Design and Fabrication of a Hydrogel Based pH Sensor Array for Physiological Applications

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Design and Fabrication of a Hydrogel Based pH Sensor Array for Physiological Applications

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Ayesha Gulzar

Dedication

Dedicated to my exceptional parents whose tremendous support and cooperation led me to this wonderful accomplishment.

Abstract

This study focuses on the design and fabrication of a hydrogel based pH sensor array for physiological applications. pH levels in the physiological environment is an important parameter in assessment of the normal functioning of the body. pH sensors such as pH glass electrodes, fiber optic pH sensors and sensors made from metal oxide has many limitations. To address these limitation, there is a need for planar, biocompatible, flexible and point of care pH sensor array that can conform with body organs and provide a map of pH levels. The development of this conductometric pH sensor array has been carried out on the basis of measurement of the conductance of a pH-responsive composite (Chitosan/Gelatin) hydrogel. The final design of the pH sensor array is dependent on the electrode density. Electrode density in a matrix is calculated and design is made using Proteus design suite 8.1 software. For fabrication copper conductive layer on a flexible polyimide substrate is used. Conductometric tests are carried out on pH sensor array and results show good sensitivity and resolution in the physiological pH range (pH 4 - 10). Sensor array is also able to provide a map of pH level for physiological monitoring.

Key Words: *Conductometric sensor, Flexible array, pH responsive hydrogel, Physiological monitoring*

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List of Abbreviations

mm	Millimeter
mV	Millivolts
µm	Micrometer
TiO2	Titanium dioxide
PANI	Polyaniline
HCl	Hydrochloric acid
PECF	Pseudo extracellular fluid

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CHAPTER 1: INTRODUCTION

The use of pH sensors is widespread in many fields such as biology, chemistry, medicine and material science and are currently used in applications such as blood pH measurements, wound pH, pH measurements in laboratory and many others (Korostynska, Arshak, Gill, & Arshak, 2007). pH homeostasis is very important for the normal functioning of human body. pH variation can be an indication of a disease or an alteration in human physiology such as inflammation, ischemia, tumor formation and atherosclerotic plaque development. In research, pH of the ex-vivo tissues and bulk environment of cell cultures are monitored to calculate the metabolic state of their preparation. Instability in levels of pH is associated with metabolic abnormality (Chung et al., 2014).

Surface of the human skin shows acidic milieu in normal conditions and ranges between 4-6 that varies depending on anatomical region and age of the person. This acidic pH of the skin acts as first line of defense against microorganisms (Khan, Ansari, & Ali, 2015). In medicines, wound healing is still an important concern. Many factors affect the process of wound healing, such as pH, moisture level, enzymes, temperature and oxygen level. Among these factors, monitoring the pH variations during different stages of the healing process is considered as the most critical factor. Wounds can be categorized in two broad classes namely acute and chronic wounds and pH alteration depends on the type of the wound during healing process (Omidi, Yadegari, & Tayebi, 2017) .

A lot of data has been reported on range of the pH levels of wound. The reported pH range of the wound is 5.45 – 8.9 (Milne, Connolly, Al Hamad, & Seoudi, 2014). The large pH range is significant as it effects all the stages of the wound healing. The process of wound healing comprises several stages, namely hemostasis, inflammation, migration, proliferation and maturation (Baum & Arpey, 2005). However, this range of pH mainly depends on the type of the wound (Omidi et al., 2017) i.e., either acute or chronic wound.

The pH levels in chronic wounds is considered as an important marker for assessing the healing process. Healthy skin and healing acute wounds often exhibit acidic pH in the range of 5.5-6.5, whereas values higher than 7.4 are often exhibited by infected chronic wounds. This high alkaline pH of chronic wounds is due to the byproducts secreted by the bacterial colonies proliferating in such wounds (Rahimi et al., 2016). Research have shown that healing process starts by lowering the pH in chronic wounds. Reduction in wound pH towards acidic range results in reduced levels of MMP and bacterial proliferation along with an increase in oxygen level in wounds (Milne et al., 2014).

The field of wound treatment and management using analytical measurement tools for assessing healing parameters such as pH of the wound, moisture level, wound size and protease level is still in its infancy. These analytical measurements techniques will help in effective and efficient wound healing process.

Peppas defined hydrogels as "hydrophilic, three-dimension networks, which are able to imbibe large amounts of water or biological fluids, and thus resemble, to a large extent, a biological tissue". Hydrogels are capable of swelling in aqueous media and can thus absorb large quantities of water. Since the major component of human body is water, therefore polymer hydrogels are considered to have great scope for biomedical and pharmaceutical applications. A broad range of research has been going on recently on hydrogels and their application into different fields such as biosensors, tissue engineering, drug delivery, hemostasis bandages etc. Hydrogels have increased advantage of biocompatibility, biodegradability, porous structure, tunable and can be manufactured in desired shape, mechanical strength and so on, as compared to other types of biomaterials (Chai, Jiao, & Yu, 2017).

Hydrogels are sensitive to a wide range of physical factors such as pH, temperature, salt concentration, electrical voltage and concentration of organic compounds in water. These hydrogels are known as stimulus responsive hydrogels and such hydrogels can be used in development of biosensors (Guenther & Gerlach, 2009).

Stimulus responsive hydrogels exhibit desirable response by changing their shape or volume when exposed to specific stimulus. One such group among them is pH responsive hydrogels. Desired chemical and physical properties are exhibited at specific pH ranges by such hydrogels. pH responsive hydrogels have acidic and basic groups in their polymer chains which undergoes protonation and deprotonation. At high pH, acidic groups undergoes deprotonation whereas at low pH, basic groups protonate. Hydrogel swells in an aqueous media via association and dissociation along with the binding of other ions to polymer chain (Chai et al., 2017).

During swelling of pH-responsive hydrogels, these functional groups dissociate and there is an influx of counter-ions which causes an increased ion concentration inside the hydrogel than in the surrounding aqueous medium. This ionic imbalance creates an osmotic pressure difference and as a result solution flux into the hydrogel (Gerlach et al., 2005). Electrostatic repulsive forces are generated as a result of ionization of pendent groups and development of fixed charges on polymer network causing swelling and de-swelling of pH responsive hydrogel in an aqueous media (Koev et al., 2010).

During hydration, while the polymer chains are expanding, there is a counter retractive force or an elastic restoring force that is generated by the polymer chain to maintain its 3D structure. When the expanding force balances the restoring force, an equilibrium is attained known as “Donnan equilibrium” or Quasi-equilibrium” and the hydrogel is said to have achieved maximum swelling (Gerlach et al., 2005).

As already has been discussed that pH level in a physiological environment is an important parameter for assessing the normal functioning of the body, wide range of sensors are being developed for measuring pH. These include glass pH electrode, metal oxide sensors, fluorescent sensors and conductometric sensors. However, these sensors have their drawbacks such as glass pH electrode are fragile being made of silicon glass. Its fragility hinders it being miniaturized for biological purposes (Milne et al., 2014). It also provide single point measurement thus limiting real time efficiency and 2D mapping of the wound.

We have proposed a more practical approach towards pH monitoring in the physiological environment. A flexible array of conductometric pH sensors have been developed based on Chitosan/Gelatin composite hydrogel. This pH sensor array is a point of care technology that is flexible and can generate a map of pH levels in a physiological environment providing real time therapeutic feedback.

CHAPTER 2: LITERATURE REVIEW

2.1 Glass pH electrodes

pH sensors have been developed using conventional glass electrodes. pH electrode ranges are assessed by correlating the values in a sample with the values of buffers whose pH has already been determined. A potential is changed across the surface membrane whenever the pH electrode is in contact with sample. Thus a reference electrode is used as it provides a uniform and constant potential to assess the variations of the membrane potential. Thus pH electrodes consist of a reference electrode along a sensing electrode incorporated into the one electrode body. Combine, the reference and the sensing electrode contribute to the selectivity and the response that is similar to that of hall cell system but in addition provides the benefit of handling and sustaining one electrode body. A meter measures the difference among the potentials of the reference and the sensing electrode and give values in millivolts (mV). The meter also functions as a converter from millivolts to pH values and displays them on the meter screen (Thermo Scientific, pH Electrode Selection Handbook).

Glass electrodes have several above mentioned advantages but on the other hand it has some disadvantages also. Glass electrodes have high sensitivity towards alkaline or HF solution. Moreover, they need a high input impedance pH meter and sometimes they repeatedly display a slow feedback. Glass electrodes are also challenging to be used in several application including the *in vivo* biomedical or food monitoring applications because of the limitations of size miniature, planarization and polymerization (Huang, Cao, Deb, Chiao, & Chiao, 2011). Thus as a resultant increased number of the extensible pH sensor with size scaled down have been accomplished to overcome the restrictions caused by the glass electrode.

2.2 Metal Oxide Conductometric pH Sensors

Metal-metal electrodes upon their immersion into a solution change their potential with changing pH. According to the description by Ives, these metal-metal pH electrodes are considered to be of second kind because only one simple reaction is not sufficient for hydrogen ion response but there is a second reaction that takes place after the primary electrochemical process. The first metal-metal oxide pH sensor introduced as a substitute to the conventional glass pH electrode is the antimony electrode after which palladium and iridium metal-metal oxide electrodes are also reported (Głab, Hulanicki, Edwall, & Ingman, 1989). A relation between pH and potential of antimony electrodes have been investigated by many workers. According to the reports a linear pH-potential response is found. However, significant variations about the slope and as well as the standard electrode potential have been found in the reported values. Recent metal oxide conductometric pH sensors include titanium dioxide (TiO2)/cellulose/multiwall carbon nanotube (MWCNT) hybrid nanocomposites (Chen, Mun, & Kim, 2013).

However, sensors made of metal oxide have limited use in biomedical or biological application because of their toxicity (Isa, Hamzah, Sabian, & Ab Ghani, 2012).

2.3 Hydrogel based pH sensors

Ruan et al. presented another type of pH sensor based on a reversible, mass-changing pH responsive hydrogel. By increasing the amount of acrylic acid in the copolymer, poly ((acrylic acid-co-isooctyl) acrylate, increased sensitivity is achieved. Polyurethane layer is pre-coated over the sensor along with the addition of 1-(3-dimethylaminopropyl)-3 ethyl-carbodimide into hydrogel solution to enhance adhesion. Output is recorded in form of frequency of the output signal.

An extremely sensitive micro-mechanical pH sensor is presented by Bashir et al. The basic working principle of the sensor is the measurement in the deflection using an atomic force microscope. The sensor is able to detect a change of 5×10^{-5} pH. Cantilever is covered by a layer of a polymer. Changing pH, changes the surface stress and as a result polymer expands. This expansion of polymer results in bending of the cantilever and the deflection is recorded (Deligkaris, Tadele, Olthuis, & van den Berg, 2010).

A passive wireless sensor has been reported by Sridhar and Takahata for real time pH monitoring of the wound. This consist of an inductive transducer system which can employ hydrogels belonging to different class. A coplanar dual spiral coil is folded for the design of the transducer. For the transducer fabrication a thick copper (50 µm) covered polyimide film is used. The inductance is influenced by the distance between the coils whereas a hydrogel is placed between the two coils for the gap modulation. pH variations results in change in hydrogel dimensions. Displacement versus inductive response of the transducer is found to be linear (Khan et al., 2015).

2.3.1 Hydrogel based piezoresistive sensor

M. Guenther and G. Gerlach established a hydrogel based piezoresistive sensor. The working principle of this sensor is based on pressure sensor chips to which a thin flexible silicon bending plate is attached. A piezoresistive Wheatstone bridge is integrated into the system as a mechano-electrical transducer. A deflection of the plate is then transformed into an electrical output signal. Liquid solution that is to be measured is introduced in the chip cavity via inlet channels. This aqueous solution results in either swelling or shrinkage of the hydrogel which results in bending of the flexible silicon plate. This bending corresponds to electrical output voltage of the sensors.With the combination of micro scale pressure sensors in a hydrogel, the system can monitor the swelling of the hydrogel when submerged in an aqueous medium. This systems enable for a continuous recording of readings as the hydrogel undergoes swelling process. Studies are conducted for finding a correlation between the ionic compositions of the aqueous medium and the swelling ratio of the hydrogel. It has been shown in the studies that both composition of polymer and the degree of crosslinking effects the sensitivity (Guenther & Gerlach, 2009) .

2.3.2 Conductometric pH sensors

The development of conductometric sensors, based on a pH responsive hydrogel is pioneered by Norman. F. Sheppard, who is successful in designing a conductometric sensor on the basis of swelling/de-swelling phenomena of pH responsive hydrogels. Conductometric results of this work correspond to the fact that ionic mobility is induced into the hydrogel when it is immersed into an aqueous solution and the conductivity values are as a result of that ionic mobility and the pH responsive nature of the hydrogel. Different compositions are tested and their conductivity over pH graphs are recorded (Sheppard, Tucker, & Salehi-Had, 1993).

2.3.3 pH sensor array

The next advancement in pH sensory arrays is just not to be flexible but to provide better 2D and 3D resolution as well as real time results. The work done by Chung-We Pan and his teammates in “Development of real-time pH sensing system for array sensor”, makes use of films made of tin oxide as the base membrane for the fabrication of sensor array.

This method applied for the real time monitoring of the system. The additional use of sensory array provided better local point and multi point pH results, which can be further used to compare the result between different regions and provide statistical analysis. The paper showed the successful use of tin oxide sensor array as it provided signals from 8 point simultaneously which reduced significant time consumption. Electronic circuit integration is added at the back end to reduce noise and to amplify the signal coming from the sensors. The surface characteristics are studied using X-ray diffraction and Scanning probe Microscopy. However, these real time sensors still have a few flaws which needs to be addressed such as the effect of drift and hysteresis as well as temperature effect (Pan, Chou, Sun, & Hsiung, 2005).

2.3.4 Sensor array on flexible substrates

For the past few years’ research has been focused on sensor fabrication on flexible substrates and their miniaturization. Sensors that are majorly studied are potentiometric and ion sensitive field effect transistors (ISFETs) (Lee & Cui, 2010). Multiple polymeric substrates like polyimide, parylene and polyethylene terephthalate (PET) are used for their fabrication. Sensitivity, resolution and properties like dynamic range are acceptable for majority of these sensor. But the time consuming and costly processes of cleanroom-based fabrication are still an issue (Chin et al., 2001).

In 2011, Schreml and his colleagues used luminescence imaging of fluorescein isothiocyanate (FITC) and ruthenium (II) tris-(4,7-diphenyl-1,10-phenanthroline) for two dimensional pH mapping .A 5µm spatial resolution is used for this technique. Formulation and immobilization of indicator dyes for this technique required intricate and multistage chemical manipulations; which is a major issue with this process (Harrison & Walker, 1979; Schreml et al., 2011).

In 2012, a group of researchers used chemo-mechanical transduction force to generate a pH sensor. A sensitive hydrogel is wrapped around a metallic wire and changes in pH of region resulted in either shrinkage or swelling of the hydrogel which led to a change in impedance of wire. This combination is significantly compliant and wearable but it's conversion into an array posed major issue of damage to hydrogel while array formation (Nocke, Schröter, Cherif, & Gerlach, 2012).

2.3.5 Paper based sensor array

A cost effective and flexible sensor array has been developed by Rahimi and his coworkers. Each sensor is a pair in this array consisting of two electrodes. An Ag/AgCl reference electrode and a carbon electrode. Carbon electrodes also contained a coating of a polymer known as polyaniline (PANI). The working principle for these sensors is based on the protonation/de-protonation of generated emeraldine salts form of PANI. This form has more electrical conductivity. This surface charge leads to an increase in the electrical potential of the sensing electrode as compared to the reference electrode. In case of alkaline environments, acquired hydrogen ions are neutralized leading to a reduced charge on the polymer surface. When emeraldine salts are de-protonated, they become emeraldine bases and lose their electrical conductivity. This equilibrium between emeraldine salts and emeraldine bases is pH dependent and it leads to an inverse relationship electrochemical gradient of sensing electrode and pH of the surrounding environment.

Fabrication process of this sensor involves attachment of an adhesive tape on palette paper. Through laser cutting technique, it is formed into a stencil mask. This led to ablation of cellulose acetate backing of the tape. The tape covered regions of palette paper are screen printed with carbon and silver inks. After curing at 100°C for 30 minutes, nine carbon and three sliver electrodes are obtained. Silver electrodes are subsequently coated with a layer of silver chloride. A mixture of KCl and a UV curable epoxy is used as reference electrode electrolyte. This is cast onto Ag/AgCl reference electrodes and cured in ultravoilet light. PANI solution is casted onto carbon electrodes and are left to dry cured. After this, it is vaporized with HCl to add hydrogen ions into film of polyaniline emeraldine base. The substrate is folded to form an array containing 9 pH sensors. Using a hot roll laminator, the interconnections between them are sandwiched between two layers of palette paper. Thicker PANI is good for sensor working but it also increased the intricacy and duration of fabrication. In addition, there is micro scale roughness on surface due to KCl particles and leakage of electrodes in this system (Rahimi et al., 2016).

We have proposed a more practical approach towards pH monitoring in the physiological environment. A flexible array of conductometric pH sensors have been developed based on Chitosan/Gelatin composite hydrogel. This pH sensor array is a point of care technology that is flexible and can generate a map of pH levels in a physiological environment providing real time therapeutic feedback

Chapter 3: METHODOLOGY

Design of the hydrogel based conductometric pH sensor array is dependent upon the electrode density which is the maximum number of sensors in a matrix. To calculate the electrode density, size of the conductive sensor port and separation between conductive sensor ports in a sensor pair, are optimized. A high electrode density results in high resolution and better pH monitoring.

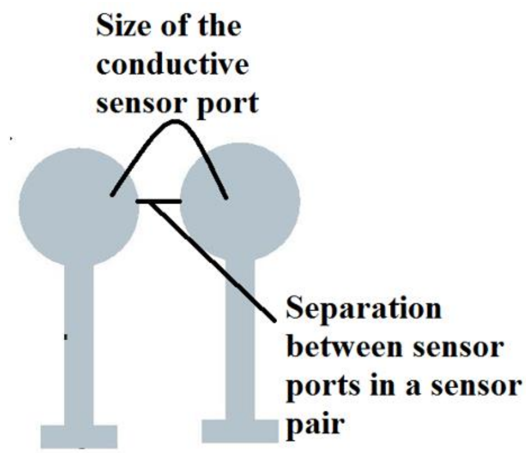

Figure 3. 1. Shows conductometric sensor

3.1 Size optimization

Different sizes of the conductive sensor port are tested i.e. 2.5mm, 4mm and 5mm sensor size. Sensor port size is selected based on the best sensitivity and response time.

3.2 Separation optimization

Separation between conductive sensor ports in a sensor pair are tested. Different separations from 3mm-1mm are tested and results are recorded.

Separation between sensor port in a pair is selected based on the sensitivity, response time and fabrication constraints.

3.3 Design of pH sensor array

Input obtained from size and separation studies led to the final design of the pH sensor array. Final design of pH sensor array is made using Proteus 8 professional. The pH sensor array consists of 8 pairs of sensors in planar configuration.

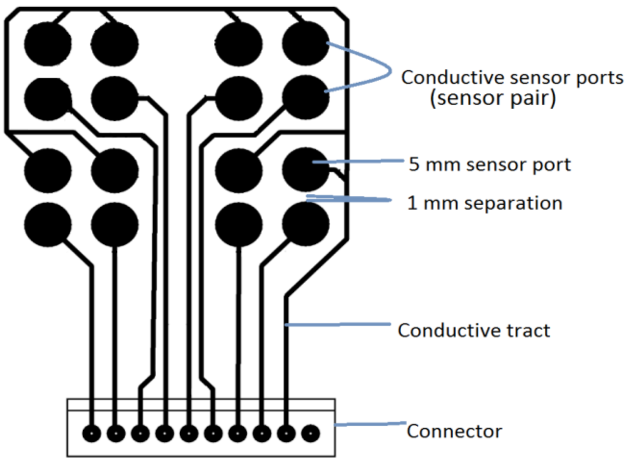

Figure 3. 2. Shows the design of conductometric pH sensor array

3.4 Fabrication of pH sensor array

Initially pH sensors in planar configuration are made using adhesive copper conductive layer on polyimide substrate. 3D printer and laser machine are used for stencil preparation. Size and separation studies are carried out on these sensors.

3.4.1 Rigid pH sensor array

This final design of the pH sensor array is then replicated on a hard pcb for the testing of the pH sensor array. Etching technique is used for fabrication of hard pcb of sensor array. Masking of the tracts is done via polyurethane to protect the conductive sensor tracts from the oxidation and exposure to immersion medium. The circular port is the active part of the hydrogel and contains pH responsive hydrogel.

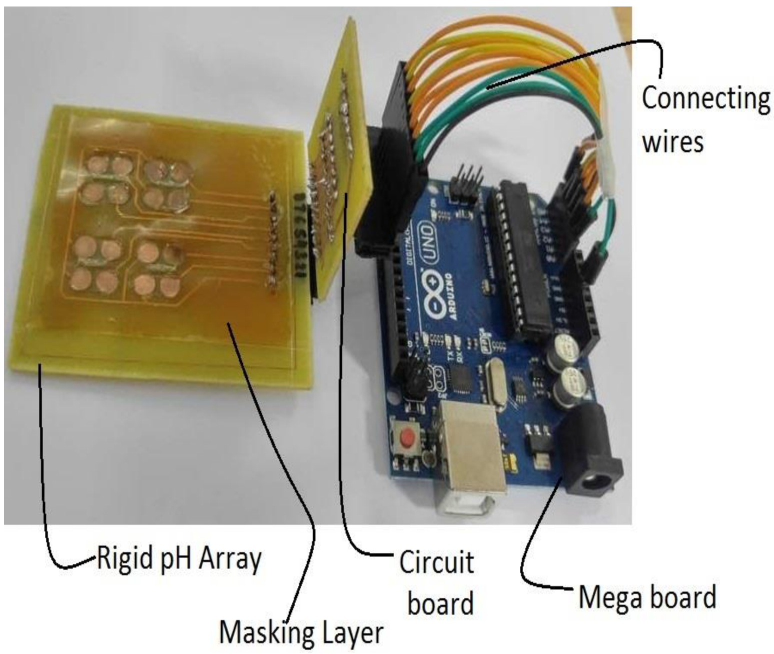

Figure 3. 3. Shows Chitosan/ gelatin rigid pH sensor array with polyurethane masking layer

3.4.2 Flexible pH sensor array

After rigid pH sensor array is tested, the next step is the fabrication of flexible pH sensor array. Same design is printed on flexible substrate. The flexible pH sensor array is fabricated on polyimide substrate which is biocompatible material. The flexible pH sensor array is tested and results are obtained.

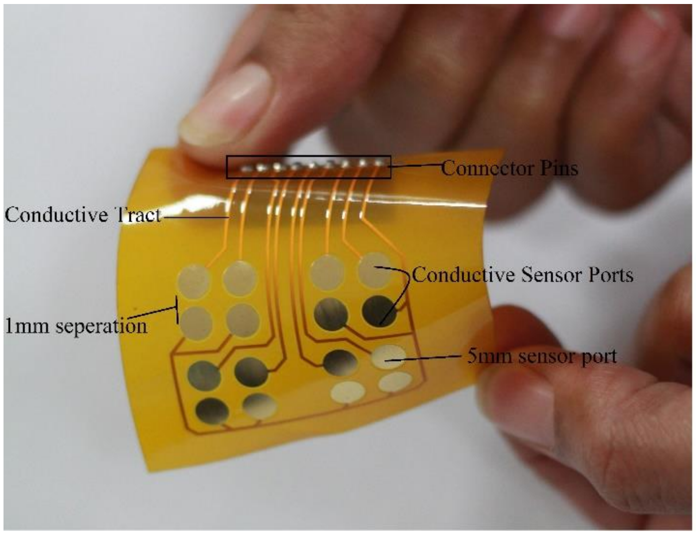

Figure 3. 4. Shows Chitosan/ Gelatin conductometric pH sensor array on flexible substrate

3.5 Synthesis of Chitosan/Gelatin Composite Hydrogel

Chitosan solution (2% w/v) is prepared by dissolving pre-weighted amount of Chitosan in 1% acetic acid (adjusted at pH 4) and stirring the solution for 2 hours at 30°C on hot plate. 7% gelatin solution is made in distilled water at 55°C. After preparation of both the solutions, 5ml chitosan is mixed in 10 ml of Gelatin. Pre-calculated values of Gluterladehyde (0.25%) is mixed into the 1:2 Chitosan/Gelatin solution at stirring. Glutaraldehyde is added as a cross linker (Yamada et al., 2000). Casting of the composite hydrogel is done via metered syringe over planar sensors in a semi cured form and left for curing for 8 hours at room temperature.

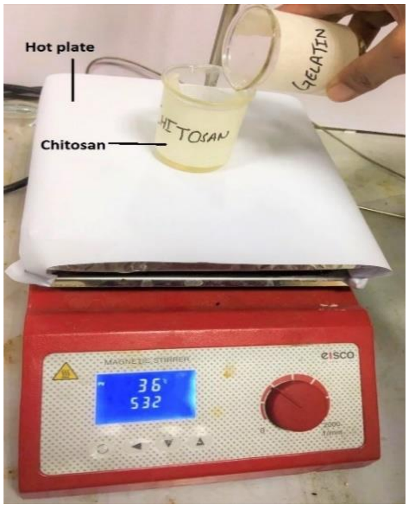

Figure 3. 5. Shows gel preparation

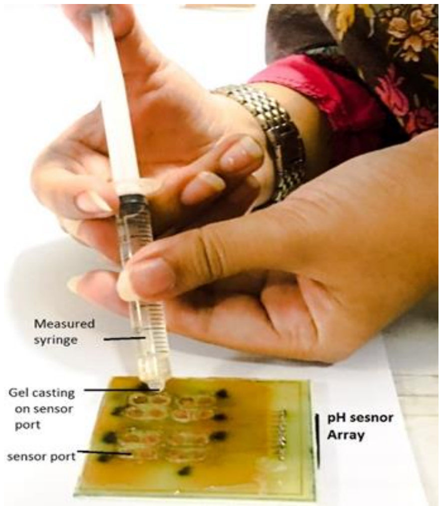

Figure 3. 6. Shows casting of hydrogel on conductive sensor port of pH sensor array

3.6 Conductometric Analysis

Pseudo Extracellular Fluid (PECF) solution is used for the conductometric tests of the pH sensor array. The PECF solution consisted of 1.1g KCl, 3.4g NaCl, 12.5g NaHCO3, 1.75g NaH2PO4 in 500 ml of de-ionized water (Lin, Chen, & Run-Chu, 2001; Şen & Avcı, 2005). Each sensor is immersed in 1ml PECF solution of specific pH (4, 6, 8, 10). Conductance is measured at 1 sec after immersion into PECF between each pair of sensor. Sensors are configured with a variable resistor circuit; an Arduino mega microcontroller is used to record the conductance values. Each experiment is repeated three times to minimize error. The graphical interpretation of the results is shown in figures (conductance versus pH box-plots) for size, separation and array studies.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Size optimization

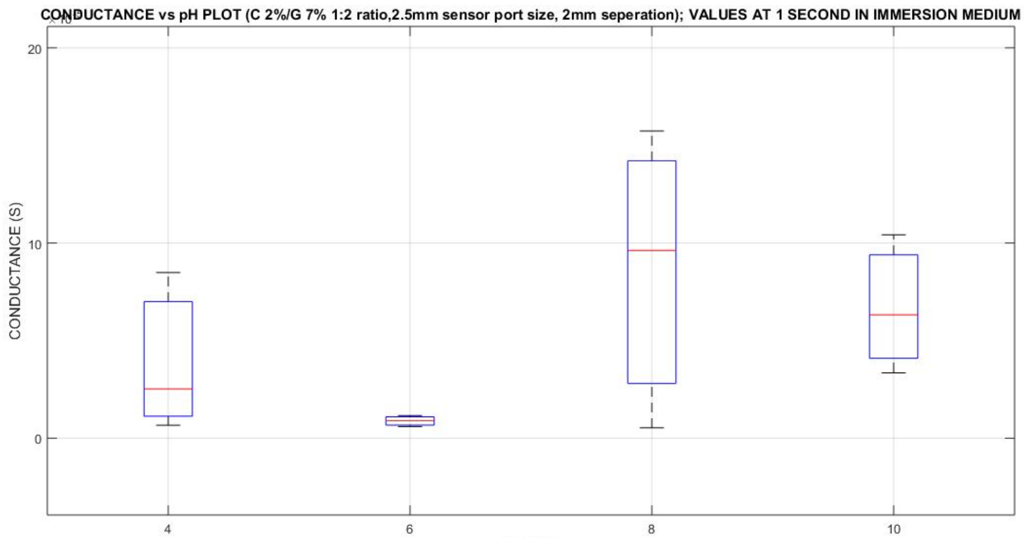

Figure 4. 1: Conductance box plot for pH 4, 6, 8, 10; Conductance against pH values are shown for sensor size 2.5 mm

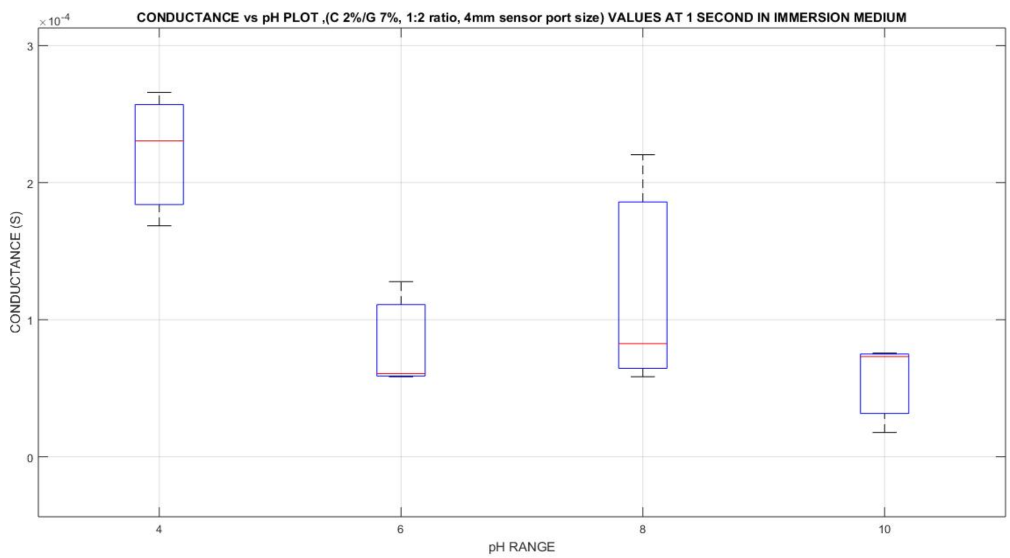

Figure 4. 2: pH – Conductance box plot for pH 4, 6, 8, 10; Conductance against pH values are shown for sensor size 4 mm

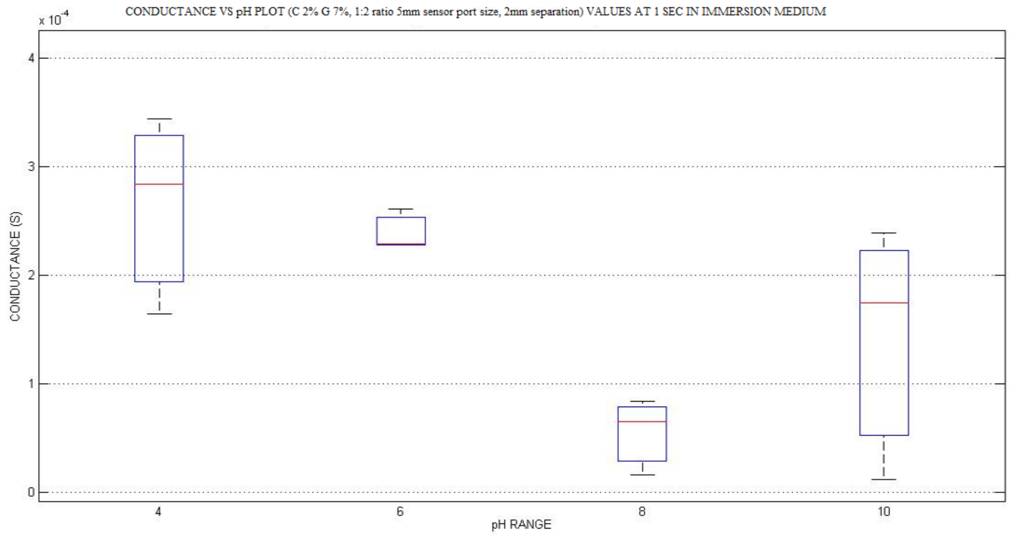

Figure 4. 3: pH – Conductance box plot for pH 4, 6, 8, 10; Conductance against pH values are shown for sensor size 5 mm

Conductometric analysis results for different sensor sizes have been shown in the box plot between the pH on x-axis and conductance on y-axis in figure 4.1- 4.3. For the design of the sensor array, the first parameter that needed to be optimized is the size of the sensor. For this purpose, different sizes are tested to find the sensor size that has best sensitivity and response time. Figure 4.1- 4.3 shows the boxplots for sensor size 2.5mm, 4mm and 5mm respectively. This conductometric sensor exploits the changes in electrical properties of a hydrogel film deposited on electrode. The box plot shows the spread of data variation and distribution. The standard box plot graph obtained from previous work done by Mariam Mir et al on the hydrogel composition shows high conductance values at acidic pH whereas low conductance values at lower pH. The results obtained from sizes 2.5mm and 4mm do not follow the general trend i.e. there is no linearity or sensitivity in these two sizes and overlapping of data can be seen in these box plots. The fig 4.3 shows the box plot for 5mm sensor size. This sensor size follow the standard trend i.e. high conductance at pH 4 whereas the conductance values are reduced as the pH moves towards alkaline. The box plot of 5mm sensor size shows no overlapping of data and conductance values are measureable for each pH input.

4.1.1 Sensor sensitivity for size

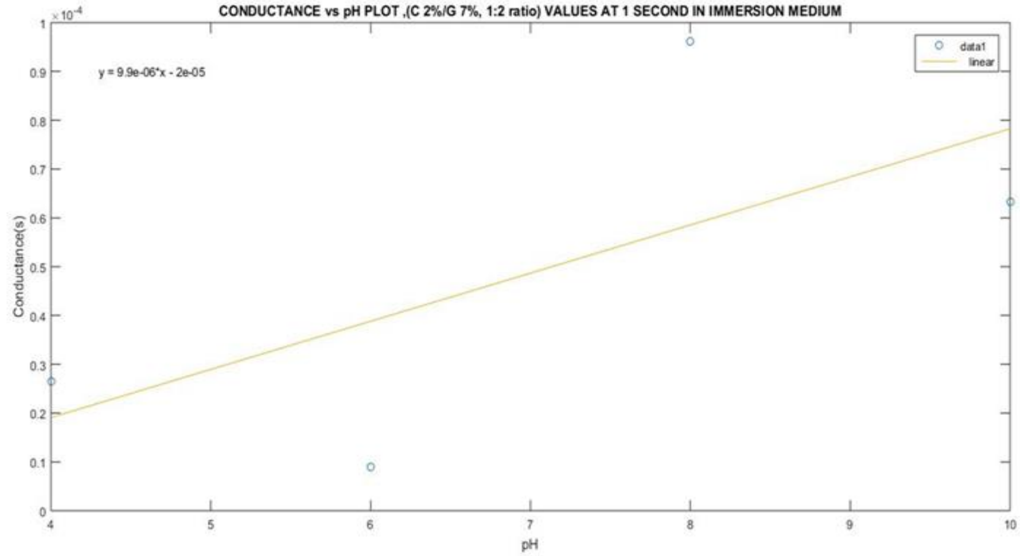

Figure 4. 4: pH-Conductance linear function for pH 4, 6, 8, 10; Sensor sensitivity for 2.5 mm sensor size

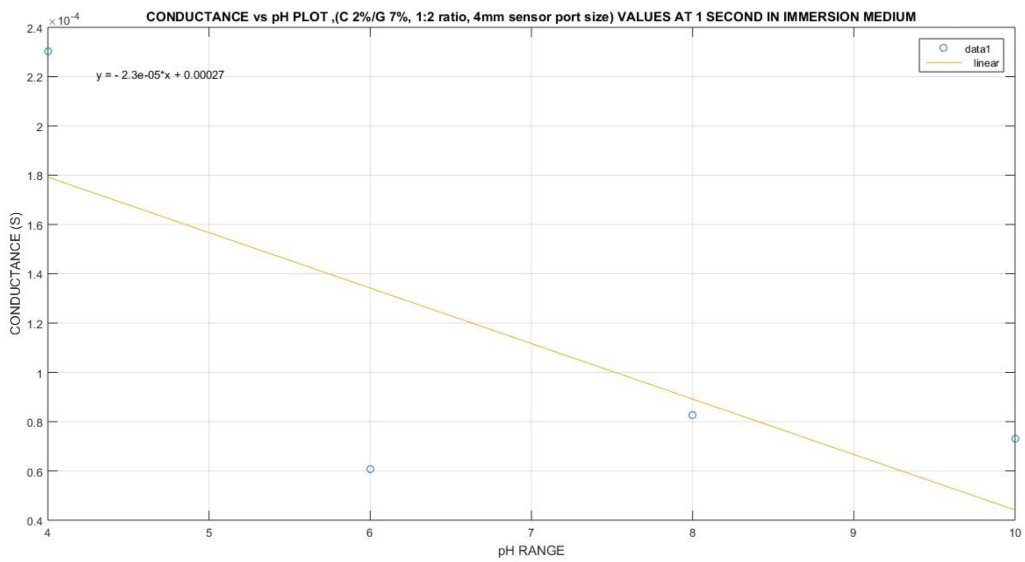

Figure 4. 5: pH-Conductance linear function for pH 4, 6, 8, 10; Sensor sensitivity for 4 mm sensor size

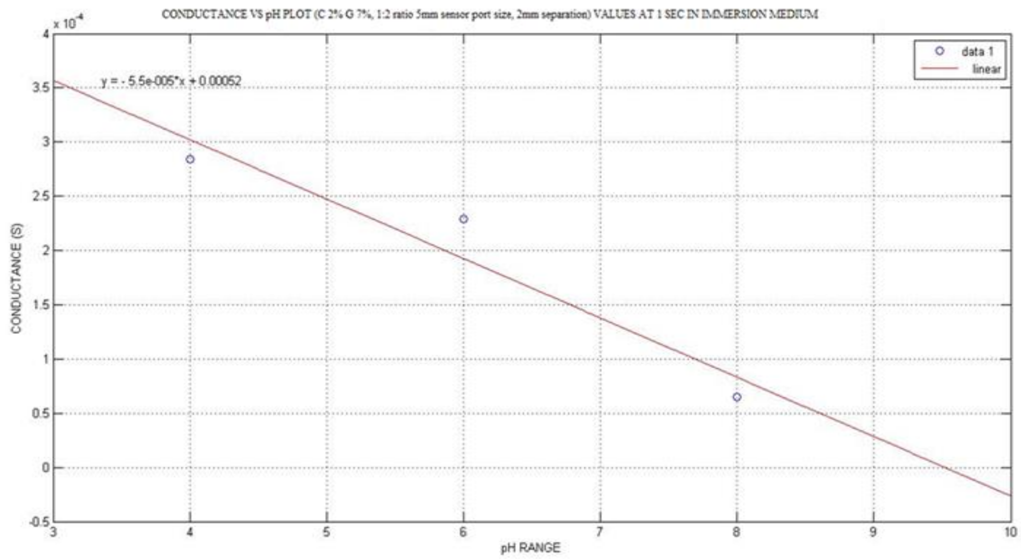

Figure 4. 6: pH-Conductance linear function for pH 4, 6, 8, 10; Sensor sensitivity for 5 mm sensor size

Figure 4.4, 4.5 and 4.6 represents the linear functions for sensor size 2.5mm, 4mm and 5mm. These linear functions gives the sensitivity of data sets for different sensor sizes. The linearity of the slope shows the sensitivity of the sensor. Regression coefficient is obtained for all data sets. The sensitivity of sensors depends on slope of regression line. In figure 4.4, 0.4294 regression value shows very low sensitivity for 2.5mm sensor size. Fig 4.5, shows the sensitivity for 4mm sensor size with 0.5325 regression value. In fig 4.6, a high regression value of 0.9233 shows best sensitivity for 5mm sensor size. 5mm sensor size shows the highest change in output in response to the smallest change in input which lead to a higher slope for the regression line.

Table 4-1: Comparison of sensor sensitivity and linearity of response

BATCH	SIZE(mm)	SEPARATION (mm)	SPAN(pH-range)	SENSITIVITY	Regression Coefficient
A	5	2	4-10	0.000055	0.4294
B	2.5	2	4-10	0.000023	0.5325
C	4	2	4-10	0.0000018	0.9233

This table shows the comparison of sensor sensitivity and regression coefficient of different sizes of sensors. 5mm sensor size shows best sensitivity and has a high regression value. As the sensor size dictates the electrode density in array, this size is selected on the basis of high sensitivity, shortest response time which is 1sec.

It has been reported in previous works that both response time and sensitivity of the signal is dependent on sensor size (port size, amount of gel). A reduction of sensor size leads to enhanced signal and response but loss in sensitivity at smaller sizes. This is due the fact that at smaller sensor sizes, Quasi equilibrium state of the hydrogel is attained at a faster rate. As a result the difference between the conductances for respective pH are not measurable and hence sensitivity is lost.

4.2 Separation optimization

Next study in the designing of sensor array is the optimization of distance between the conductive sensor ports in a sensor pair. Separation between sensor ports dictates the electrode density in designing of the pH sensor array. Different separations are tested and results are obtained.

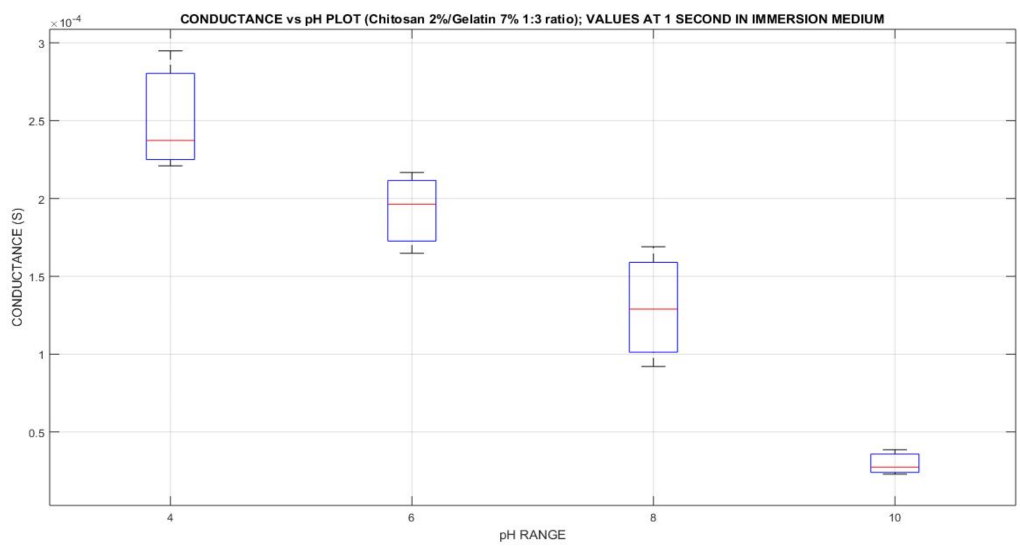

Figure 4.7: pH-Conductance box plot for pH 4, 6, 8, 10; Conductance against pH values are shown for 3mm sensor port separation

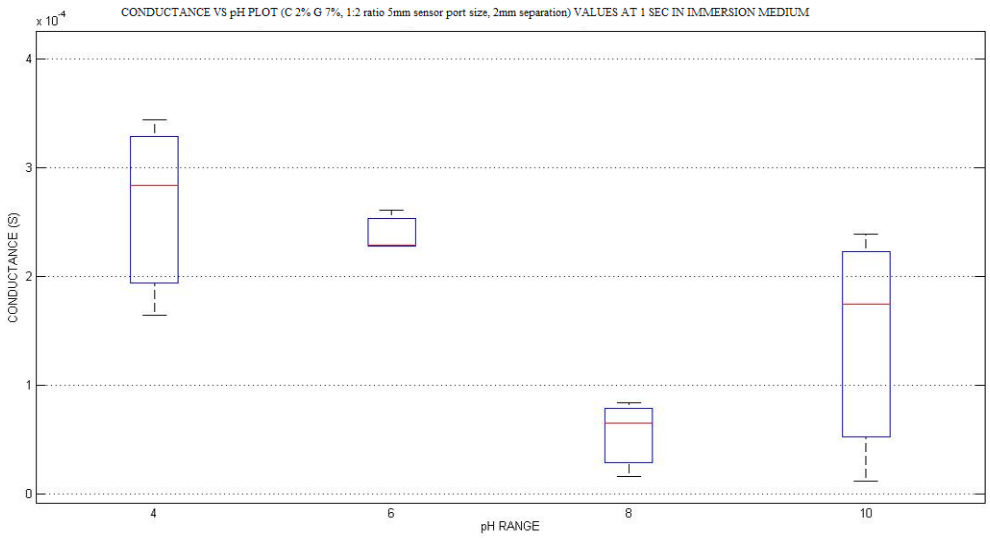

Figure 4.8: pH-Conductance box plot for pH 4, 6, 8, 10; Conductance against pH values are shown for 2mm sensor port separation

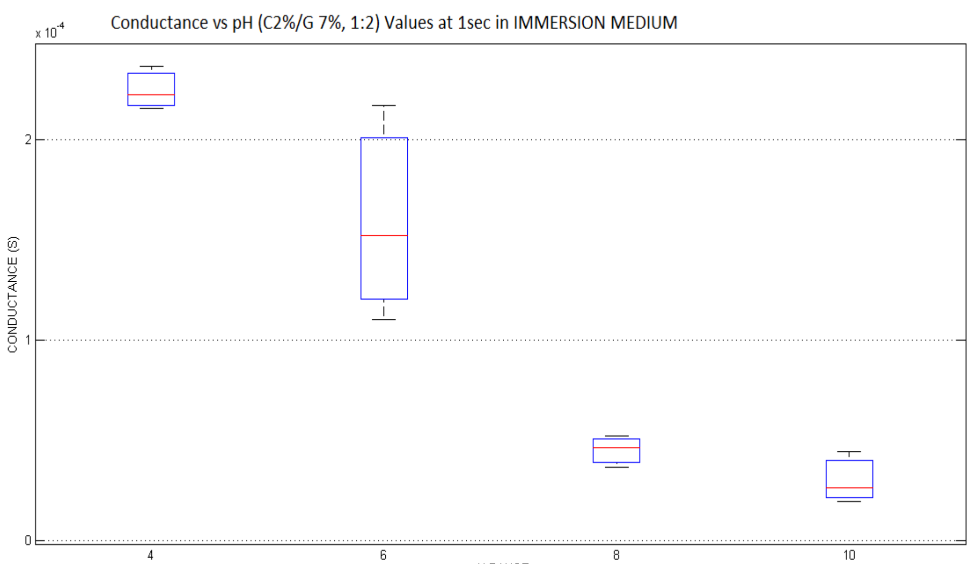

Figure 4.9: pH-Conductance box plot for pH 4, 6, 8, 10; Conductance against pH values are shown for 1mm sensor port separation

Fig 4.7 - 4.9 shows the conductometric analysis results in box plots for different separation between the conductive sensor ports in sensor pair with conductance on y-axis and pH on axis. Three different separation are tested i.e. 3mm, 2mm and 1mm. Figure 4.7,4.8 and 4.9 represents box plots for 3mm, 2mm and 1mm separations respectively. Box plots obtained for all three separation shows good conductance values versus pH. 1mm separation between conductive sensor ports in a sensor pair show best sensitivity and response shown in fig 4.9. There is no overlapping of data and a linear response is shown.

4.2.1 Sensor sensitivity for separation

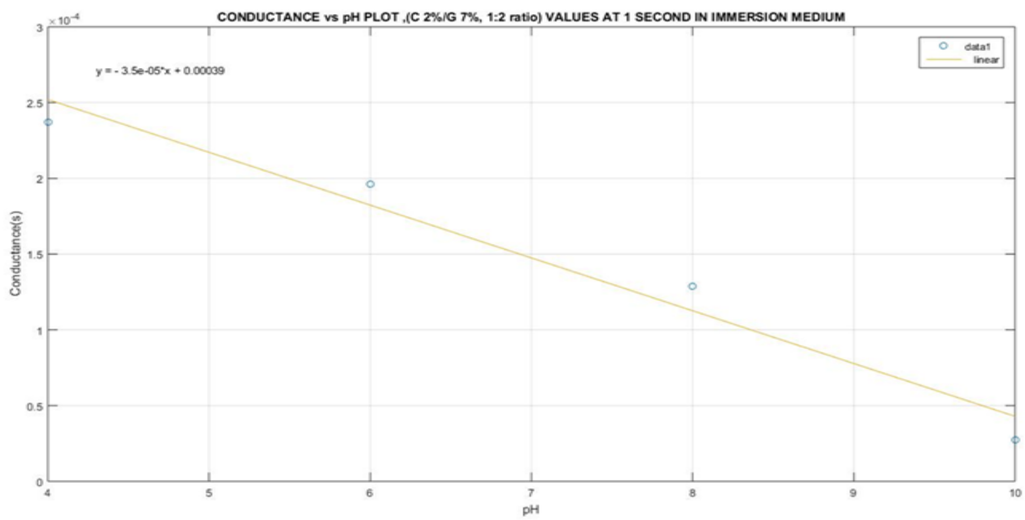

Figure 4.10: pH-Conductance linear function for pH 4, 6, 8, 10; Sensor sensitivity for 3mm sensor separation

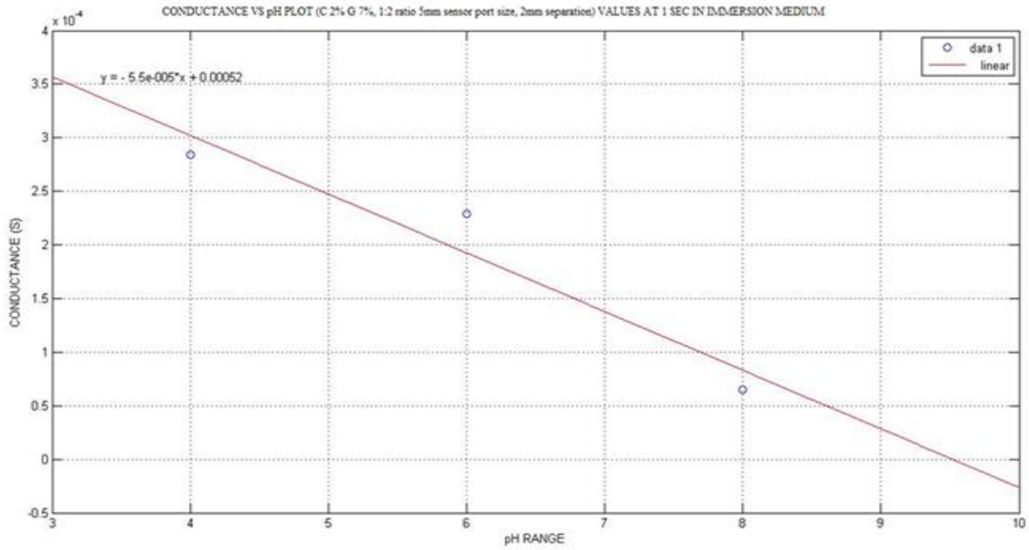

Figure 4.11: pH-Conductance linear function for pH 4, 6, 8, 10; Sensor sensitivity for 2 mm sensor separation

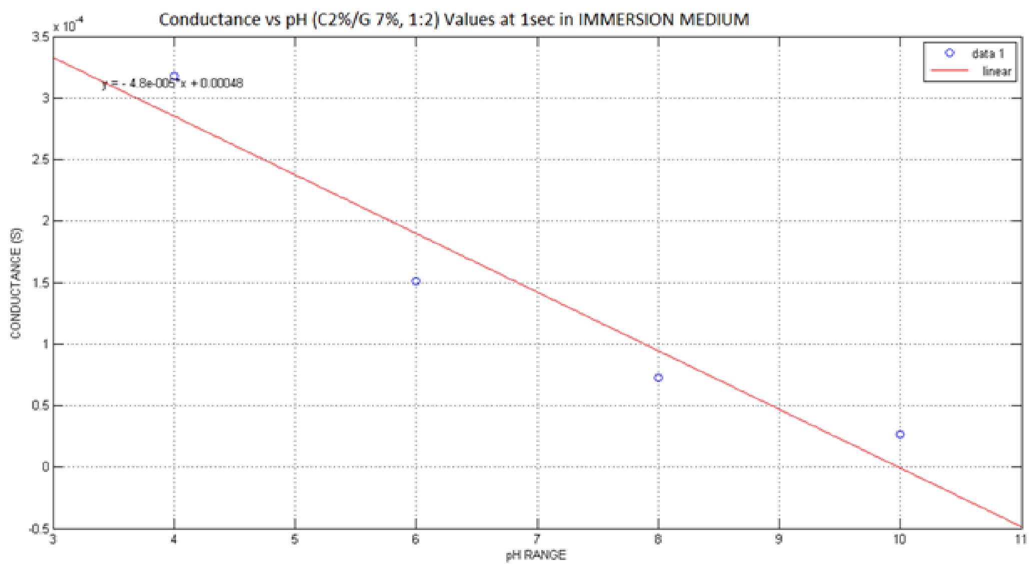

Figure 4.12: pH-Conductance linear function for pH 4, 6, 8, 10; Sensor sensitivity for 1 mm sensor separation

Fig 4.10-4.12 shows liner function for 3mm, 2mm and 1mm separation between sensor ports respectively. Comparison between the sensitivities of these three separation shows that 1mm sensor has best sensitivity and fast response time with a regression value of 0.9635.

Table 4-2: comparison of separation between sensor port based on sensor sensitivity and electrode density requirement

BATCH	Separation(mm)	Size(mm)	SPAN (pH RANGE)	SENSITIVITY	Regression coefficient
A	1	5mm	4-10	0.000048	0.9635
B	2	5mm	4-10	0.000055	0.9233
C	3	5mm	4-10	0.000035	0.9254

Lowering the distance between the conductive sensor ports, lowers the resistivity if applied voltage is constant and lowering resistivity increases current according to Ohm's law. Results of separation study shows that increase in separation between sensor ports in a sensor pair results in reduced conductance and slower response of the sensor. Therefore, optimum separation of 1mm between sensor ports is selected for the sensor array design on the basis of best sensitivity, better response time of 1sec and ease of fabrication.

4.3 Rigid pH sensor array

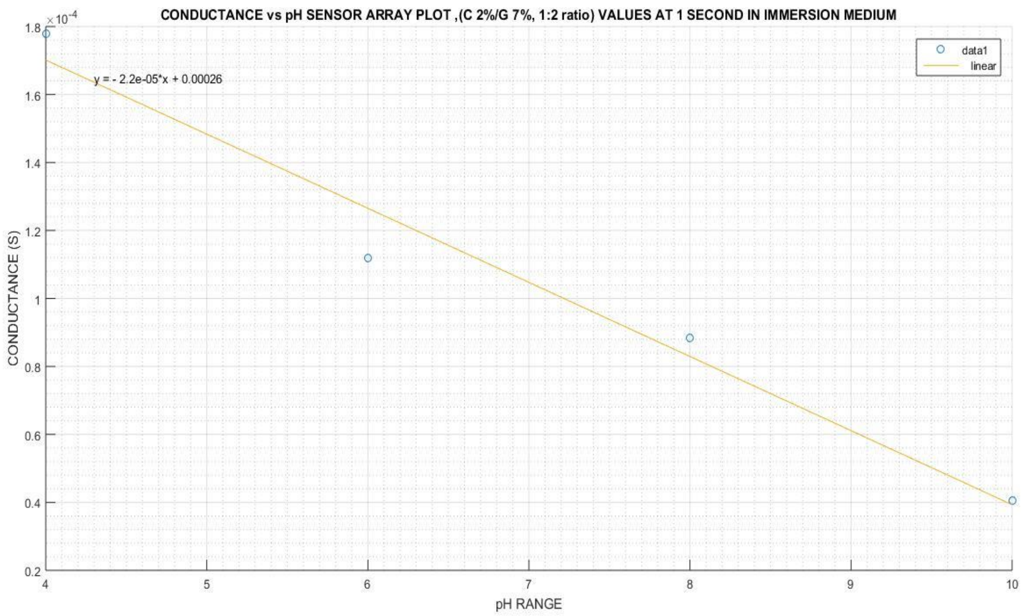

Figure 4.13: pH-Conductance sensor array plot for pH 4, 6, 8, 10

Fig 4.13 shows linear function for a rigid sensor array with conductance on y-axis and pH on x-axis. The rigid sensor array results shows good sensitivity of 2.2 x 10^-5 and a regression value of 0.969. Graph shows high conductance at acidic pH whereas conductance values are reduced at lower pH. The linearity of regression line shows greater sensitivity.

4.4 Flexible pH sensor array

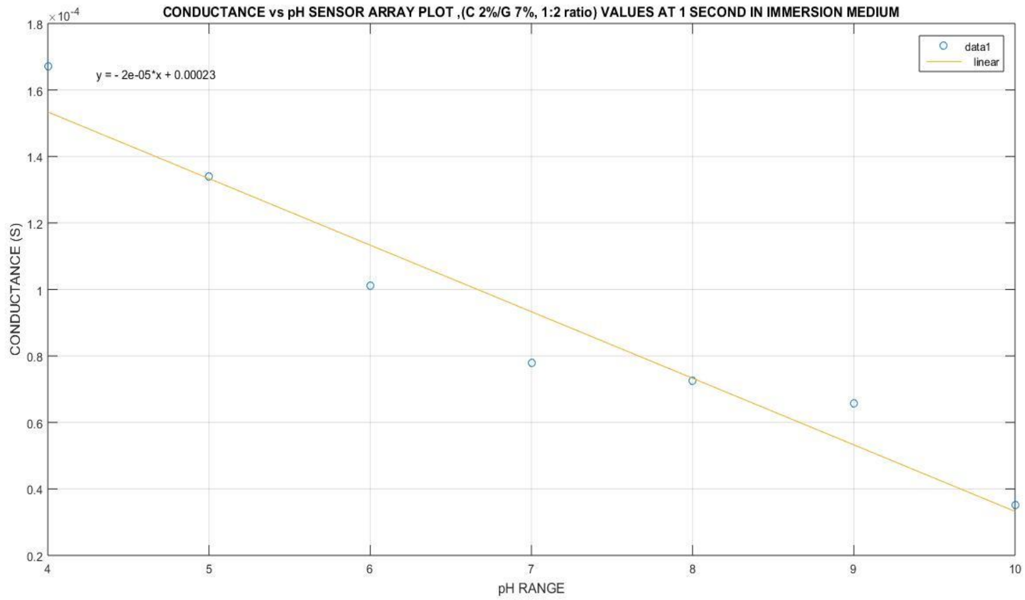

Figure 4.14: Conductance-pH linear function for the full relevant pH range, from 4 to 10; Intermediate values, at pH 5, 7 and 9 have been tested. Conductance against pH values are shown for flexible pH sensor array

The conductometric analysis result for flexible pH sensor array have been shown in fig 4.14 with conductance on y-axis and pH on x-axis. Full range of pH from 4-10 has been tested on flexible sensor array. The graph obtained shows a linear response with good sensitivity. A high regression value of 0.9389 shows high sensitivity with a response time of 1 sec.

4.5 Applications of sensor array

Both the flexible PCB and Rigid PCB can be used in applications such as:

- Rigid pH array can be used for laboratory and in-vitro pH analysis of physiological solutions.
- Flexible pH array can be used for real time measurement of wound pH.
- Measurements of pH of anatomical regions of the body as flexible array can conform with the contours of the body.
- pH mapping.
- Can be integrated with endoprothesis devices for endovascular measurements.

Chapter 5: CONCLUSION AND FUTURE PROSPECTS

This study focus on the design and fabrication of Chitosan/ Gelatin composite hydrogel based pH sensor array for physiological applications. Successful design and development of flexible array of conductometric pH sensors has been achieved in this study. The conductometric sensor array is fabricated on biocompatible (Polyimide) substrate for use in pH monitoring of physiological solutions. The sensor array achieved targeted sensitivity and fast response time.

This pH sensor array being biocompatible can be used for remote patient monitoring in future. It can also be used for prolonged patient monitoring by integrating with implantable devices.

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PUBLICATIONS

Submitted Article

Mariam Mir, **Ayesha Gulzar**, Murtaza Najabat Ali, Afifa Barakullah, Munam Arshad, Shizza, Maliha Arshad. (2017). POLYMERIC BIOMATERIALS FOR WOUND HEALING-A REVIEW. Journal of Applied Polymer Science.

Original article

Ayesha Gulzar, Mariam Mir, Murtaza Najabat Ali, "Design and fabrication of a hydrogel based pH sensor array" (Manuscript in writing).

Review artcle

Ayesha Gulzar, Munam Arshad, Mariam Mir, Murtaza Najabat Ali. "Review on pH responsive Chitosan based composites for controlled drug delivery".

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