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# Fabrication of Interdigitated Electrodes based biosensor and Prediction of Covid-19 cases using Linear Regression

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***Abstract***

*Novel coronaviruses (CoVs) are pathogens pertaining to zoonosis but their interaction with humans is recently found. The CoVs is well known as one of the great genome of RNA viruses which was recently found to exhibit mutations. The first case recorded by the WHO was at Wuhan, China on 31 December 2019. It started as a case of absurd pneumonia condition. The use of serum of patients is being used of the patients that have been affected by Covid-19 and now have been cured. Patients those have been cured with the disease are seen to develop a Covid-19 polyclonal antibody to different viral antigens. This paper aims in developing a biosensor based interdigitated electrodes which can be used for detection of the Covid-19. IDEs based biosensors are showing progress in the field of medicine for the detection of biomolecules like bacteria and viruses. For optimization purpose, the corresponding electrical circuit with particular number of fingers, width and spacing of IDE was designed using AutoCAD. The designed electrical circuit is simulated using Gamry Echem-analyst software from Gamry Instruments. The study includes the future prediction analysis of number of Covid-19 cases in India using linear regression in machine learning. The prediction showcases the predicted values on day 31 and day 50. The simulation is done in Matlab environment. The sensitivity of the model for prediction on 31$^{st}$ day is 94.25% and on 50$^{th}$ day is 76.58%.*

***Index Terms—*** *Anti-SARS-CoV2, interdigitated electrodes, biosensor, Covid-19, biomolecules, machine learning.*

## I. INTRODUCTION

In recent times, novel corona virus (Covid-19) has become known to be as one of the most threatening situation in the world [1]. The Centers for Disease Control and Prevention initiated to monitor the spread of the novel Covid-19 virus and found that it belongs to the family of β-coronavirus cluster causing respiratory illness in December, 2019 [6]. The authority reported the first case of the virus in Wuhan, China in December, 2019. [2]. The virus were the pathogens belonging to the category of zoonotics but their interaction with humans was found recently [5]. In the late December 2019, some patients came up with cases of pneumonia with no

specific cause in Wuhan. The detected virus initially came to be known as 2019 novel corona virus (2019-nCoV). The World Health Organization (WHO) declared it as a severe respiratory illness and renamed it as Syndrome corona virus-2(SARS-CoV-2). Therefore, the infection caused by the specific virus is known as Coronavirus disease-2019 [1]. The Director-General of the World Health Organization distinguished the COVID-19 condition as a pandemic [7].

Coronaviruses are made up of various single-stranded, positive sense RNA genomes that are non-segmented in nature [2]. The CoVs are composed of peplomers of nano size having the polarity acting positively based on their characteristics [5]. Human coronavirus causes infections related to the respiration system of humans with low affecting to acute consequences [2]. The basic symptoms reported to show up as the virus attacks the human body are mostly fever, fatigue and intense cough. Other

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symptoms to get develop in patients includes shortness of breath, body aches, dry cough, and ageusia [3]. These viruses are found to be one of the pandemic situations responsible for the outbreaks across the world including the Severe Acute Respiratory Syndrome (SARS) pandemic of 2002-2003 and the Middle East respiratory syndrome (MERS) pandemic of South Korea that occurred 5 years ago. While some coronaviruses have caused severe epidemics, others caused mild to acute respiratory illness [2]. Physical distancing is the major objective that should be fulfilled during the pandemic situation and it has been implemented across the world to prevent the dispersal of SARS-CoV-2. Physical distancing aims to prevent a rapid increase in SARS-CoV-2 infections in a particular place aiming to minimize the number of cases [3]. The genome of corona virus having the length range from 26 to 32 kilo-bases are known to be the largest viral RNA. Earlier there were six corona viruses which can be classified into high or low pathogenic properties that affected human race. Amongst them, 229E, HKU1, OC43 and NL63, are considered as low pathogenic types and they causes respiratory illness upto 10% to 30% of upper respiratory. However, the Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) coronavirus are considered to be highly pathogenic and affected lower airways of respiratory tract and may lead to fatal pneumonia [8]. A report stated that the patients infected with Covid-19 in the early stage had a travel history or were residents of Wuhan (first generation patients), and the patients infected in later stage were the ones who had some sort of contact with the first generation patients. These patients are known as second generation patients [4]. The researchers are continuously studying various ways of developing the biosensors that have the ability to detect the virus SARS-CoV-2 causing Covid-19. Jing Wang et al. reported at the Swiss Federal Laboratories for Materials Science and Technology and ETH Zurich the development of a reliable biosensor for the detection of Covid-19 virus. The National Institute of Virology, Pune have successfully developed a sensor for the detection of SARS-CoV-2 virus based on ELISA technique [30]. Remdesivir is considered as one of the most efficient example that has the ability to interfere with the viral polymerase. It shows efficiency with the MERS to detect the presence of the novel Covid-19 virus. The use of serum of patients that have been affected and now cured is proven to be the most effective method. Patients that have been cured with the diseases are seen to develop a Covid-19 polyclonal antibody to different viral antigens. Some of them neutralises the virus and thus the patients can recover with the uses of the serum [8].

### *A. Significance of IDE in the field of Medical Science*

Generally, IDEs are used in transducers for lab-on-chip (LoC) devices [9].In recent year, most of the biosensors depend on fluorescent labels with the help of data imaging. However, these techniques require highly sensitive equipments and produce uncertain interference in the system causing non-reliable results. These drawbacks can be removed using an interdigitated electrode based impedance biosensor due to the ease caused by miniature size, simplified operation and effective cost [10]. Interdigitated electrodes (IDEs) are most widely applicable sensors, and are effective in gas sensor, humidity sensor, biosensor, and many others. The IDE's are used to detect the electrical signals that are produced by the sensing system [11]. There is quick need in the medical and healthcare fields to use a sensor that can have label free operation and obtain reliable results [10]. Most effectively, IDE based biosensor can be used to detect the presence and quantity of a particular template i.e., biomolecule [12]. IDEs can be used to detect the presence of cancer cells using impedance spectroscopy and detecting how the electrical properties of the IDE change. Interdigitated electrodes (IDEs) are utilized in various sensors including surface acoustic wave sensors, chemical sensors [2] and MEMS based biosensors [13]. IDEs based sensors have gained a lot of recognition as biosensor due to its structure of design as a sensing device [14]. IDEs have the ability to work as a portable biosensors detecting biomolecules including DNA, RNA and protein [15]. The major merits of using an IDE based biosensor is its low ohmic drop, quick steady state establishment and high signal-to-noise ratio (SNR). The electrode gap width of the IDE plays a significant role in the structural design as it affects the sensitivity of IDE and its electrical field strength [16]. IDE based biosensor do not require any additional biomarker to fulfill the purpose of detection of a particular

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biomolecule as it follows the basic principle of impedance spectroscopy showing impedance change as soon as the interaction of template and biosensor is recorded.

*B.IDE based biosensor for diagnosis*

The technology of biosensors has completely revolutionized the conventional chemical analysis and optimization using the bio analytical methods and assay based terminologies. The biosensors offer various merits over other conventional techniques such as sensitivity, specificity, accuracy, and rapid control [14]. IDE based sensors are generally implemented for the detection and quantification of biomolecules [15]. Interdigitated electrodes (IDEs) or interdigitated capacitors (IDCs) are the most widely used types of electrodes including quality control [17], determining the dielectric properties of thin films fabrication of electronic equipments, such as microwave filters and supercapacitor [18]. IDE consist of two individually placed electrodes design resembling to structure of a comb [10] usually recommended as a highly sensitive biosensor [14]. The geometry of the IDE and structures affect the impedometric as well as capacitive biosensor [10]. Interdigitated electrodes have been fabricated and optimized for the detection of biomolecules and their pH concentration [19]. The IDEs are designed to record conversions and alterations of the indicator microorganisms [20]. For optimization purpose, IDE with specific width, spacing between fingers is fabricated [21]. Interdigitated electrodes having a combination of parallel microband of electrodes are used in electrochemical impedance spectroscopy to fabricate immunosensor based on immunoglobulin-A (IgA) [22]. The measurement IDE consists of a pair of twin electrodes arranged in a comb like structure consisting of a gap between the electrodes in which there is a gap that is formed between the two electrodes. As the size of the gap between the electrodes is decreased, the value of capacitance will increase [19].

*C. Antibodies of Covid-19 for Diagnostic Purpose*

1. Anti-Covid-19 and SARS –CoV S Glycoprotein [CR3022]

Novici Biotech fabricated lab-scale amounts of the CR3022 anti-coronavirus spike glycoprotein antibody that was originally explained by Crucell. The antibody is capable of detecting both SARS-1 and SARS-2. The antibody is monoclonal in nature and was produced in the form of pure Ig-G. It was obtained in 24 days utilizing a plant based transient system which had the ability of producing quick turn-around of the antibodies and proteins [25].

2. Anti-SARS-COV S Glycoprotein [S227]

The rabbit IgG is the isotype of the antibody anti-SARS-CoVS glycoprotein and the the fomat is Kappa. S227 refers to the clone number of this antibody. SARS-CoV-neutralizes the hmAbs and obtain binding with the SARS-CoV S glycoprotein. This results the antibody to lose binding of the glycoprotein to human ACE2. S227 was examined prophylactically at 10 and 1 mg/kg in 12-month-old BALB/c mice infected with a little dose of SARS-CoVUrbani, GZ02 and HC/SZ/61/03. All the animals were found to be recovered treated with S227 by day 5 [25].

3. Anti-SARS-COV S Glycoprotein [S230]

The isotypeof the antibody Anti-SARS-COV S Glycoprotein S230is Human IgG1 and the format is Kappa respectively. The clone number of the antibody is S230.The size of the antibody is 200 μg. The pure form of antibody where the purification is done in Protein-A affinity and then application with PBS with 0.02% Proclin 300. The directions of storage for the antibody states that it should be stored at 4ºC for up to 3 months. For longer period of time, the storage should - 20ºC and 1 mg/ml should be the concentration [25].

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4. Anti-SARS- COV S Glycoprotein [S109]

The isotypeof the antibody Anti-SARS- COV S Glycoprotein S109 is Human IgG1 and the format of the antibody is Lambda respectively. The specificity of this antibody has SARS-CoV-neutralizing hmAbs having the binding towards SARS-CoV S glycoprotein. It inhibits the binding of the glycoprotein to human ACE2. The clone number of the antibody is S109 [25].

## II. METHODOLOGY

Interdigitated electrode-based impedance biosensor is widely used due to its simplistic miniaturization, label-free operation and low cost. They are used while using an impedance sensor or electrical cell substrate impedance sensing [24] for the purpose of monitoring the attaching, spreading, growth rate and death rate of the cells. An insulating dielectric membrane appears as soon as the cells deposit on the surface of electrode. This insulating membrane impedes the current flow. The measurements of the impedance are provided by the number of cells, the interaction between the cells and the morphological change in the cell.

The fig 1 demonstrates the methodology of the designing and fabrication of the interdigitated electrodes as biosensor that can be used for the detection of Covid-19.

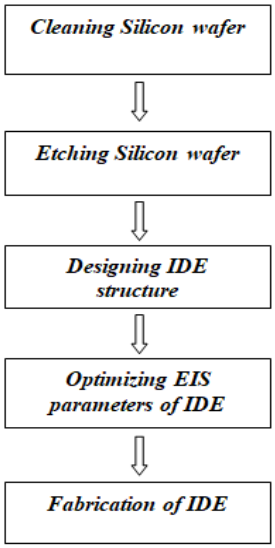


Fig 1: Methodology of the proposed work

The circuit model in fig 2 & 3 illustrated the interdigitated electrodes-based biosensor and its equivalent electrical circuit. The induction of impedance occurs from double layer capacitance of the electrodes ($C_{dl}$) and the resistance of the solution, when no attaching of the cells is recorded. On the attachment of the cells on the surface of electrode, the electrical circuit adds the impedance component as R and $C_{dl}$. The impedance of the attached cells is signified by the resistance (R) and a capacitance ($C_{cell}$) placed in parallel. As the cells are attached, the impedance due to the interference increases gradually due to high insulation that eventually reduces the surface of the electrode. The ionic environment around the electrode or solution interface is altered due to the presence of the cells. This happens as there is a significant change in the $C_{dl}$ and R which eventually increases the impedance. It is proved by various studies that capacitance component contributes to the impedance dependent of the frequency and the resistance component contributes to the impedance which is independent of frequency [10].

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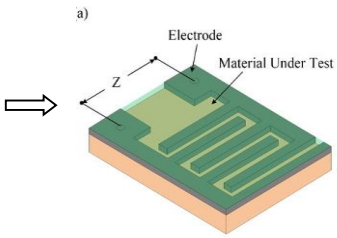


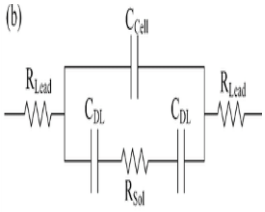


Fig 2: Interdigitated electrode Fig 3: Circuit of Interdigitated

## III. EXPERIMENTS PERFORMED

### *A. Cleaning of silicon wafer substrate*

The silicon wafer used in the experiment was P-type oriented with a resistivity about 1-10 Ω.cm approximately is used as a base material. Although the wafer has an insulator layer on the surface of about 1.5cm x 1.5cm [2], it is of great significance to avoid deterioration of the silicon wafers [15]. The Pirhana process can be used in wafer cleaning. Piranha solution, also known as Piranha etch, is a mixture of sulfuric acid ($H_2SO_4$), water ($H_2O$) and hydrogen peroxide ($H_2O_2$). It is used to clean the organic residues form the substrates [19]. Most of the organic matter is removed as the mixture is a strong oxidizing agent because the mixture is a strong oxidizing agent. This results in making the surface extremely hydrophilic.

$$H_2SO_4 + H_2O_2 \rightarrow H_2SO_5 + H_2O \qquad (1)$$

The sulfuric acid is mixed with hydrogen peroxide in the absolute ratio of 3:1 by volume. 75ml is added and transferred. 25 ml hydrogen peroxide ($H_2O_2$) is added to the acid ($H_2SO_4$).
The process of Piranha etching has the ability to remove the organic components form the silicon wafers. In order to improve the strip speed, the temperature of the mixture is leveled up to 130 degrees centigrade. The controlling on the mixing is quite difficult as the process is exothermic which produces heat. The temperature and the strip rate is maintained consistently therefore requires heating on a timely basis. Piranha solution is used frequently in the fields of microelectronics. Piranha solution should be prepared by adding hydrogen peroxide to sulfuric acid and never in reverse. The resulting solution is extremely exothermic in nature [29].

Fig 4: Pirhana Cleaning of Silicon Wafer

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*B.Etching of silicon wafer substrate*

The main aim to etch the cleaned silicon wafer is to make it porous that eventually increases the surface area of the substrate. The structure of the porous silicon is prepared by electrochemical dissolution of doped silicon wafers in hydrofluoric acid (HF) solution. The etching cell is used for the electrochemical etching. The silicon wafer acts as the anode. However, it is situated on the back-side of the contact, while the front side consists of an O-ring which is exposed to the anodising electrolyte. The cathode is made of platinum. The anodization cell is composed of a highly-acid resistant polymer i.e. polyvinylidene fluoride (PVDF) or polytetrafluoroethylene (PTFE). For the preparation of the highly resistive porous silicon substrate, it is very important to have an evaporated metal backside contact that ensures the Schottky contact between the semiconductor and the plate [26]. The most widely used application of an electrochemical etching cell is the fabrication of nanocrystalline porous silicon layer on a single crystal Si wafer. It fits perfectly with the aqueous (EPDM O-Rings) and organic solvent (FFKM O-Rings) electrolyte necessities.

To make an electrochemical process operate, a square conducting sample is placed on the cell base with a piece of aluminum foil as a back contact. The equipment setup is sealed with the help of an O-ring from preventing any sort of leakage from the electrolyte. The cell consists of an electrolyte and the electrode is immersed into the solution acting as a counter electrode. The electric current passes between the aluminum back contact and the electrode. As soon as the process gets completed the cell elements are washed several times with ethanol and dried under the nitrogen flow [27].

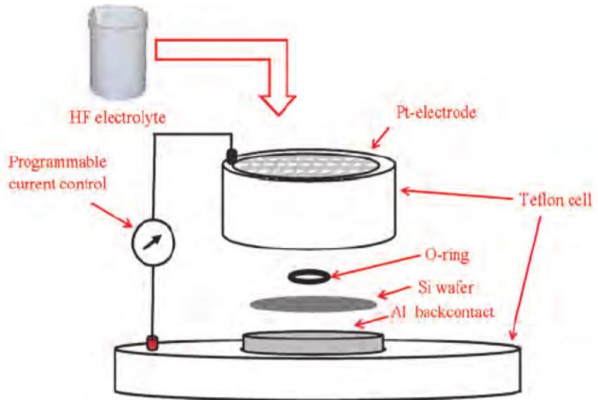


Fig 5: Electrochemical Etching Cell Setup

*C.Designing of IDE mask using AutoCAD*

AutoCAD is a commercial computer-aided design software application. It was developed and endorsed by Autodesk. The structure of interdigitated electrode has been designed using AutoCAD software. The parameters of electrode are shown in the figure, where L (15mm) is denoted by length of the IDE, d (0.5mm) represents the distance between the finger, W (8mm) is denotes the width of the electrode N represents the number of finger in the IDE.

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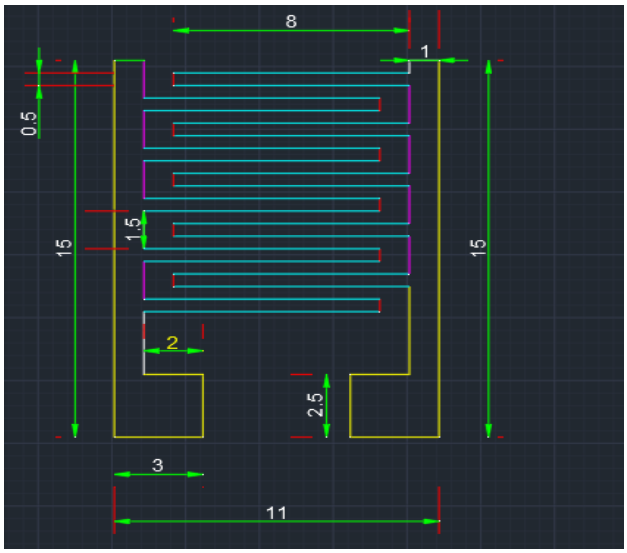


Fig 6: Designing of IDE Mask using AutoCAD Software.

*D. Fabrication of IDE using Photolithography Technique*

Other than the conventional techniques containing the optical and mass based properties of analyte, the technique of impedimetric transducer based on the interdigitated electrode (IDEs) results in an operation that is label free and quick identification of bacterial cells. The IDEs utilized as sensors have various advantages due to the design. Biosensors are of great significance in health care [23].

The traditional photolithography method is widely for the fabrication of IDE on the silicon wafer that acts as an electrochemical biosensor. Silicon wafer used is P-type. The removal of dust and dirt from the silicon wafer by RCA1, RCA2 and BOE and then cleaned by ethanol and then before dried air blown rinsed with distilled water [15]. It is really significant that the surface of silicon wafer is not polluted. The performance of the final device is enhanced through the insulating layer on the wafer [19]. Silicon wafer is treated under a high temperature. The process is called oxidation. The basic aim of this procedure is to develop an oxide layer on the surface of silicon wafer. Before the synthesis of IDE, mask has to be designed as it is useful for the better edge, finer resolution [15]. Photo mask are produced using AutoCAD software and converted to the commercial chrome mask [19].

The layer of aluminium settled down on surface of silicon via Thermal evaporator (PVD) vacuum coater. The process of lithography is used to produce well-defined pattern on a surface during this the synthesis of microelectrode sensor is done via photolithography technique. Under the process of photolithography the coating on positive photoresist is done on Al surface, with the help of the spin coating technique and pre heating at 100ºC for 1minute. This process is known as a Soft Baking. The aim of the mask aligner is to allow the UV light to pass through the Chrome mask for 10-15 sec. Further the silicon wafer substrate is dipped in RD6. The parts of the photoresist that is brought in contact of UV light were etched. Further, the sample is heated using a hot plate at a temperature of 100ºC which is known as Hard Baking, which inhibits the solidification of photoresist layer of aluminium surface. The sample is observed with HPM (High Power Microscope) for the verification of the supply of current between the two electrodes. Then, the sample is dipped in an aluminium etched solution. Finally the sample spin on acetone and IDE gets fabricated [15]. The fig 8 shows the fabricated interdigitated electrode on a silicon wafer using conventional photolithography technique. Due to its label free service, miniature structure and economic cost interdigitated electrode impedance biosensor is widely used in medical applications [10].

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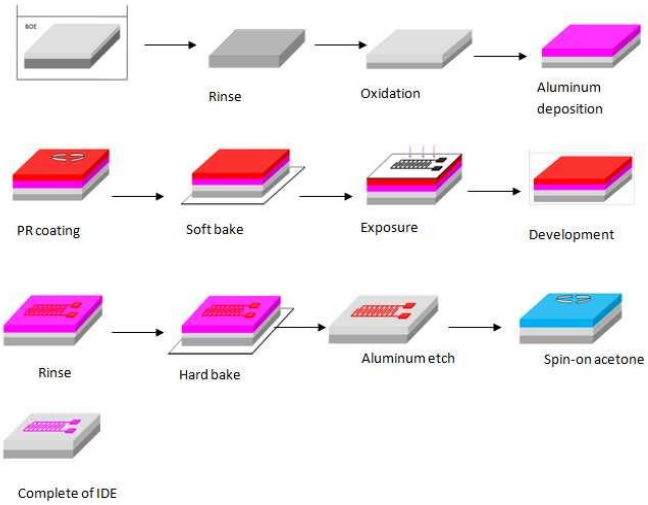


Fig 7 : Process flow of Fabrication of IDE Using Photolithography.

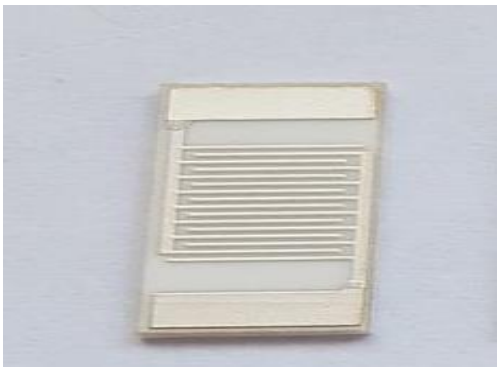

Fig 8: Fabricated IDE on silicon wafer

*E.Optimizing the EIS parameters of the IDE*

The Echem Analyst is data analysis software from Gamry Instruments. The experiment based on the generated data files in Gamry Framework then can be analyzed in the Echem Analyst. The Echem Analyst is a program that runs data analysis of the data for experiments like Electrochemical Impedance Spectroscopy and Physical Electrochemistry [28]. The equivalent electrical circuit of the corresponding interdigitated electrode was formed using the software.

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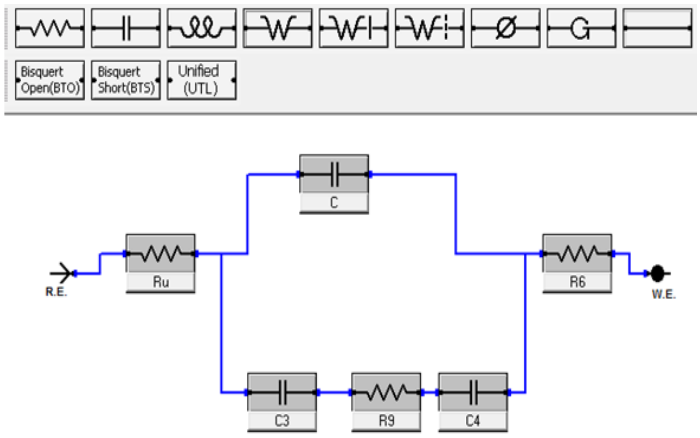


Fig 9: Designing of electrical circuit of the IDE

The electrochemical impedance spectroscopy plot consists of the impedance vs. frequency plot. The EIS plot was obtained as the circuit is simulated.

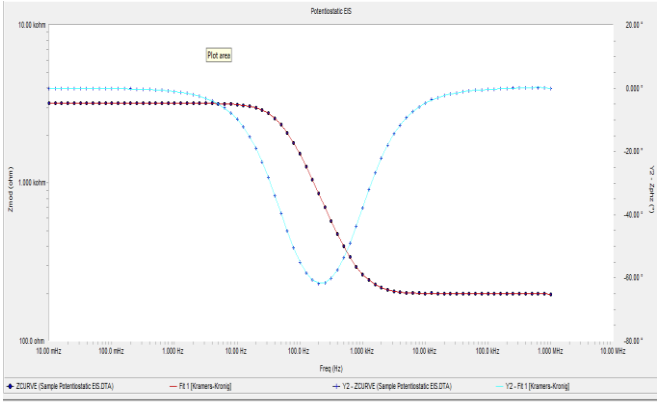

Fig 10: Impedance vs Frequency EIS plot of IDE

The Bode plot obtained in fig 10 signifies the relationship between impedance and frequency of the interdigitated electrodes. The logarithm of total impedance is plotted against the logarithm of the frequency. The plot is very crucial as it clearly shows the frequency dependence of the model designed.

## IV.PREDICTION ANALYSIS OF COVID-19 CASES USING MACHINE LEARNING

*Step1- Preparing dataset as input:*

The database of number of cases of Covid-19 with the days is obtained from [31]. The data taken is of Covid-19 cases in India from 27 February 2020 to 27 March 2020.

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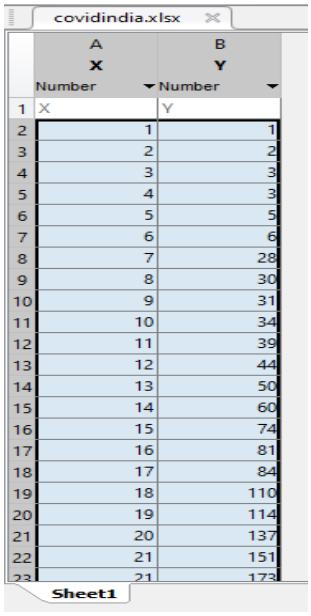

covidindia.xlsx

| | A<br>X<br>Number | B<br>Y<br>Number |
|---|---|---|
| 1 | X | Y |
| 2 | 1 | 1 |
| 3 | 2 | 2 |
| 4 | 3 | 3 |
| 5 | 4 | 3 |
| 6 | 5 | 5 |
| 7 | 6 | 6 |
| 8 | 7 | 28 |
| 9 | 8 | 30 |
| 10 | 9 | 31 |
| 11 | 10 | 34 |
| 12 | 11 | 39 |
| 13 | 12 | 44 |
| 14 | 13 | 50 |
| 15 | 14 | 60 |
| 16 | 15 | 74 |
| 17 | 16 | 81 |
| 18 | 17 | 84 |
| 19 | 18 | 110 |
| 20 | 19 | 114 |
| 21 | 20 | 137 |
| 22 | 21 | 151 |
| 23 | 21 | 173 |

Sheet1

Fig11: Raw Dataset.

*Step2- Applying Linear Regression:*

The data is fed into the system as input. Linear Regression is applied to the input data. The curve fits best into fourth degree polynomial curve. The regression equation is:

$$y=0.0026x^4-0.072x^3+0.68x^2+ 1.9x-6.5. \quad (2)$$

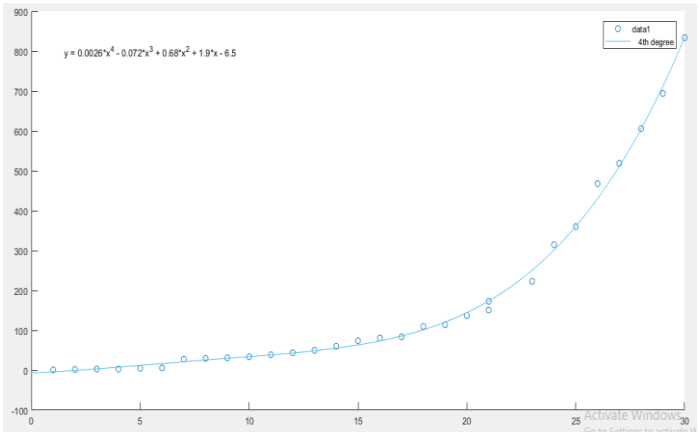


Fig12: Training the Data.

*Step3- Testing and Prediction analysis*

The test is performed by predicting number of Covid-19 cases in India after 31st day and 50th day from the 1st day initialized as input. The predicted number of cases after 31 days is 974 and after 50 days is 9139.

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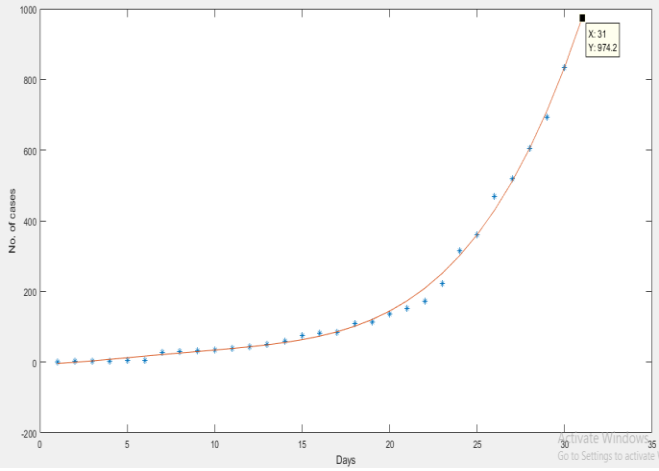


Fig 13: Testing on 31th Day.

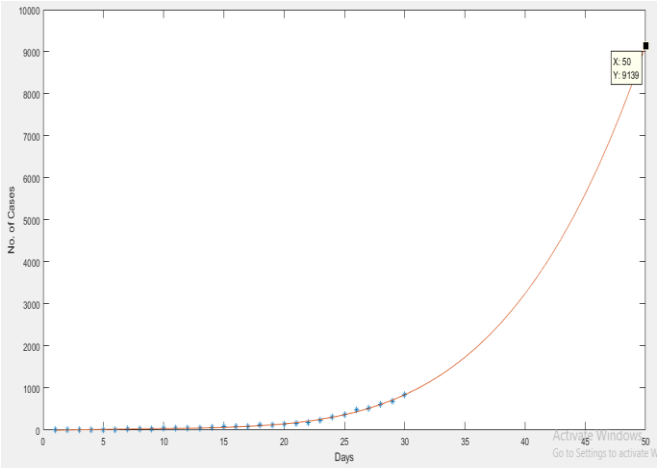


Fig 14: Testing on 50th Day.

## V. RESULTS AND DISCUSSION

The fabricated interdigitated based biosensor can be used in diagnosis of various abnormalities. The linear regression applied to the input dataset worked with great accuracy and predicted the number of Covid-19 cases on 50th day to be 9139. According to the report, the actual number of cases of Covid-19 on the 50th day i.e. 15April 2020 were found to be 11,933. This number is irrespective of the lockdown scenario and the number of recovered cases. This data can be used to estimate the number of cases that could be present if the lockdown of the country would end. The sensitivity of the prediction analysis on 31st day i.e. 28 March 2020 is 94.25% and that on the 50th day is 76.58%. The prediction analysis of Covid-19 cases in India using linear regression showcases the great performance of the model created. The sensitivity of the system can be increased if the input dataset is large and contains different attributes.

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Table 1: Prediction Analysis

| Day | Predicted No. of Cases | Actual No. of Cases |
|---|---|---|
| 31 day (28 March 2020) | 974 | 918 |
| 50day (15April 2020) | 9139 | 11,933 |

## VI. CONCLUSION

Interdigitated array electrode (IDE) sensor is fabricated by using conventional photolithography method. The material used for IDE sensor is silicon. The mask of the IDE is designed on AutoCAD. The Fabricated IDE based biosensor is used to detect various bacteria and viruses when the corresponding antibody is applied on the surface on IDE. The IDE as a biosensor has the ability to be more effective and cost-efficient when comparing to the traditional techniques. Further, biosensor can be used for detecting the pharmacogenetic biomarkers, genetical pathogens and nucleic acid based applications. There are various clinical applications of a biosensor that has the ability to detect the abnormalities like bacteria and viruses. The fabricated IDE can be used for the detection of E. Coli bacteria by applying the corresponding antibody.

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