Optimization of pH Sensor Characteristics:

An exploration of sensor characteristics and reusability of hydrogel based pH sensors

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A thesis submitted in partial fulfillment of the requirements for the degree of

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Thesis Supervisor:

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MASTER THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by: **Afifa Barakullah** regn no. 00000118832 Titled: **"Optimization of hydrogel based pH sensors: an exploration of the sensor characteristics and reusability of hydrogel based pH sensor"** be accepted in partial fulfillment of the requirements for the award of **MS Biomedical Sciences** degree. (Grade A)

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

Abstract

Hydrogels prepared from natural polymers such as gelatin/chitosan composite. This composite is unique because of its abundance, nontoxicity, biocompatibility, and biodegradability. Gelatin is a matrix of polypeptide hydrolyzed from collagen. Its rapid biodegradation and low mechanical strength restricts further usage of this material. For this reason, the blending of biodegradable polymers has been employed. Chitosan is a polysaccharide derived from chitin. Therefore, chitosan was introduced for fabricating gelatin/chitosan composite. Chitosan and gelatin are being used because they are pH responsive gels and are feasible for measuring pH of any physiological environment. Following parameters were optimized for the conductometric hydrogel based pH sensors: size, resistance, simulated physiological conditions and reusability. These hydrogel based pH sensor was small enough for point of care measurement. On the basis of these optimized parameters, it is concluded that the shortlisted sensor shows good sensitivity, high accuracy, and shortest response time in the physiological pH range and can be used in the medical devices.

Key words: *pH responsive hydrogels, conductomteric sensors, chitosan, gelatin composite, wound healing*

Contents

List of Abbreviations

G Gelatin

C Chitosan

pK Acid exponent a

PECF Pseudo extracellular fluid ms

millisecond

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CHAPTER 1

1 Introduction

Volume of the pH responsive hydrogels can vary in response to the minor changes in the external environment. Hydrogels such as stimuli responsive polymers tend to change their volume in a significant proportion depends upon the swelling and de-swelling of the polymer. Stimuli include the electric field [1], light [2], temperature [3], pH and composition of solvent [4-7]. In previous literature, researchers resembled the hydrogels working like muscle and suggest that these polymers have great promising material [8-11]. These polymers have been used in different biomedical applications such as drug delivery, sensors, actuators and bio separation [12].

pH values shows the negative logarithmic scale for the concentration of H^{+-} ions in solution. It ranges from 0-14 hence characterizing the concentration of H^{+-}ion 10^{0} -10^{14} mol/1. Biochemical reactions require specific pH value [13]. pH value is the key factor for the metabolism [14]. The properties of solution can be investigated through the measurement of the pH. Monitoring of the pH values used in many fields such as biology, medicine, chemistry etc [15,16]. pH balance is important to human health. Slight change in pH is indicative of a change in physiological parameters in the body. Tumor, infection, wound healing, gastroesophageal reflux disease are the examples where pH monitoring is used for the diagnosis. pH is marker of both analytic and theranostic interest. The change in physiological pH values caused by the fatty acids, amino acids and other byproducts released by keratinocytes layer which shift the bicarbonate-lactate buffer system of the body into acidic environment [14]. Healing of wound is very intricate process which comprises three phases: inflammation, proliferation and tissue remodeling [17]. In the process of the wound healing, the pH value of the infected area affects directly or indirectly all biochemical reactions [14] such as endogenous and tissue remodeling [17]. It is so far neglected factor. For the proper recovery of the wound requires that wound metabolically active for which pH value is a important factor [14]. Wound healing process most likely occur in acidic environment. Optimum pH values require for the healing which inhibits the bacterial growth and suppress the proteolytic activity and enhance the fibroblast development is the indication of the self-healing. The pathogenic bacteria require pH value above 6 for their growth which stop by lowering the pH value [18-21].

The diagnostic concept of sensors can apply on both chronic and acute wounds. The better understanding of the wound healing process enhance by the detection of the wound environment with help of sensor [22].

Until today, the glass electrode remains the best quality level for the clinical estimations of the skin surface or wound pH despite the fact that a similar procedure has just been utilized decades ago [23]. But is offers the few disadvantages which are its mechanical delicacy, inflexibility and large size. Because of its large size, it just measures pH at a particular spot, which renders it tedious procedures to assess the spatial pH value of the entire wound. Unfortunately, different methodologies for the pH measurement are appropriate in vitro [24]. So these difficulties of current glass electrode based pH observing device are being cater by the assistance of material science and biomedical building which incorporates fiber-optic pH sensors [25] and metal oxide conductometric pH sensors [26].

Recently, great research has been carried out in polymeric substance and composite. Hydrogel based pH sensors were made. Natural polymers were used for the preparation of the hydrogels such as cellulose, chitosan, and gelatin, HEMA are of great interest because of biocompatibility, biodegradability which are used in many biomedical applications [27]. The first conductometric hydrogel based pH sensors were developing by Norman [28]. These sensors based on the swelling and de-swelling of the pH responsive hydrogel. Based on the following conductometric results some important observations were made, when is immersed in an aqueous medium; the movement of the ions inside the gel matrix are measured as conductance. We have proposed the development of a conductometric sensors based on chitosan and gelatin composite. Chitosan is a polysaccharide derived from the chitin, are of great interest because of its biological and physiological properties [29]. Chitosan consists of hydroxyl groups and reactive amino that can chemically and physically modified depending upon the application. One of the intriguing effects of chitosan on wound healing is the development of the granulation tissue with angiogenesis [30,31].Gelatin is the polypeptide hydrolyzed from collagen. It is the most promising material because of its biodegradability and biocompatibility. Though, its fast biodegradation and low mechanical strength limits the use of this material [32].

Due to this reason, the combination of biodegradable polymers has been used to produce collagen-based scaffolds [27]. Different parameters were optimized for the development of the sensors and check the reusability of this composite.

CHAPTER 2

2 Literature Review

2.1 Hydrogel based Conductometric sensor

Conductometric sensors are the type of sensors which sense the change in the conductive properties of a material exposed to the environment. Conductive properties of the layer changes in response to the ions to be sensed are exhibited as a change in the electrical resistance of the electrode [28].

Hydrogels while their response to pH comprise of (co)monomers that consist of ionizable side groups being weak acidic or weak basic. The charge carried by these groups' terms as aspect of the pH. As the interplay of the pH takes place, it makes hydrogel's sensitive part to swell and ultimately affects the solution's ionic strength to which hydrogel was exposed. The monomers that tend to be ionizable get dissociated as a function of the pH in the hydrogel and these results to free counter ions which then get exchanged with salt particles from solution. A definite counterion amount gets developed inside the hydrogel, that causes a thus causing an osmotic pressure difference between solution and the gel. Subsequently, swelling occurs till equilibrium is achieved by the elastic forces inside the hydrogel. This is called Donnan quasi equilibrium state. [33].

One of the conditions to be taken account of, in swelling such hydrogel is maintenance of charge neutrality present in hydrogel. Thus it cannot donate ion to a solution without getting an appropriate ion in return. Hydrogels with an co monomer acidic in nature, if exposed to pure water, osmotic swelling does not happen in gel despite of the fact that solution's pH is way more different in value than the p*K*a of comonomers (acrylic acid). It has the ability to donate protons to the surrounding solution thus ensuring the electro neutrality a hydrogel needs to receive counterions from the solution. In the conditions of pH (neutral), low ionic strength, ions that can be available to hydrogel are protons produced when auto protolysis of water takes place. In response to this, pH inside the hydrogel gets lower down and acrylic acid groups tend to be in an uncharged, state. No concentration that term out to be free of counter ions, would take place in hydrogel thus causing no osmotic swelling. On increasing ionic strength of solution, an exchange can happen between ions and the solution. As a result of this exchange, the hydrogel ensures the charge neutrality and increases the quantity of free counter ions. A rise in the osmotic pressure difference occurs between the hydrogel and the solution thus swelling the gel. On increasing ionic strength, the hydrogels will lead to shrinkage. This happens as a result of loss in osmotic pressure difference [33].

To understand chemical sensors, significant work has been done using the sensitivities and considering the alternate behavior in hydrogels' properties [34]. In addition to a straightforward design, these sensors are operational devices working directly with a high sensitivity and selectivity. The recent developments in the research have enabled the description of sensor's characteristics qualitatively and quantitatively.

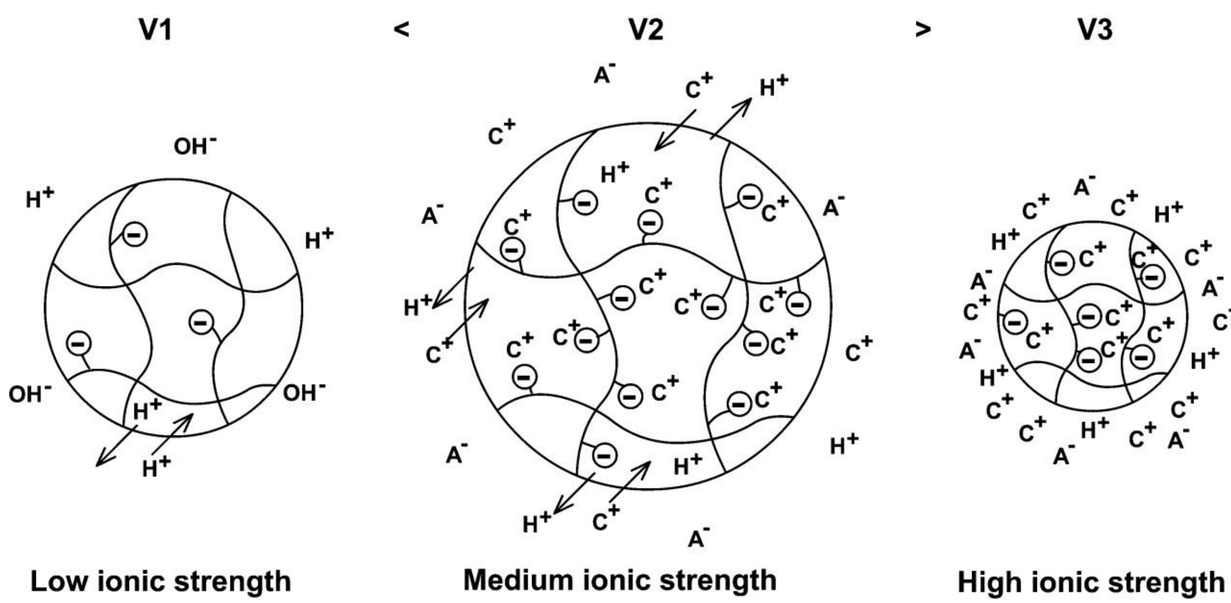


Figure 2.1 Swelling of pH based sensitive hydrogel

2.2 Various hydrogel based sensor

Norman Jr. carried out another research using hydroxyethyl methacrylate membrane acting as inter layer for ISFET to ensure an interface that is defined on thermodynamic basis between ionsensitive membrane and gate oxide. The conditioning of this membrane was done in pH buffer so that interference could be eliminated from CO_2 besides reducing noise levels [35]. This membrane also found its enormous use in the making of ISFET sensor (calcium based) [36], an amperometric sensor used in case of chlorine, and a conductometric pH sensor [37]. The immersion of buffers is made potassium chloride solutions with various pH followed by peel test of adhesion. Testing of sensor was carried out in a pear flask regulated on thermal basis at 25°C. Resistive part was measured by HP4192A Impedance Analyzer with properties such as 0.1 V amplitude of peak to peak value and 100 kHz frequency. Buffer used was there to change the values of pH [38].

Hydrogels under a higher value of 9 pH medium display extensively worse bond than ones tested in 6.0- 7.4 pH medium. Hydrogels experimented at higher temperature (40°C) demonstrate equivalent bond to ones at room temperature (23°C). This aspect shows that restoration of siloxane bonds. At cost of these bonds, sensor failure terms out to be a major factor, performance of failed sensors thus can be regenerated by making them dry in a vacuum oven. Design implications and formation of micro fabricated sensors thus tend to have certain findings thus making use of sensing layer of polymer. In identifying the pH sensitivity of the bond, sensor failure can be avoided if pH of testing experiment environment can be restricted to values maintaining the siloxane bond [38].

Sheppard announced a conductometric sensor defined as an electrode array with coating of a hydrogel layer [28,37]. Values of 100 Hz to 100 kHz of frequencies measuring the resistance displayed a resistive behavior. The hydrogel layer swellings thus marked to raise the conductivity of hydrogel with a reduction in the resistance [28].

Norman's work was based on conductometric hydrogel in which the sole subject was microsensor. These hydrogels were prepared by polymerization of 2- hydroxymethyl methacrylate and N,N – dimethylaminoethyl methacrylate. Tetraethylene diaacrylate's main purpose in the experiment was crosslinker [28].

2.3 Optimization of the different parameters

2.3.1 Sensitivity

Sensors prefer to be working with phase range of transition of the hydrogel tested. Figure 2.2a depicts the range lies between $pK_a \approx 4.7$ of the poly (acrylic acid) and pH of 9 because at this point of 9 pH, the ionization process of the acidic groups gets finished. This extreme volume change within the phase transition leads to a surprising sensitivity per pH unit following an order of 10^{-3} to 10^{-5}. This aspect is illustrated in Figure 2.2b that range follows a linear pattern. pH measurements residing out of the hydrogel phase transition range don't find any recommendation. Once the value is lowered by the range, shrinkage of gel occurs alongwith marginal sensitivity. Figure 2.2a shows this swollen behavior of the gel. Some important observations to be made at this point are impact of the ionic strength and bad sensitivity [12].

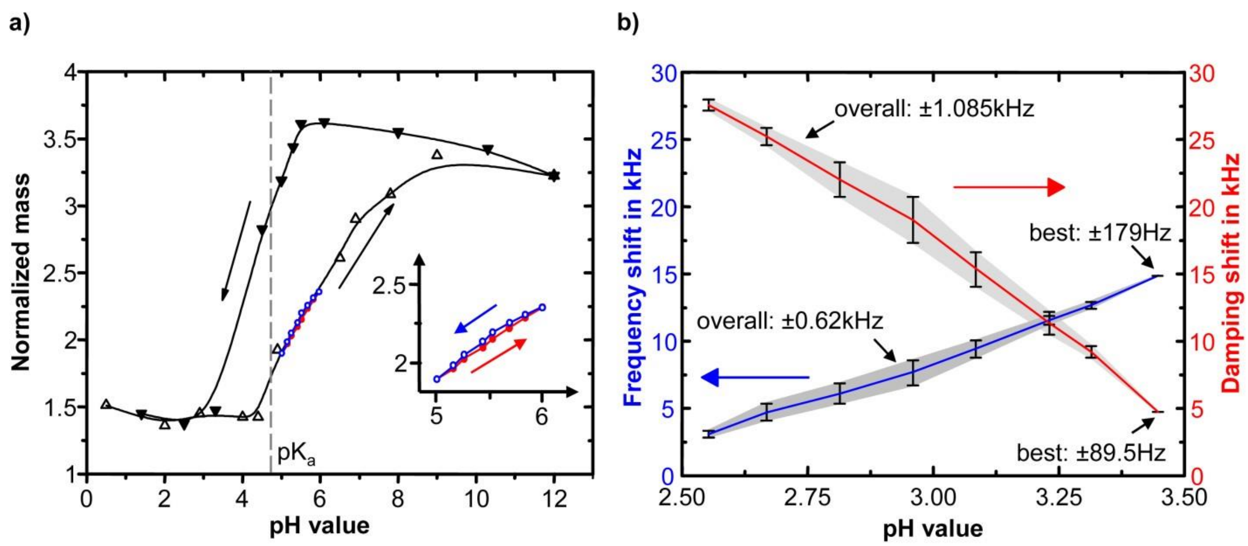


Figure 1.2 a) Swelling response of bulk gel; b) Specifications of micro balance pH sensor

Norman et al undertook another experiment for thickness of the finite gel, the fact that the resistance increases with increasing pH as the conductivity of the gel layer decreases. At a fixed pH, gel's thickness increases and in return, it leads to an increase in the resistance, because of less conductivity of the gel than the solution. Diffusion controls the resistance of this gel's response to ensure that response time might be improved apart from decreasing the gel thickness.

As the resistance winds up plainly autonomous of gel thickness, there is no requirement for a gel thicker than 2/3. Nonetheless, the sensitivity will rely upon thickness. The analysis carried out on sensitivity ignores resistance between the layer of gel and metal electrode the interfacial impedance tends to have a resistive component that follows in series with the resistance as a result of which, sensitivity is reduced [28].

Close to reaction time, the sensitivity is a standout amongst the most significant parameters for an effective sensor usage. It is usually is measured by the polymer arrangement and also due to its cross-connecting degree. The last relies upon the substance of the cross-connecting operator in polymer and on UV light characteristics for the photo cross-linkable hydrogel [39].

2.3.2 Measurement Accuracy

On a whole, measurement accuracy of most sensors, lies in the order of $\pm 10^{-2}$ pH unit and this aspect is similar to follow a standard deviation of 95% confidence level. Reasons behind this are processes that make a typical swelling behavior as illustrated in Figure 2.2a [40]. The trademark fundamental to acidic curve is very not the same as the acidic to basic response. This is shown in hydrogel and mechanical properties [12]. Suzuki clarifies as with both a substitution of the H+ counter ion by a sufficient particle, for example, Na+ and an abundance of these particles in the gel making screened ionized gel groups [41].

As appeared in figure above, this set can be essentially brought down by solid confinement of the working reach inside the stage change go. For instance, quartz micro balance sensor (Figure 2b) tends to find its working range as 0.9 of pH with a general precision of ± 1.085 kHz and this criteria follows for damping shift. Additionally decline of the working reach to brings about an expansion of the precision. As specified counterforces influence the affectability of a sensor by bringing down the volume change of the hydrogel [12].

Another experiment was conducted by Norman. 30% of the water contact was achieved by weight at pH 10. With a decrease in pH, the water ratio of the gel goes high, reaching 50% at pH 4. Swelling process follows a mechanism as tertiary amines present in the gel get protonated with a reduction in pH, so to maintain electro neutrality; counterions are drawn into the gel. This results in an osmotic driving force. Conductivity of the gel changes with pH following a same pattern as of water content. In this experiment, the conductivity is observed to be about 2% at the bathing solution value at value of pH 10, and it goes up to 5% at pH value of 4. The increase in the conductance of the gel with respect to the solution imitates principally the expanded water substance of the gel. Because of the nearness of ionized amines in the gel at bring down pH esteems, there is a chance of Donnan allocating of particles into the gels. A gauge utilizing Donnan balance hypothesis [42] recommends that the conductivity of the fluid arrangement inside the gel is expanded by at most 10% in respect to that of the buffer solution outer to the gel [28].

2.3.3 Working Range

pH sensor find a working range with such a specification while selecting a hydrogel component. This working range is directly equivalent to the ionic pK value. In case of acrylic acid, this $_a$ process can be carried out below 400 nm, and in case of poly (*N*-isopropyl acrylamide) the shift was also observed for values below 500 nm [43,44].

2.3.4 Response Time

The reaction time relies upon the swelling resulted. The impact of ionic quality on reaction time is inspected by two sensors with thicknesses values in the order as 300 nm. The micro scale sensor was examined in an acidic nature and got a most limited reaction time as low as 500 ms [45]. The working of refractometric sensor takes place inside the impartial pH extend portrayed by a low ionic quality. Along these lines, the reaction is tedious with around 80 s [12].

Moreover, reaction time is influenced by powers that have an impact on the swelling procedure of hydrogel. These sensors utilizing mechanical aspect of work of the hydrogels demonstrate a huge distinction between time of contracting and swelling. In case of bending plate sensor [46] the plate compels swelling because of counterforce bringing about a reaction time of 7.6 hour after an expansion of pH 1-6. The other way around contracting process is supported by the flexible power of the bending plate, which prompts a reaction time around 40 min. The shrinkage appears to be fast and is more than eleven times speedier than swelling [12].

Devices in light of free swelling gels have a tendency to be faster and demonstrate no critical distinction in terms of swelling and contracting time. For instance, the quartz crystal small scale adjust sensor has a 500 ms reaction time of swelling and 800 ms for contracting by a difference in pH 1.84 to 3.19 and the other way around [45].

Since the gel reaction is normally diffusion, time reaction of the volume change roughly takes after the square of the example measurement. Scaling to small scale measurements improves the time reaction. A decrease of the example measure enhances the sensor's working. In any case, a decrease in the gel thickness is restricted by the need to get an adequately high sensor signal and therefore, an adequate sensitivity. Keeping in mind the end goal to accomplish an ideal between the sensor sufficiency of the signal and the sensor reaction time, hydrogel’s parameters of swelling time was explored [47-49].

Various processes take place during swelling of the hydrogel:

- Added substance’s diffusion into the gel. Additive’s amount relies on a distance that gets increased as time increases [50].
- Additive’s amount triggers off process of ionization in gel follows a specific rate.
- Additional water uptake. Absorbed water’s concentration increases at a steady rate In response to increasing amount of ionized polymer groups of the additive [47,48].

The polymer chain prompts the impeded swelling of gel which is constrained by a relaxing procedure with the time steady and is slow than rate of diffusion. It was discovered that the aggregate sum of the moreover retained water and in addition the rate of the underlying quick swelling and the slow long-time drift's rate relies upon the concentration gradient of the added substance [51].

2.3.5 Calibration Calculations

In the working range of the sensors, considering their good, follows the application of a s mple two-point calibration [37]. This calibration is to be done individually for each of the process solution.

An offset must also be considered while calibrating and hydrogels that tend to have a specification of double-sensitivity, can undergo a calibration to be electronically for offset basis [52].

2.3.6 Stability

Hydrogel sensor usually depicts poor measurement accuracy during the initial operations. The reason behind this being the microscopic structure of the polymer network comprising the breakage of short polymer chains and thus rest of the chains need to manage according to an alternate pattern [53].

Hydrogel layer is delaminated and it is one of the problems faced at stage of the experiment. To avoid this delamination, an adhesion promoter can be used [54], film-developing microgels [55] or enclosing of the hydrogel element [56].

Solutions consisting of high ionic strength gather up in hydrogels as a result of which an irreversible malfunction of the sensor takes place. These ionic groups can be accumulated in a very short span of time usually within hours except those of hydrogels with very thin films. In order to cater this problem, the sensor must be liquidated on regular basis and its storage must be ensured in deionized water. The lifetime of a hydrogel-based sensor can long for several years [12].

2.3.7 Resistance

For the measurement of electrode impedance in a range of frequencies with 100 Hz to 100 kHz in case of hydrogel based pH sensors, the time span tends to be greater than dielectric relaxation time of the materials to be brought under consideration. The impedance output comes out to be primarily resistive, so the modelling to be carried out is performed considering only resistive component of the impedance [28]. The resistance and capacitance depend on the frequency and an inverse relation follows between them to the square root of the frequency. The nature of the metal (electrode) is an important factor in this regard on which interfacial impedance relies. The electrolyte comprises of a polished metal surface tends to have an interfacial capacitance of roughly 10 uF/cm* at a frequency of 1 kHz, whereas in case of a roughened metal surface (e.g., platinum black) this value lies at 1000 uF/cm^2. An interdigitated electrode array of 2 mm by 3 mm, interfacial capacitance lies within a range of 20 for the bare electrode at 1 kHz, to 2 R for a platinized electrode at 100 kHz [28].

2.3.8 Reproducibility of Signal

Reproducibility is an important aspect of repeated measurements of pH under a following certain conditions. It also has its impact on reproducibility of the signal [57,48,49]. Following key factors must also be considered in this regard: Structure of the titration curve cgi =f(pH) is affected by gel ionization and hydrogel swelling. Thus only a conditional process can ensure high signal reproducibility to be performed before carrying out each measurement so that a confident reference sensor signal can be accomplished. These pH measurements if carried out in buffered solutions with a specification of constant ionic strength, low molecular salt formation takes place in the gel and value of the pH is then adjusted by addition of an acidic or basic substance. This leads to a variation in the ionic strength having its impact on the osmotic pressure and ultimately on the resulting water flux (positive or negative) [39].

The content of ionized groups depends on the immersed duration in solution. This can only be reduced with help of a constant t_{ex} and it follows the same way for all the pH measurements undertaken [39].

2.3.9 Adhesion/ Reusability

The investigation of adhesion of photo lithographically patterned membranes was carried out in terms to assess the stability of pH sensors and sensing elements in this work were the patterned polymer membranes. Exposure of these membranes to buffered saline at pH value of 9 was eliminated using a peel test, whereas those with a pH value of 6 stayed adherent. One of the conclusions to be made from this aspect was that sensor operation must be restricted to a specific range pH range of 6 to 8 so to ensure a low rate of base-catalyzed hydrolysis of siloxane bonds.

If temperature is increased to 40° C from 23°C, it didn't turn out to have its impact on adhesion. The removed membranes had a lifetime from the aqueous testing environment. Reformation of these bonds takes place when the membrances get dried. This hypothesis additionally upheld by the recovery of the sensor execution watched following drying. To summarize all of this, peel tests give a period and appropraite methods for improving microsensor lifetime where loss of diffusion is a noteworthy method for the sensor to fail [38].

Adhesion failure is imperative affecting lifetime of sensor. The confirmation of reorganization of siloxane securities after drying is authenticated by the capacity to recover the affectability and broaden the lifetime of sensors. Bond transformation requires that the silanol stays proximal to the surface with the goal for recondensation purposes. Investigation demonstrates that the affectability does not come back to its actual dimension [38].

The above mentioned parameters are needed to be taken under consideration before further fabricating conductometric pH sensors.

CHAPTER 3

3 Methodology

3.1 Synthesis of Chitosan/Gelatin Composite Hydrogel

The preparation of 2% Chitosan was done by dissolving the pre measured volume of Chitosan in 1% acetic acid and stir the solution on hot plate at 30 C for 2 hours. 7% Gelatin was prepared by mixing the pre measured value of gelatin in distilled water at 50 C for 20 minutes. Mix the solution of 2% Chitosan and 7% Gelatin in ratio of 1:2. Then 0.25% Glutaraldehyde was added in the solution as a cross linker and solution was stirred for 1 minute. After the homogenous mixing of the solution, composite was cast into the mold and left for curing at room temperature.

a

b

Figure 3.1 Preparation of hydrogel: a) 2%Chitosan b) 7% Gelatin

3.2 Fabrication of pH sensors

3.2.1 Cylindrical Configuration

C/G hydrogels have adhesive quality which is due to addition of the cross linker Glutaraldehyde (Yamada et al., 2000). Silver electrodes were embedded into the semi cured hydrogel. Gel upon complete curing shrink and wrap around the electrodes. The electrode with pH sensing portion as shown in the figure below

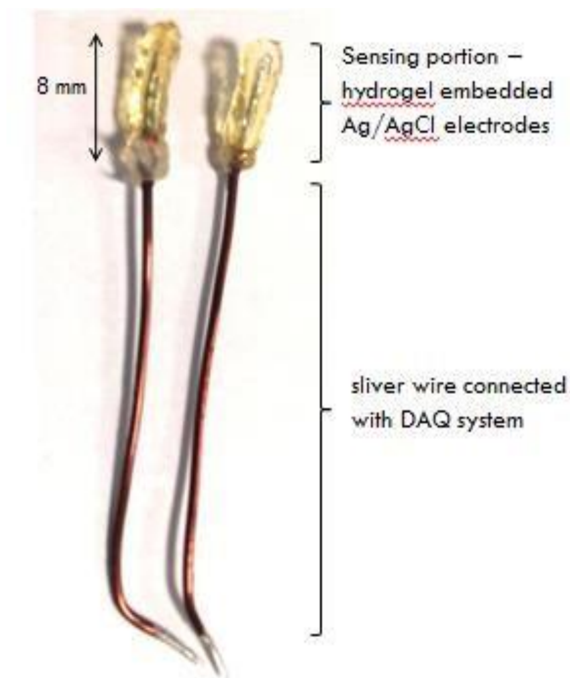
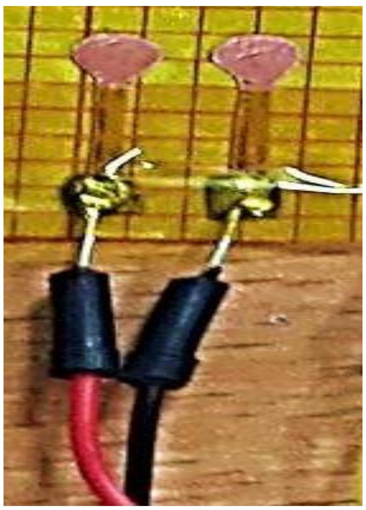


Figure 3.2 Preparation of hydrogel: a) 2%Chitosan b) 7% Gelatin

3.2.2 Planar Configuration

For better flexibility, cylindrical configuration was converted into planar configuration. Fabrication of planar sensor was done by using the conductive tape for making the track. Hydrogel was poured on the circular port of the electrode. This conductive tape was attached to the polyamide substrate which is biocompatible.



a **b**

Figure 3.3 Planar configuration of chitosan/gelatin conductometric sensors: a) Electrode; b) pH sensitive hydrogel poured on the electrode

3.3 Conductometric Analysis

Conductometric tests of the hydrogel pH sensors were done in Pseudo Extracellular Fluid (PECF). The PECF solution was done by mixing the 3.4g NaCl, 1.1g KCL, 1.75g NaH_2PO_4 and 12.5g $NaHCO_3$ in 500ml of de- ionized water [58,59]. Took 10ml PECF solution of specific pH (4, 6, 8, 10) and each sensor immersed in it. After immersion of sensor in PECF, conductance was measured between each pair of sensor for 1 minute. Pair of sensor was configured with Arduino uno. Microcontroller was used for the recording of conductance values. Each experiment was repeated for three times to minimize the error. Conductance versus pH box plots was created for the graphical representation of the results.

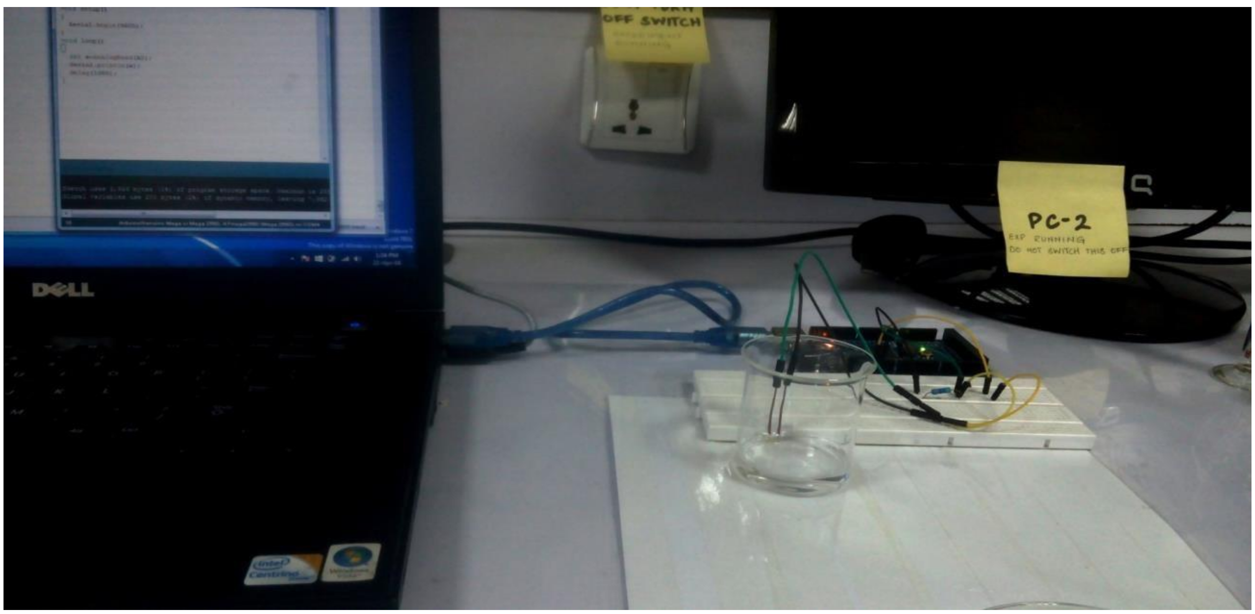


Figure 3.4 The conductometric static study set up (for immersion medium pH 4,6,8,10) for the cylindrical configuration of conductometric chitosan/gelatin sensors

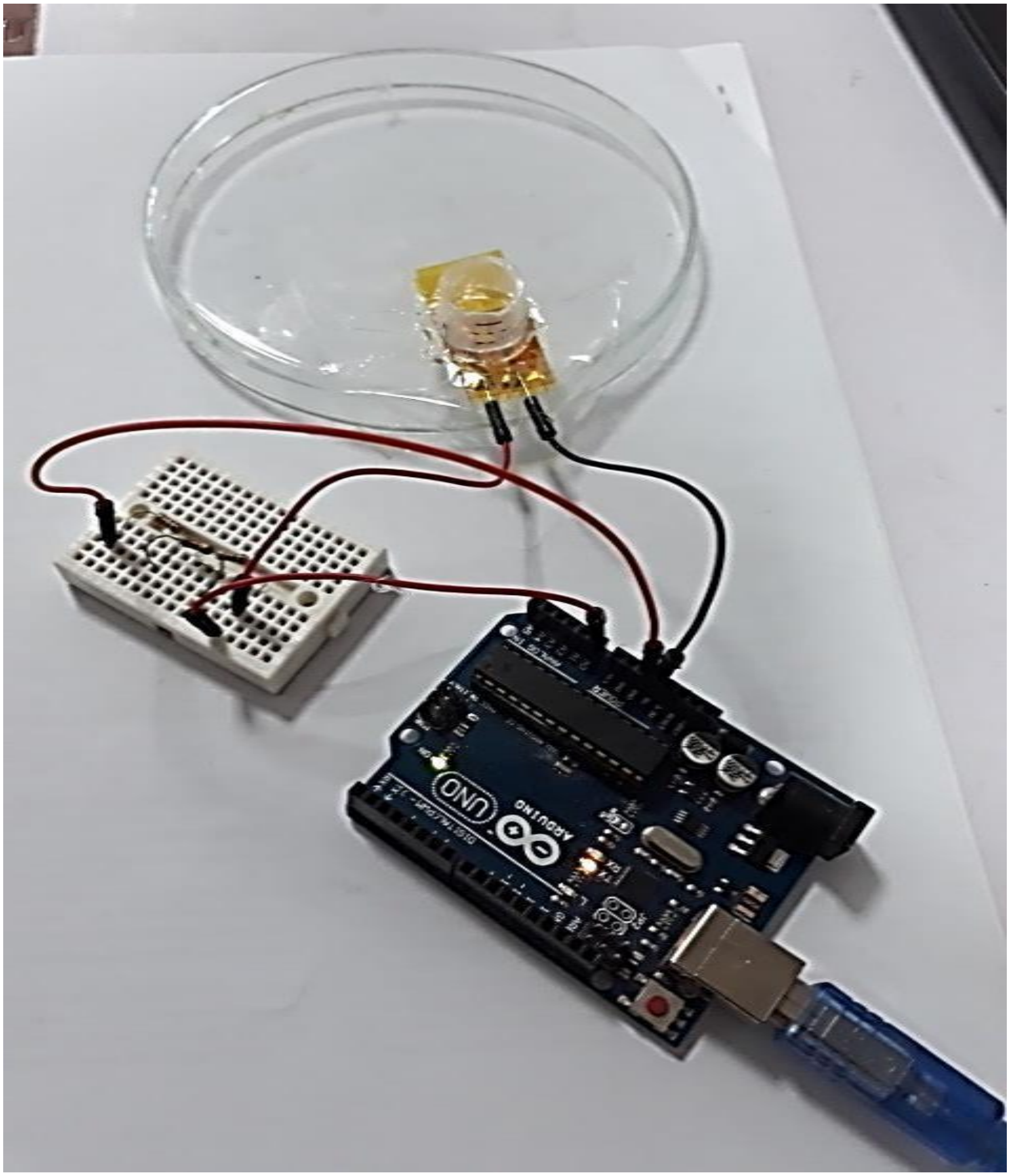


Figure 3.5 The conductometric static set up(for immersion pH 4,6,8,10) for the planar configuration of the conductometric chitosan/gelatin sensors

3.4 Optimization of sensor characteristics

3.4.1 Size

The maximum pH response of the hydrogel was measured by using different sizes. The size optimization was done in cylindrical configuration. This parameter was very important because pH response time vary with size. The conductometric testing of different sizes of sensors were done with same procedure as mentioned above

3.4.2 Resistance

Resistance of the sensors was measured to ensure the maximum life of the electrodes. Testing of the sensors was carried out at 10k, 4.6k and 2.7k. Adjust the pH of the solution of specific value such as 4, 6,8,10. After testing checked the pH of the solution again. The minimum change of the external pH was measured. For the better flexibility, mode of fabrication changed and conductometric testing was done in planar configuration of the electrode.

3.4.3 Physiological simulated conditions

Initially tests were carried out in PECF solution which is basically an ionic solution. Gum acacia was added into the PECF solution in order to increase the viscosity of the solution. Viscosities of different concentration i.e. 30%, 50%, 70% were checked with Ostwald viscometer. Different concentrations of the viscosity were tested in order to mimic the natural exudate.

3.4.4 Reusability

The already tested sensors were tested again to check whether they can be used more the one time. This testing was done on pcb as shown in the figure. Another study was done on the hydrogel swelling behavior. This study was actually done to address the reusability of the hydrogel based pH sensors. Different solvents were used in this experiment such as PECF, Distilled water and Reverse osmosis water (RO). In this experiment, measure the dry weight of the hydrogel piece then immersed in a medium for 1 min. Took out the piece of hydrogel, check its wet weight. Leave the hydrogel for overnight. Repeat this procedure for consecutively for three days.

CHAPTER 4

4 Results and Disscusion

4.1 Size Optimization

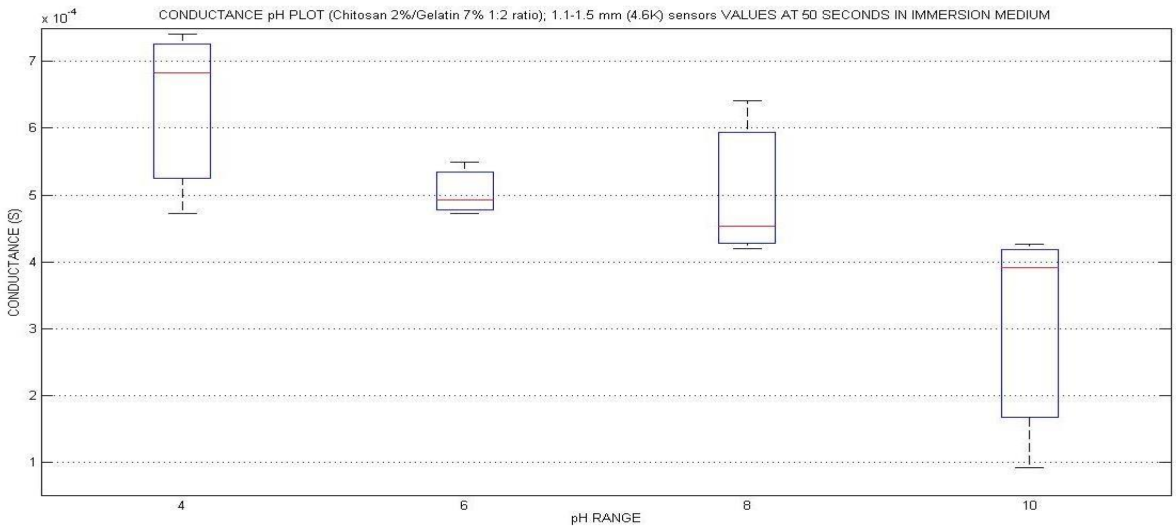


Figure 4.1 pH – Conductance box plot for pH 4,6,8,10; Conductance against pH values are shown for sensor size 1.1-1.5mm

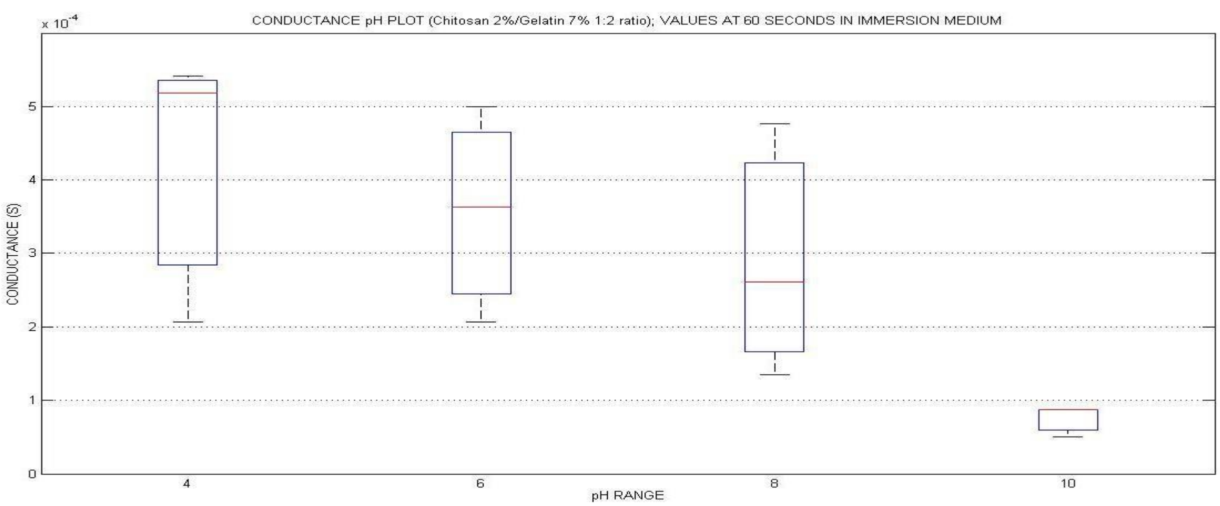


Figure 4.2 pH- Conductance box plot for pH 4,6,8,10; Conductance against pH values are shown for sensor size 1.5-2.0mm

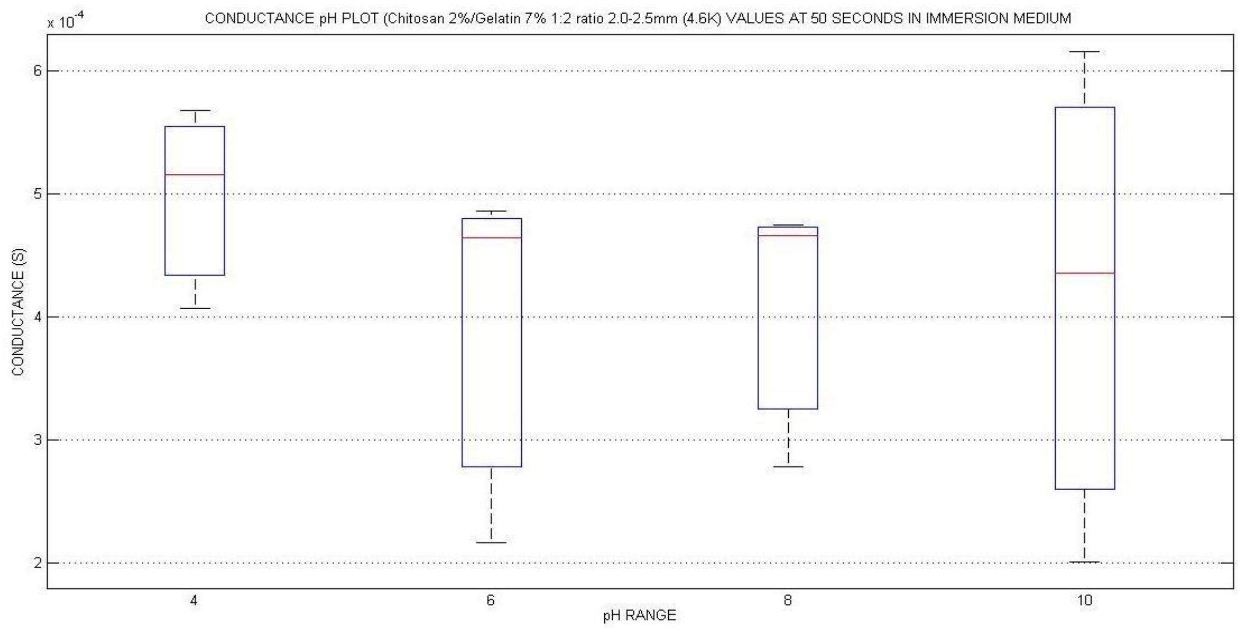


Figure 4.3 pH-Conductance box plot for 4,6,8,10; Conductance against pH values are shown for sensor size 2.0-2.5mm

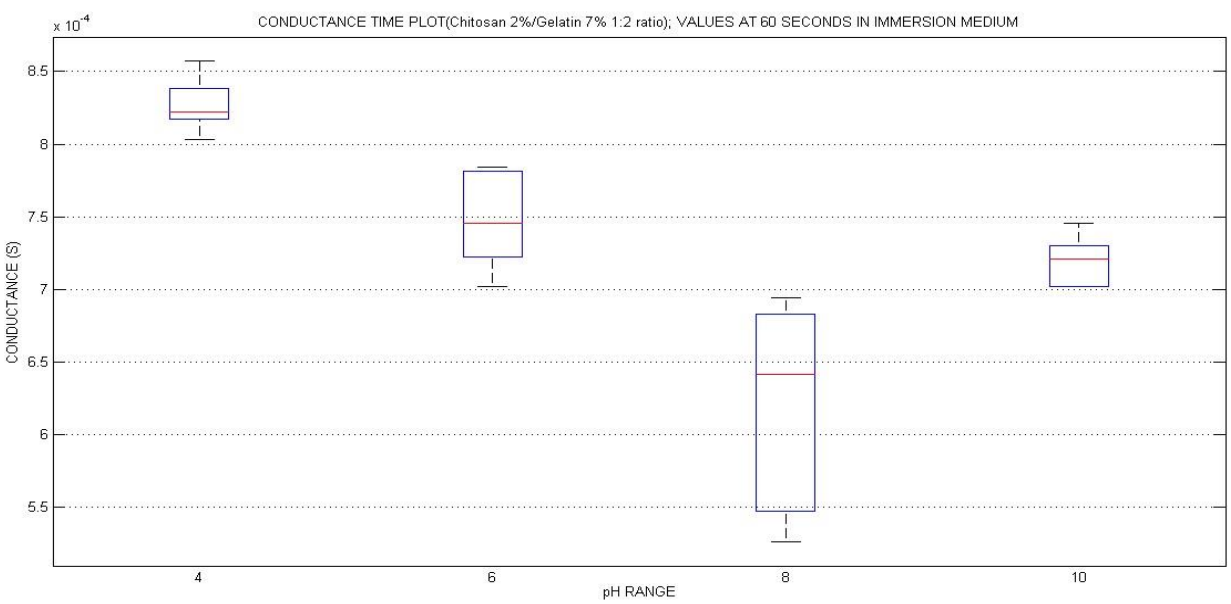


Figure 4.4 pH-Conductance box plot for 4,6,8,10; Conductance against pH values are shown for sensor size 2.5-3.0mm

The first parameter which was needed to address the optimization of size .Conductomteric analysis results have been shown in the box plot between the pH on x-axis and conductance on y-axis in figure x-x. Different sizes were tested to check the maximum response time. In figure 4.1, 4.3, 4.4; 1.1-1.5mm, 2.0-2.5mm, 2.5-3.0mm sizes were tested respectively. Box plots represent spread of data variation and distribution. The results of these test shown that from pH 4-pH 10, conductance value was reduced. The results of the size 1.1-1.5mm show somewhat linearity while other two sizes range from 2.0-3.0mm does not show linearity. Linearity of data was checked on the results of regression coefficient for all data sets. The highest regression coefficient shows the highest sensitivity of sensors. The sensitivity of sensors was depending on slope of regression line so we plot the sensitivity graphs accordingly. In figure 4.5, the size 1.1.1.5mm has 0.9504 regression value. In figure 4.7 and 4.8, the size 2.0-2.5 mm and 2.5-3.0mm has 0.8523 and 0.5366 regression value which is very low. The lowest regression value shows that these sizes of sensors have low sensitivity. In figure 4.2 and 4.6, 1.5-2.0mm size was tested. This size has 0.9528 regression value which means that this size of sensor has highest sensitivity among all other sizes which were tested. This size of sensor shows the highest change in output in response to the smallest change in input which followed to a higher slope for the regression line. On this basis, we have chosen this size of sensor that gives the highest accuracy, highest sensitivity and shortest response time which was 60 seconds. Further reduction of size causes the loss of sensitivity because the Quasi-equilibrium was attained faster. So there is the minimum difference between the conductance values which is not measurable. The optimization of size was done in cylindrical configuration. Next parameters testing were done in planar configuration for the better flexibility.

4.2 Sensor Sensitivity

Figure 4.5 pH-Conductance box plot for 4,6,8,10;Sensor sensitivity for the size 1.1- 1.5mm

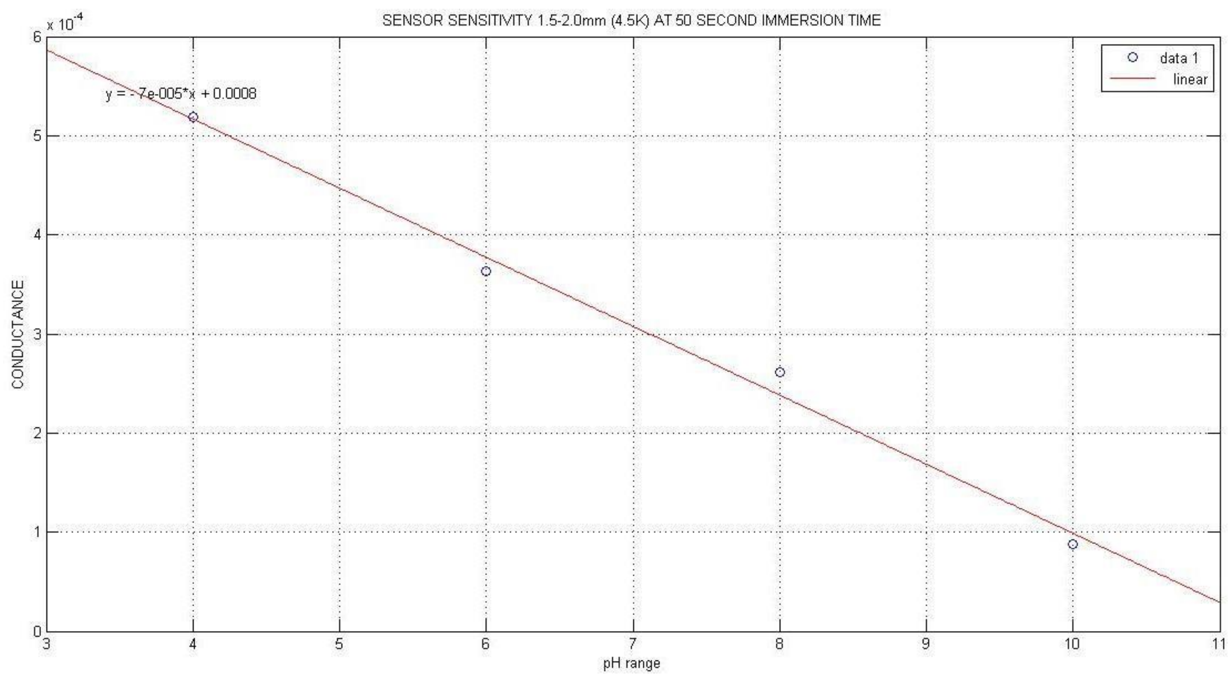


Figure 4.6 pH-Conductance box plot for 4,6,8,10;Sensor sensitivity for the size 1.5-2.0mm

Figure 4.7 pH-Conductance box plot for pH 4,6,8,10;Sensor sensitivity for the size 2.0-2.5mm

Figure 4.8 pH-Conductance box plot for 4,6,8,10;Sensor sensitivity for the size 2.5-3.0mm

BATCH	SIZE(mm)	SPAN(pH RANGE)	SENSITIVITY	REGRESSION COEFFICIENT
A	1.1-1.5	4-10	-0.000046	0.9504
B	1.5-2.0	4-10	-0.000070	0.9528
C	2.0-2.5	4-10	-0.000012	0.8523
D	2.5-3.0	4-10	-0.000021	0.5366

Table 4.1 Comparison of sensor sensitivity and linearity of response

This table shows the comparison of sensor sensitivity and regression coefficient of different sizes of sensors. We have chosen this size of sensor that gives the highest accuracy, highest sensitivity and shortest response time which was 60 seconds. Further reduction of size causes the loss of sensitivity because the quasi equilibrium was attained faster.

The separation of the ionic groups and the inflow of counterions cause the increase in the concentration of ions in the hydrogel. Due to this osmotic pressure changes and results in a solution inflow and hydrogel swells. The interaction of the charges with the polymer chain also leads to an increased swelling. When the elastic restoring force of the polymer network balances the osmotic forces Quasi-equilibrium state is achieved. In the Donnan-equilirum state, the diffusion flow of the ions in one direction is equal to the electromigrational flow in the opposite direction, this results in a net zero charge and mass transport.

4.3 Resistance optimization

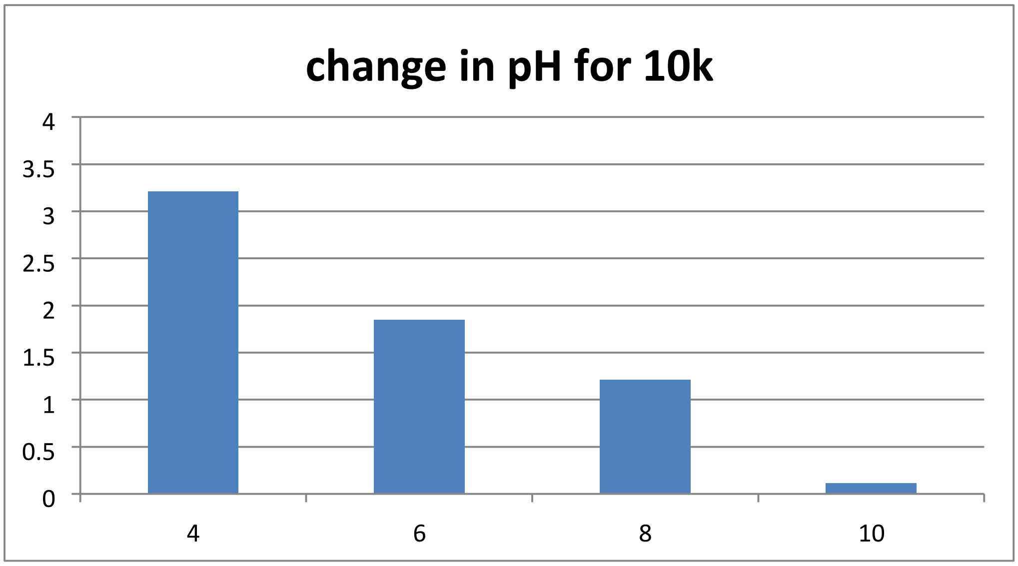


Figure 3 pH verses change in pH graph were plotted for the 10k value

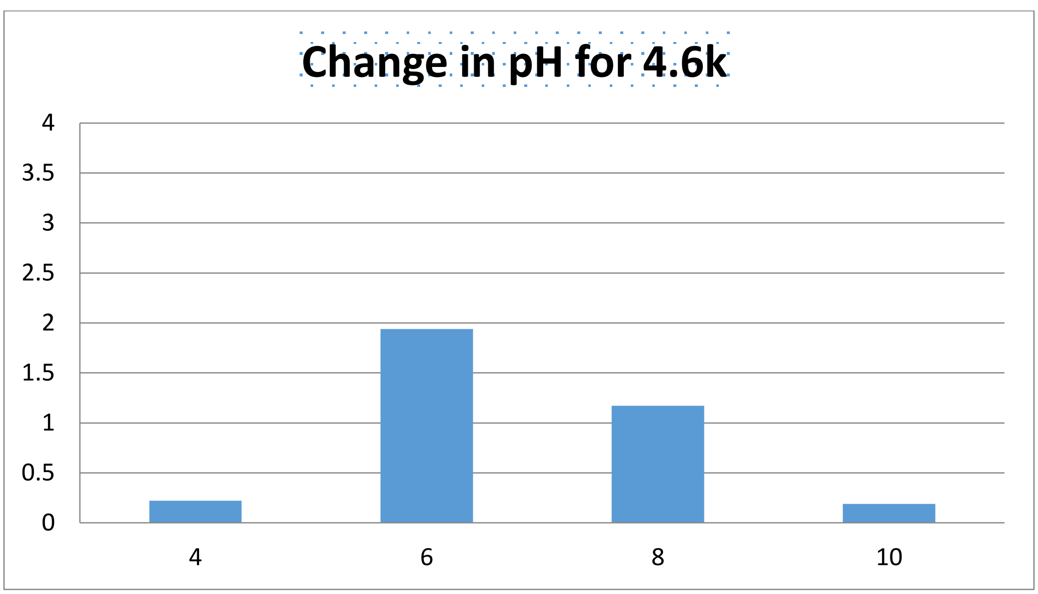


Figure 4.10 pH verses change in pH graph were plotted for the 4.6k value

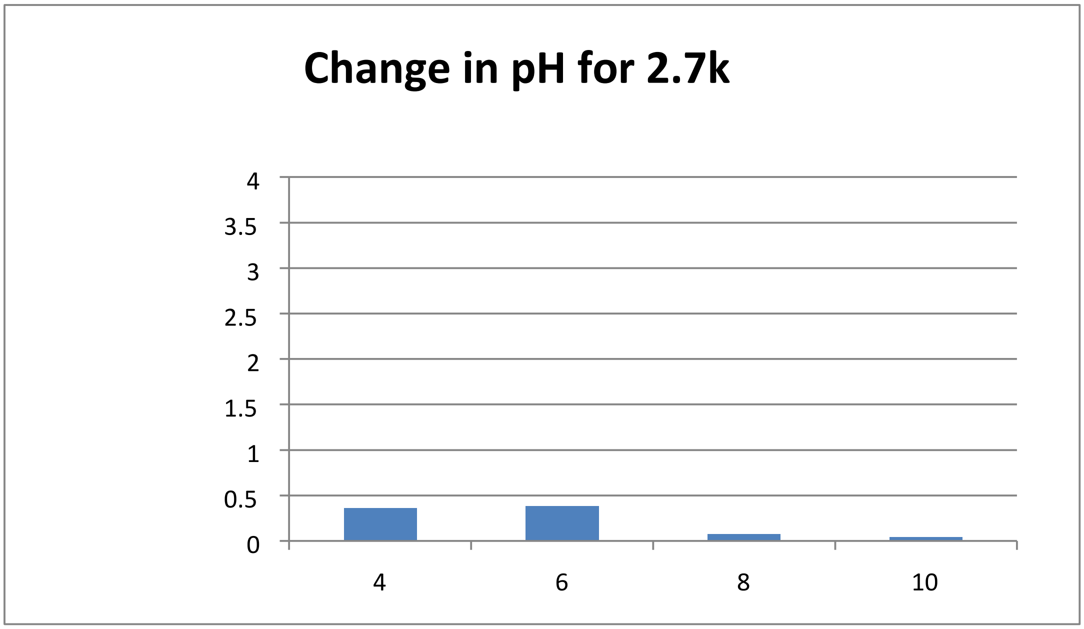


Figure 4.11 pH verses change in pH graph were plotted for the 2.7k value

These following graphs show the comparison of the resistors of the different values used to minimize the change in external pH. Graphs were plotted between pH on x- axis and change in pH on y-axis. In Figure 4.9, at first the test was conducted with 10k value resistor, large difference in change in pH of the solution was observed, which was not desirable. Another test was conducted with 4.6k value resistor; the change in pH observed was less than it was previously observed in 10k test (figure 4.10). The test was repeated with the 2.7k resistor which gave the desirable minimum change in the pH of the solution (figure 4.11). This optimization was done in planar configuration of sensors. In planar surface, the more surface area was exposed which lead to the decrease in response time which was 01 second.

4.4 Optimization of Simulated Physiological Conditions

The third parameter which needed to be address was the optimization of simulated physiological conditions. Gum acacia was added to the PECF solution to mimic the natural wound exudate. Different concentration of viscosities was tested. Conductometric tests have been shown in box plots between pH and conductance in figure 4.12. For 30%, 50% and 70% concentration of gum acacia the viscosities measures were 27.26 cst, 111.4cst and 184.7cst respectively. The optimized viscosity was 184.7cst, which was near to the natural wound exudate, gave the best sensitivity as shown in figure 4.13 [60,61]. These test showed that these sensor can work properly in a viscous medium. This optimization was done on the pcb prototype.

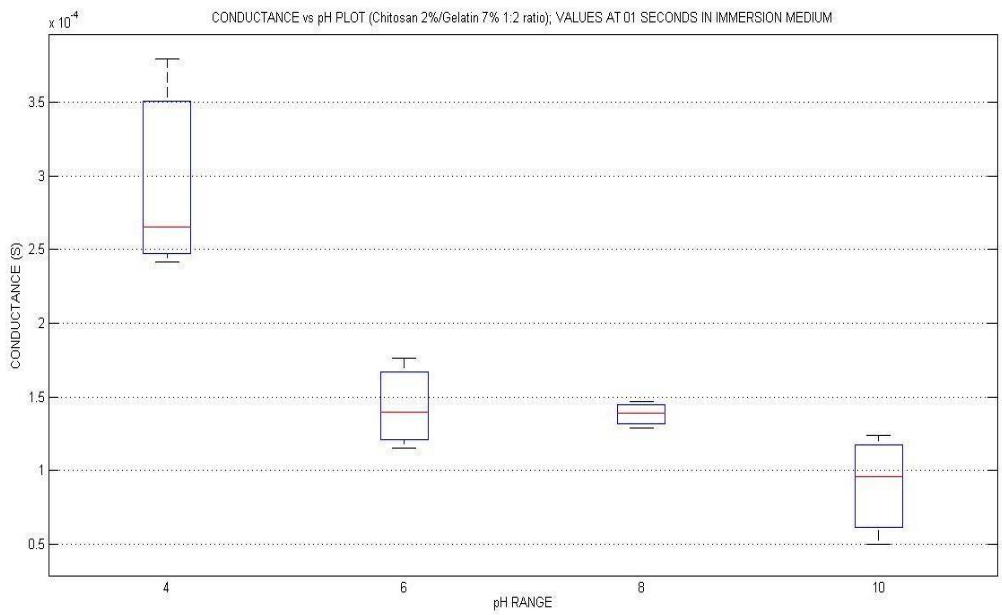


Figure 4.12 Conductance against pH values are shown for the 70% concentration of gum acacia

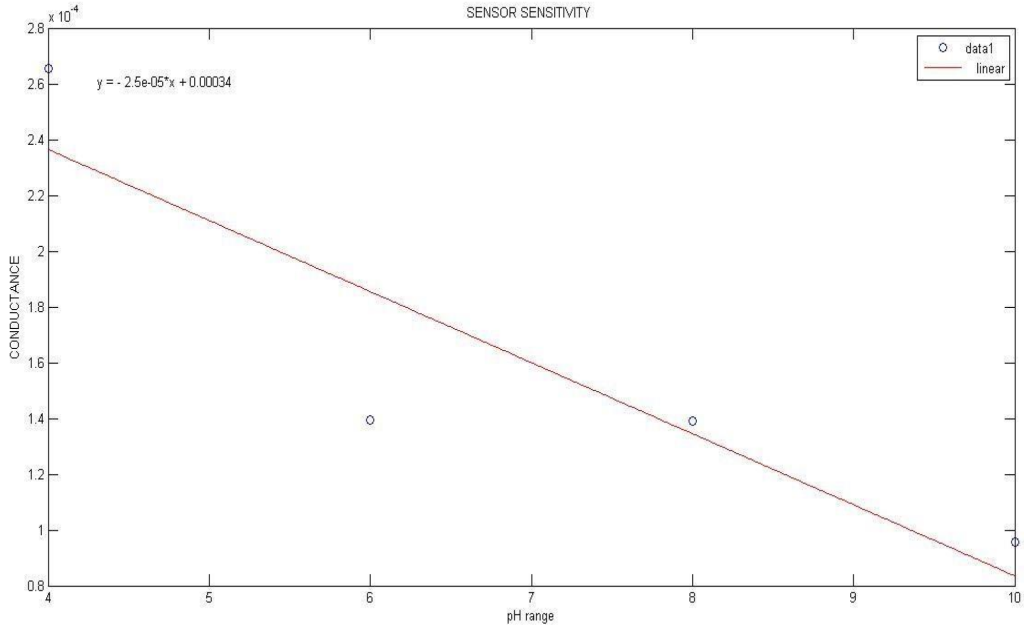


Figure 4.13 Sensor sensitivity are shown for the 70% concentration of gum acacia

4.5 Reusability

This study was done to address the reusability of the hydrogel based pH sensor. In the previous literature, it has been reported that the hydrogel dissolve in solvent with time [62]. Another study was done to check the hydrogel swelling behavior in different solvent as shown in figure 20,21,22. On this basis we conclude that due to deposition of salts the hydrogel geometry changes which in turn changes the hydrogel bond strength. Change in bond strength reduces hydrogel adhesion with time. Due to the change in morphology of the hydrogel, dry and wet weight varies as shown in the figure 4.14, 4.15, 4.16. In figure 4.17, there is no linearity which shows that these sensors loss its sensitivity. The regression value is very low i.e. 0.3208 which means that applied curve has not best curve fitting. On this basis we conclude that the sensor of this composite has not reusable due to the change in morphology of this composite.

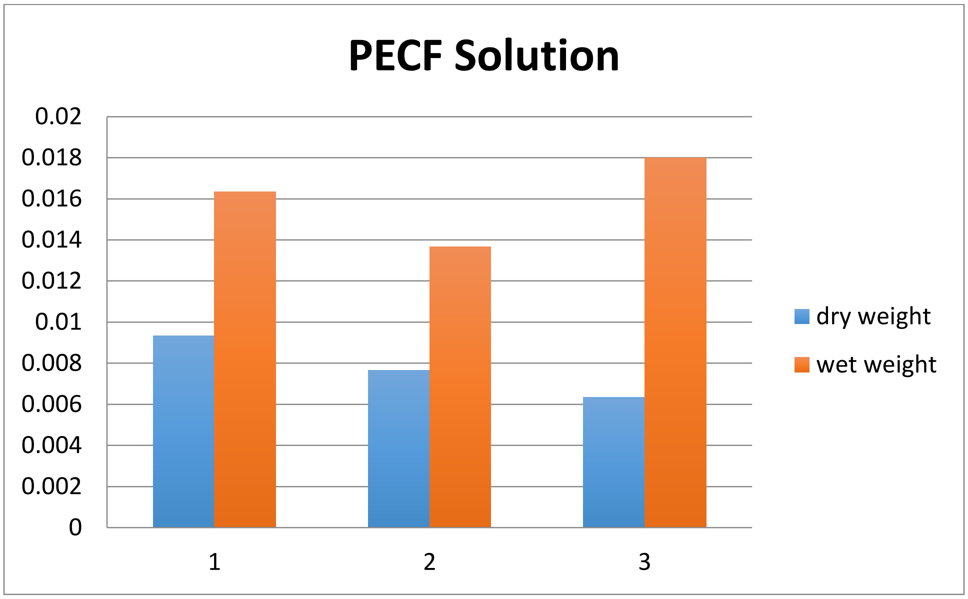


Figure 4.14 C/G composite swelling behavior in PECF solution

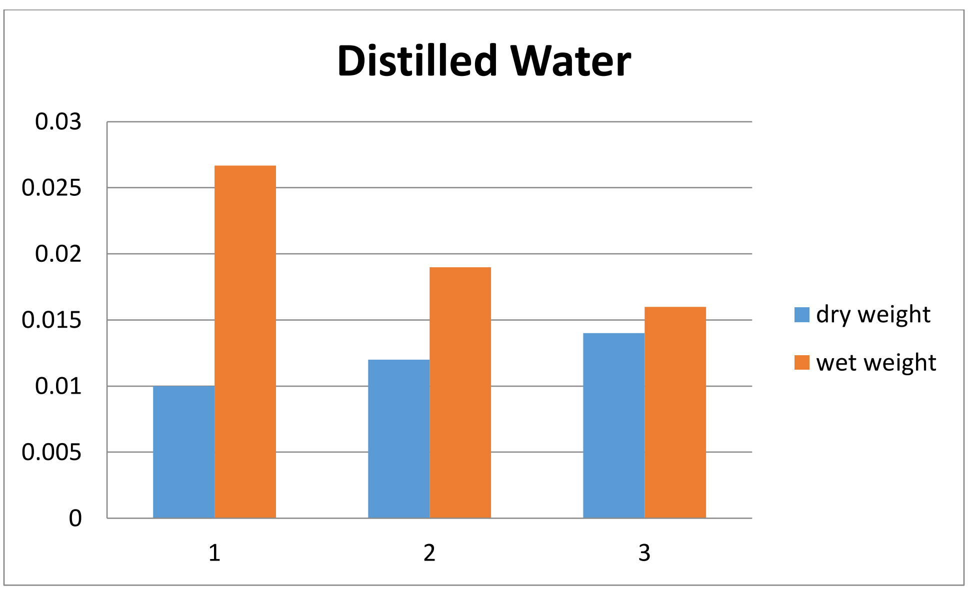


Figure 4.15 C/G composite swelling behavior in distilled water

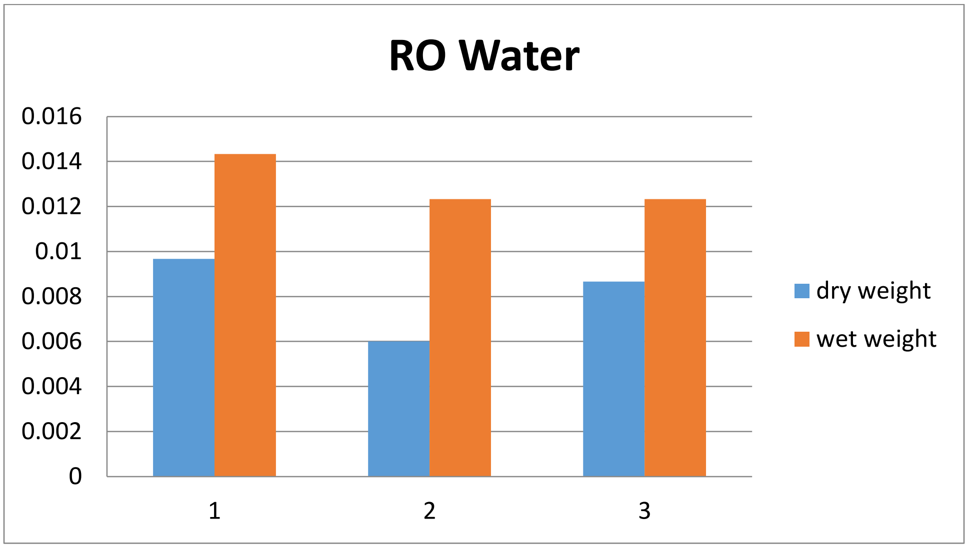


Figure 1 C/G composite swelling behavior in RO water

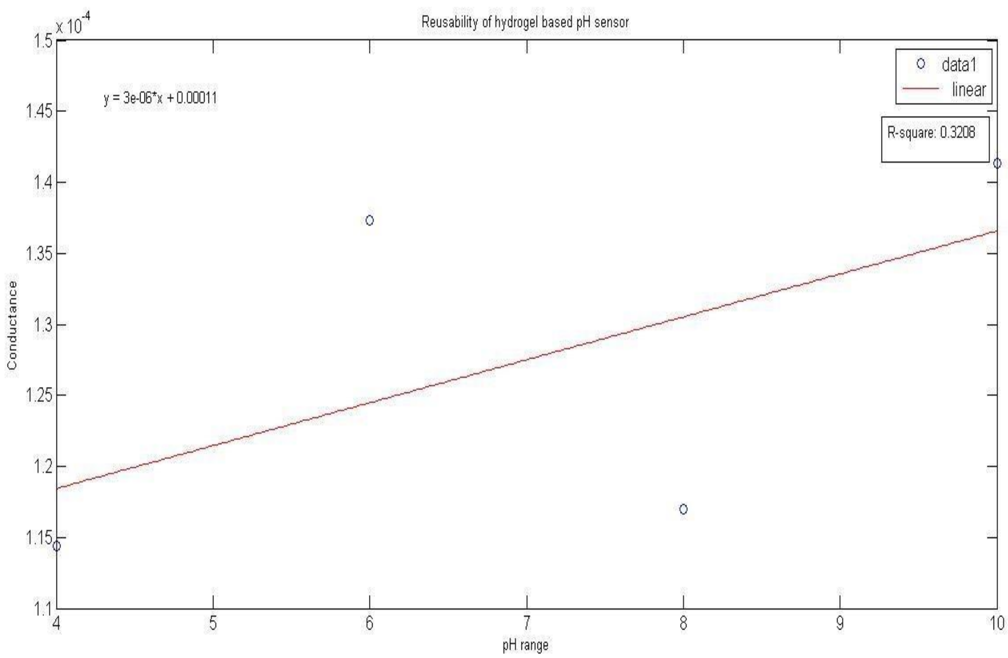


Figure 4.17 Reusability of hydrogel based pH sensor sensitivity

CHAPTER 5

5 Conclusion and Future Perspective

A detailed analysis of the conductometric properties of chitosan/gelatin composite sensors has been carried out. This study focused on the optimization of the major parameters of chitosan/gelatin composite as a pH sensor. On the basis of this study, we have been able to nominate a pH sensor that shows good sensitivity and resolution in the physiological pH range (4-10). Sensitivity can further be improved by reducing size in planar configuration. According to the following study it is concluded that the chitosan/gelatin composite cannot be used repeatedly thus, its use in vivo is restricted. Building on the current conclusions, further studies on the use and reusability of this sensor may be carried out.

CHAPTER 6

6 References

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Publications

Submitted Article

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Afifa Barakullah, Murtaza Najabat Ali, “Review on Conductometric Hydrogel based pH sensors (2017),” “Journal of applied biomaterial and functional materials”

Original Article

Afifa Barakullah, Marium Mir, Ayesha Gulzar, Ammad Ahmed,Murtaza Najabat Ali, Marium Mir “Design, optimization and fabrication of a hydrogel based conductometric pH sensor array for physiological monitoring applications” (To be submitted).

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