure nCovid-19 Antigen (ag) (Spike S1 protein), nCovid-19 Antibody (Ab) from ProSci (California, USA). All the basic other chemicals used were of analytical grade and prepared in distilled water [33].

4.2 Procedure

4.2.1 Synthesis of AuNP

Gold chloride solution, taken with milli-Q water, heated to boiling. Sodium citrate (1mL) added to boiling solution. Slowly change in the color from yellow to dark blue then to wine red appeared indicating formation. Further, they characterized prepared particles [33].

4.2.2 Labelling of AuNP with Covid-19



Antibody (Ab)

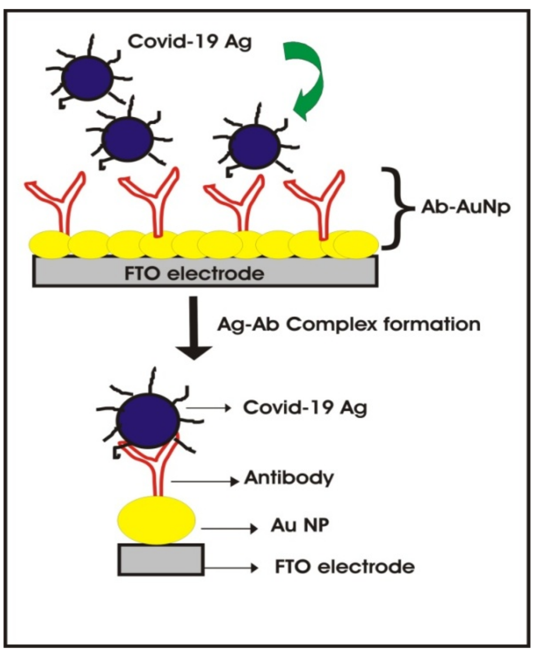
To anchor AuNP with antibody, 90 µg nCovid19 Ab was added to 1 mL of AuNPs solution taken in Phosphate Buffer of neutral pH. The mixture was kept overnight and centrifuged before use [33].

4.2.3 Fabrication of Fluorine doped Tin oxide Electrode (FTO)with AuNPs/nCovid-19 Ab

A glass plate of dimension 3*5cm is coated with Fluorine Tin oxide, 200 µL of AuNP-Ab was drop casted and kept for drying about 48 hours [33].

4.2.4 Formation Covid-19 Antigen-Antibody complex

Scheme 3, clearly depicts the mechanism of Ab-Ag complexation. The AuNP tethered Ab, when comes in contact with Covid-19 spike protein, it binds to the active site of Ab.

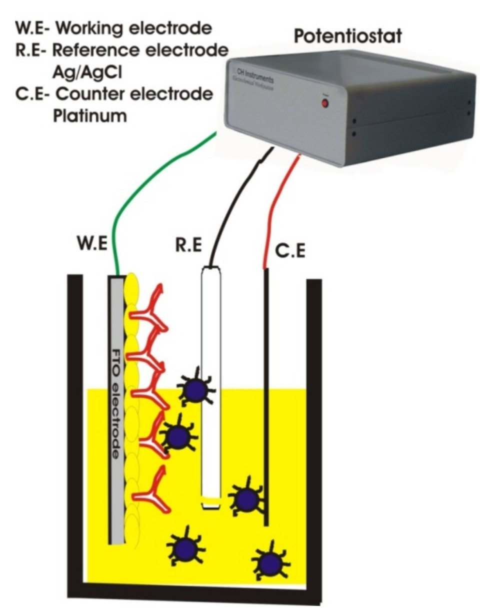


Scheme 3: Schematic representation of nCovid-19 Ag-Ab complex formation

The FTO electrode was modified by gold nano particles which act as the underlying matrix for attaching the antibody. The spike protein Ag, selectively binds to the active site of Ab forming the aforementioned complex. The formed complex gives fluctuations in current value recorded by a potentiostat.

4.2.5 Electrochemical measurements

The electrochemical measurements they carried out were using a conventional three-electrode based potentiostat. Working electrode (W.E) was the fabricated FTO/AuNp/nCovid-19, electrode, reference (R.E) was Ag/AgCl, counter (C.E) was a platinum electrode. The electrolyte was potassium ferricyanide-potassium chloride solution. Known concentration of Covid-19 Ag was spiked in the electrolyte and current fluctuations were measured with respect to increasing concentration. Scheme 4 shows the experimental set up [33].



Scheme 4: Cartoon for three-electrode based electrochemical set-up

Scheme 4 demonstrates experimental set up. The working electrode is modified with the specific antibody and used as working electrode. The two other electrodes involve Ag/AgCl and Platinum as reference and counter respectively. The electrolyte solution used is Ferricyanide-potassium chloride solution. Antigen (Spike protein) is added in the calculated concentrations into the solution. It binds to the antibody and changes the current of working electrode[33].

4.3 Results

The cyclic Voltammetry (CV) and differential pulse voltammetry (DPV) measurements were carried out with a potentiostat. The CV/DPV graph detection at various concentration of nCovid-19 Ag reflected changes in current that was explored to predict the limit of detection (LOD) of fabricated FTO electrode. Different concentrations of nCovid-19 Ag in a linear range from 1 fM to 1 μM were tested on the FTO/AuNPs/nCovid-19 Ab modified electrode via standard addition approach. The calibration plot of DPV current vs. concentration showed linearity with LOD as 10 fM for nCovid-19 Ag [33].

5. Conclusion

This article gives an overview on the recent advancement in the electrochemical detection of novel Corona virus, Covid-19. Mahari et al., very recently developed FTO chemically modified electrode with AuNP, anchored with Covid-19 antibody. This was successful to form antigen-antibody complex with Covid-19 Ag. Electrochemical measurements taken with CV and DPV, showed promising results wherein, as the concentration of Ag was changed, there was a fluctuation in current value. This method could be a potential diagnostic tool for rapid qualitative detection of nCovid-19 in real time samples. Biosensor development could proof to be a promising tool in near future for Covid-19 detection. It also has future scope wherein chip based, disposable, paper based, electrochemical sensing platforms attached with antibody can act as rapid detection tool.

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Conflict of interest

No conflicts to declare.

References

[1] https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen

[2] Kang M., Hua Z., and Zhugen Y. (2020), Can a Paper-Based Device Trace COVID-19 Sources with Wastewater-Based Epidemiology?, *Environmental Science and technology,* **54,** 3733-3735.

[3] Bard, A. J. and Falukner, L.R. (2006), *Electrochemical Methods – Fundamentals and Applications,* Wiley.

[4] Bakker, E. and Qin, Y. (2006), "Electrochemical sensors", *Analytical Chemistry,* **78**(12), 3965–3983.

[5] Wen, D., Guo, S., Dong, S. and Wang, E. (2010), „Ultrathin Pd nanowire as a highly active electrode material for sensitive and selective detection of ascorbic acid", *Biosensors and Bioelectronics* **26**, 1056-1061.

[6] Wang, J. (Eds.), Electrochemical Sensors, Biosensors and Their Biomedical Applications. *Elsevier*, San Diego, 32-50.

[7] Wilson, R. and Turner, A.P.F. (1992), "Glucose oxidase: an ideal enzyme", *Biosensors and Bioelectronics,* **7**, 165-185.

[8] Vishnu, N., Kumar, A.S. and Pillai, K.C. (2016), "Unusual neutral pH assisted electrochemical polymerization of aniline on a MWCNT modified electrode and its enhanced electro-analytical features", *Analyst,* **138**(21), 6296-6300.

[9] Palani, B. and Kumar, A. S. (2013), "Facile Electrochemical Oxidation of Polyaromatic Hydrocarbons to Surface-Confined Redox-Active Quinone Species on a Multiwalled Carbon Nanotube Surface", *Chemistry a European Journal,* **19**, 2236-2241.

[10] Gayathri, P., Kumar, A. S. and Sriraghavan K., (2015), "An Unusual Electrochemical Reductive Cleavage of Azo Dye into Highly Redox Active Copolymeric Aniline Derivatives on a MWCNT Modified Electrode Surface at Neutral pH and Its Electroanalytical Features", *The journal of Physical Chemistry C, Chemistry European Journal,* **119**, 7791-7801.

[11]Beyhan, B.Y., Yesim, T.Y., Gulcin, B., Erdoğan, Ö., Lokman, U., and SerdarA., (2020), "Molecular imprinted polymer based electrochemical sensor for selective detection of paraben", *Sensor and actuators B,* **305**, 127368.

[12] Yang, W., Lu, W., Wei, H., Ting, Z., Xiaoya, H. Jason, A.P., and Shengqian, M., (2017), "A metal–organic framework and conducting polymer based electrochemical sensor for high performance cadmium ion detection", *Journal of Material Chemistry A*, **5**, 8385 -8393.

[13] Panjan, P., Virtanen, V. and Sesay, A.M. (2017), „Determination of stability characteristics for electrochemical biosensors viathermally accelerated ageing", *Talanta,* **170**, 331-336.

[14] Thevenot, D.R., Toth, K., Durst, R.A., Wilson, G.S., (1999), „Electrochemical biosensors: recommended definitions and classification," *Pure and Applied Chemistry,* **71**(12), 2333-2348.

[15] Lowe, R.S., (2007), *Handbook of Biosensors and Biochips*, Wiley.

[16] Leland C. Clark Jr., (1962), "Electrode Systems For Continuous Monitoring In Cardiovascular Surgery", *Annals of the New York Academy of Sciences,* **102**, 29-45.

[17] Zhang, J. and Oyama, M. (2004), „A

hydrogen peroxide sensor based on the peroxidase activity of hemoglobin immobilized on gold nanoparticles-modified ITO electrode", *Electrochimica Acta,* **50** (1), 85-90.

[18] Wang, J. (Eds.), Electrochemical Sensors, Biosensors and Their Biomedical Applications. *Elsevier*, San Diego, 32-50.

[19] Kashem, M.A., Suzuki, M., Kimoto, K. and Iribe, Y. (2015),„An optical biochemical Khosla, U.M., Zharikov, S., Finch, J.L., Nakagawa, T., Roncal, C., Mu, W., Krotova, K., Block, E.R., Prabhakar, S. and Johnson, R.J. (2005), „Hyperuricemia induces endothelial dysfunction", *Kidney International*, **67**(5), 1739-1742.

[20] Bidmanova, S., Kotlanova, M., Rataj, T., Damborsky, J., Trtilek, M. and Prokop, Z. (2016), „Fluorescence-based biosensor for monitoring of environmental pollutants: from concept to field application", *Biosensors and Bioelectronics,* **84** 97-105.

[21] Rotariu, L., Lagarde, F., Renault, N. J. and Bala, C. (2016),„Electrochemical biosensors For fast detection of food contaminants – trends and perspective", *Trends in Analytical Chemistry,* **79**, 80-87.

[22] Chen, H., Tan, X., Zhang, J., Lu, Q., Ou, X., Ruo, Y. and Chen, S. (2014), „An electrogenerated chemiluminescent biosensor based on a g-c3n4–hemin nanocomposite

and hollow gold nanoparticles for the detection of lactate", *RSC Advances*, **4**(106), 61759-61766.

[23] Goretti, E., Wagner, D.R. and Devaux, Y. (2014), „miRNAs as biomarkers of myocardial infarction: a step forward towards personalized medicine?", Trends in Molecular Medicine **20** 716-725.

[24] Rattray, N.J.W, Hamrang, Z., Trivedi, D.K., Goodacre, R. and Fowler, S.J. (2014), "Taking your breath away: metabolomics breathes life in to personalized medicine", *Trends in Biotechnology,* **32**, 538-548.

[25] Mehrotra, P. (2016), „Biosensors and their applications – a review", *Journal of Oral Biology and Craniofacial Research,* **6,** 153-159.

[26] Burcu, E. and Kemal, M. (2015), "Applications of commercial biosensors in clinical, food, environmental, and biothreat/biowarfare analyses", *Commercial Biosensors,* **478** 107-120.

[27] Kojima, R., Aubel, D. and Fussenegger, M. (2015), „Novel theranostic agents for next- generation personalized medicine: small molecules, nanoparticles, and engineered mammalian cells", *Current Opinion in Chemical Biology,* **28** 29-38.

[28] Donahue, A.C. and Albitar, M. (2010),„Antibodies in biosensing. In: Zourob, M. (Ed.), Recognition Receptors in Biosensors. *Springer*, *New York*, 221-248.

[29] Conroy, P.J., Hearty, S., Leonard, P.

and O'Kennedy, R.J. (2009), „Antibody production, design and use for biosensor-based applications", *Seminar on Cell Division Biology*, **20**, 10-26.

[30] Pohanka, M. (2009), „Monoclonal and polyclonal antibodies production-preparation of potent biorecognition element", *Journal of Applied Biomedicine*, **7**, 115-121.

[31] Shankaran, D., Gobi, K. and Miura, N. (2007), „Recent advancements in surface plasmon resonance immunosensors for detection of small molecules of biomedical, food and environmental interest", *Sensors and Actuators B: Chem*ical, **121**, 158-177.

[32] Bhatta, D., Stadden, E., Hashem, E., Sparrow I.J.G. and Emmerson, G.D. (2010), „Multi- Purpose optical biosensors for real-time detection of bacteria, viruses and toxins", *Sensors and Actuators B: Chem*ical, **149**, 233-238.

[33] Subhasis, M., Akanksha, R., Deepshikha, S. , Sonu, G. eCovSens-Ultrasensitive Novel In-House Built Printed Circuit Board Based Electrochemical Device for Rapid Detection of nCovid-19 antigen, a spike protein domain 1 of SARS-CoV-2. https://doi.org/10.1101/2020.04.24.059204