Deposition Volume and Conditioning Time for Good Nernstian Response from a Novel PVC-based Mn(TPP)Cl Membrane for Histamine Sensitive FET

S.F. Mohammed Esa, Khuan Y Lee, R Jarmin

***Abstract*—** Histidine, a type of amino acid in fish muscle tissue, is capable of causing scombroid poisoning. This happens when the toxin produced by the fish due to mishandling of storage, histamine, reacts with the decarboxilased enzymes. Ingesting of histamine exceeding the FDA safety limit of 100ppm brings on symptoms of scombroid poisoning such as facial swelling, headache, vomiting, diarrhea and low blood pressure. Existing techniques for detecting histamine such as HPLC and TLC are expensive in terms of cost, labour and time, as well as in need of seasoned physicians. On the contrary, recent advances in semiconductor IC technology based manufacturing enables production of robust, simple, cost effective and miniaturized ISFETs. Literature review found majority previous works on ISFETs for detection of H+-ions. However, ISFETs can be adapted to detect other ion types. Our previous work, being the first attempt, has shown that ISFET can be adapted for detecting histamine with the use of a novel polyvinylchloride (PVC)-based membrane, plasticized with dioctyl phthalate (DOP), dissolved in tetrahydrofuran (THF), enhanced with ionophore manganese tetraphenylporphyrin chloride (Mn(TPP)Cl) and polyHEMA layer on its insulator gate.This paper intends to determine the membrane deposition volume and conditioning time for membrane to achieve high Nernstian slope of sensitivity. It is found that ISFETs that are sensitive to H+-ions also are sensitive to histamine ions. In examining the effect of deposition volume, it can be deduced that a deposition volume of 20µL is optimal for sensitivity of adapted ISFETs to histamine ions. In examining the effect of conditioning time, it is observed that adapted ISFETs with membrane conditioned for 1 hour are found with higher sensitivity than those for 3 hours, over the full range of histamine solution concentration, in 0.05 molar of histamine conditioning solution and at a constant 20uL of membrane cocktail.

***Keywords*—**ISFET; histamine; PVC-membrane; membrane volume; conditioning time

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I. Introduction

Scombroid poisoning is a food borne chemical intoxication resulted from consuming stale Scombridae fish such as sardine, mahi-mahi, tuna and mackerel. It occurs easily amongst population who consume a substantial amount of Scombridae fish[1][2][3]. In 1970-1980, 42 outbreaks involving 4122 cases of scombroid poisoning were reported by the Ministry of Health and Welfare of Japan. Also, Japan shows the largest world-wide cases of scombroid poisoning, 2702 cases, in 1973[3].

Histamine, a toxin contributing to the spoilage of fish, is produced by the reaction of decarboxylation of amino acid and histidine in fish muscle tissue[4][5]. Histamine starts to accumulate in the fish as soon as it is caught. Once produced, histamine in the fish cannot be destroyed. Freezing condition, below than -20^0C can only prevent the production of histamine.

It is difficult to distinguish between spoiled and fresh fish since they share the same appearance and odor. However, ingestion of large quantity of histamine is a causative of scombroid poisoning. The Food and Drug Administration (FDA) of USA stipulates that histamine is safe for human consumption if it is less than 100ppm [1][2]. Scombroid poisoning is always confused with a type of common food allergy which share symptoms such as headache, vomiting, diarrhea, facial swelling, thirst, skin rash and low blood pressure [1][6]. The difference is, subject inflicted with Scombroid poisoning has no allergy to the implicated food previously; the outbreak and the presence of histamine in the food in question[6]. Severity of this symptom depends on immunoglobulin, which is subject dependent. Normally, the symptoms become apparent one hour after digesting seafood containing high level of histamine. In the extreme case, it is known to cause death.

A variety of methods have been developed for histamine detection such as high performance liquid chromatography (HPLC), thin layer chromatography (TLC), gas chromatography, amperometric, chronopotentiometry and capillary zone electrophoresis [7][8][9]. The detection limit of

capillary zone electrophoresis is high. The colorimetric method visually compares the difference in color intensity. Only TLC and HPLC employ direct measurement of the primary variable, histamine, at the expense of costly equipment. Relative to HPLC, TLC is simpler to operate; measurement can be repeated; many samples can be analyzed on the same plate with only a low amount of solvent, hence more cost effective[9]. HPLC is laborious and time-consuming. Its handling requires skilled technicians. Performance wise, TLC is second to HPLC in linearity, sensitivity and repeatability[10].

Advances in sensor technology bring about the ion-selective electrodes (ISE). It is simple, durable, inexpensive and capable of reliable response over a wide range of concentration. Their fast response time, low detection limit and good selectivity make potentiometric detection suitable for direct and rapid detection of chemically and biologically compound [11][12][13]. An offshoot of this development is the Ion-Sensitive Field Effect Transistor (ISFET), a chemical field effect transistor introduced by Bergveld in the 1970's[14].

ISFET is a potentiometric sensor that accommodates a wide range of chemical, biochemical and biomedical measurements. Structure wise, ISFET is similar to MOSFET, except that the metal gate in MOSFET is replaced with reference electrode and electrolyte in ISFET. It is initially developed for detection of pH. However, after much research, it is found that ISFET can be used to detect a variety of ions in aqueous form. Adaptation of ISFET for applications such as pollutant detection, glucose level detection, protein detection, urine and blood analysis, has been reported. Unlike the ISE, the manufacturing of ISFETs is based on semiconductor IC technology, an enabler that develops small, simple and cost effective devices and sensors. It can encapsulate detection and computation on the same chip with interface for information processing and data storage. In comparison to current techniques such as TLC, HPLC, ISFET is smaller in size, higher in sensitivity, lower in cost and power consumption, as well as dispense of wet chemistry laboratory analysis. All these are favorable factors for miniaturization and portability.

This paper researches into the use of ISFETs for detection of histamine, for quality control over fish freshness to minimize occurrences of Scombroid poisoning. For ISFETs to be sensitive to histamine, it needs to be adapted with a membrane, of which deposition volume of membrane cocktail and conditioning time of membrane are important to obtain good Nernstian slope of sensitivity. Fundamental theories on adapted ISFET are presented in Section II. Method and procedure to select functional blank ISFET, prepare and depositing membrane are described in Section III. Findings from pH and histamine sensitivity test in determining the optimal deposition volume of membrane cocktail and conditioning time are discussed in Section IV.

II. Theory on ISFET

ISFET is a potentiometric type sensor. It is used to measure the electrical potential difference at the interface of ion solution. ISFET has a basic structure purely electronic analogue of MOSFET, except that its metal gate is replaced with a reference electrode and an open contact for ion detection. This reference electrode produces a charge when the ion solution makes contact with the bare gate insulator.

With reference to the site binding theory, the presence of hydroxyl (Si-OH) group at the surface of metal oxide accepts or donates a proton (H+-ion), giving a negative or positive charge in the solution, until equilibrium is achieved. A current flows through ISFET as the amount of Si-OH and the H+-ions is being balanced.

For ISFET of our design as shown in Fig.1, the gate insulator layer (silicon nitrate, Si3N4) generates an interface potential on the gate once it detects H+-ions. The potential developed across the insulator layer depends on the number of H+-ions in contact with it, which can be related to the quantity of histamine ions in the solution. The gate potential modulates the current flow from source to drain, as ISFET is switched on.

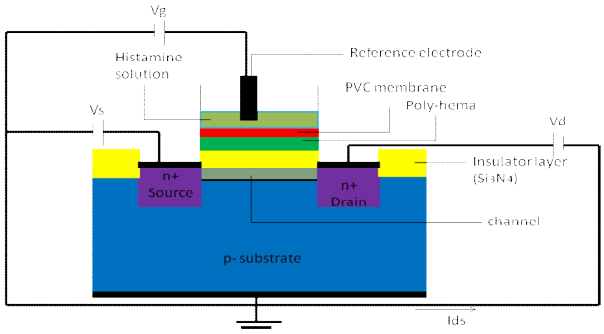

Fig.1. Structure of ISFET with PolyHEMA and PVC Membrane

ISFET can be adapted to detect ions besides H+-ions. Our research targets at histamine ion. An ion selective plasticized PVC membrane concocted with a membrane cocktail recipe, uniquely for histamine, makes the adaptation [15][16][17]. The membrane cocktail recipe that enables selectivity of the histamine ion is constituted of the following. Membrane support made of synthetic PolyVinyl Chloride (PVC (C2H3Cl)n) is the holding material. PVC is the third most widely produced plastic, after polyethylene and polypropylene. Pure PVC is a white, brittle solid. Mixed with plasticizer such as DiOctyl Phthalate (DOP, C6H4 (C8H17COO)2), the flexibility and durability of PVC membrane is improved as it softens the membrane. DOP is an organic compound. It is the most common in the class of phthalate plasticizers. It is colorless, viscous and soluble in oil but insoluble in water. Its lipophilic and hydrophobic nature restricts diffusion of membrane components to the external solution and improves membrane adhesion to the gate area. Solvent, TetraHydroFuran (THF, (CH2)4O)), dissolves the solid membrane. THF is a moderately polar, aprotic solvent for PVC with dielectric constant of 7.6 and is water miscible. Ionophore, 5-, 10-, 15-, 20-TetraPhenyl Porphyrinato

Manganese (III) Chloride (Mn (TPP) Cl), exhibits high affinity for histamine ions and acts as an ion carrier that recognizes and transports histamine ions across the membrane. Its efficiency determines the sensitivity of ISFET sensor.

III. Methodology

A blank p-type ISFET with n-channel and Si3N4 as an insulator layer was selected for detection of histamine. As shown in Fig.2, the ISFET chip was electrically bonded onto a printed circuit board and encapsulated meanwhile the sensitive gate area was left opened for contact with the electrolyte. It was here that membrane enabling ISFET for histamine detection was deposited.

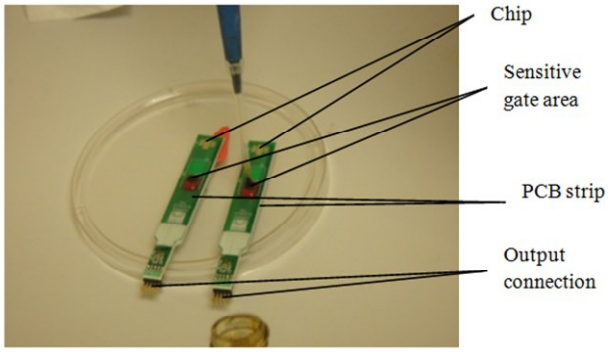

Fig.2. P-Type ISFET with n-Channel and Si3N4 as Insulator Layer

A. pH Sensitivity Test

Functional ISFET is selected using pH sensitivity test. The purpose of this process is to separate the functional ISFETs from the defective ones, produced from the fabrication process. The pH sensitivity test ensures insulator of ISFET will be sensitive to H+-ion.

Fig.3 shows the experimental setup for the pH sensitivity test. The blank ISFET is dipped into three different sets of pH buffer solution based on their level of acidity, neutrality and alkaline (pH4, pH7 and pH10). At temperature 25^0C, the gate voltage (VG) are recorded at constant drain current (ID) of 100µA for every sweep.

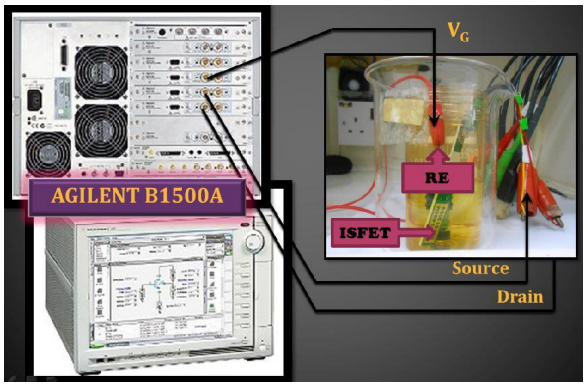

Fig.3. Experimental Setup for pH Sensitivity Test

B. Preparation of Membrane Cocktail

A PVC membrane technology based n-channel Si3N4 ISFET was prepared. Recipe for the membrane cocktail was prepared by dissolving Mn(TPP)Cl, DOP and PVC synthetic membrane, in proportion as tabulated in Table 1, to give a total mass of 50mg in 500µl of THF. All the chemicals were used as received from Sigma. It is important to ensure that all the solid particles must be thoroughly dissolved.

Table 1 Recipe of Membrane Cocktail

	Chemicals	Amount
Membrane Support	PolyVinyl Chloride (PVC)	15mg (30%)
Plasticizer	DiOctyl Phthalate (DOP)	30mg (60%)
Solvent	TetraHydroFuran (THF)	500µL
Ionophore	5-, 10-, 15-, 20-TetraPhenyl Porphyrinato Manganese(III) Chloride (Mn(TPP)Cl)	5mg (10%)

C. Membrane Deposition

After the selection for functional ISFETs and preparation of membrane cocktail, the membrane was then deposited on the gate insulator layer.

ISFETs modified with plasticized PVC membrane is usually found lacking of a thermodynamically well defined interface between membrane and solid contact. Even though acceptable stabilities and drifts have been reported, this attributes to diffusion of carbon dioxide (CO2) through membrane with subsequent formation of carbonic acid at the membrane/gate oxide interface, which effects the proton concentration that in turn determines the potential at the membrane/insulator interface. An approach is to chemically anchor the membrane to the surface of gate oxide with UV-photo-polymerized monomer, HydroxyEthylMethaAcrylate (HEMA), using photolithography. The introduction of the polyHEMA layer absorbs the aqueous buffer solution of salts at the membrane/gate oxide interface, thereby overcomes the ill-defined phase boundary between the membrane and gate oxide. Moreover, this reduces the influence of CO2 as acidic agent which penetrates through the membrane to the gate surface and thus stabilizes the potential developed in the membrane [18].

Fig.4 shows the procedural flow of membrane deposition. Firstly, 0.1µl of our HEMA cocktail was dispensed on the ISFET. PolyHEMA layer was added on. Then it was photo-cured in a N2 room for 180 seconds. After that, it was conditioned in 0.05mol/L of histamine for 30 minutes before a total of 20µl of our membrane cocktail as prescribed in Table 1 was dispensed on the ISFET with a micro-pipette, 5µl at a time. Then, the ISFET was left dried overnight at ambient room condition. Finally, the membrane deposition process was completed with conditioning of the ISFET in 0.05mol/L of histamine for 60 minutes.

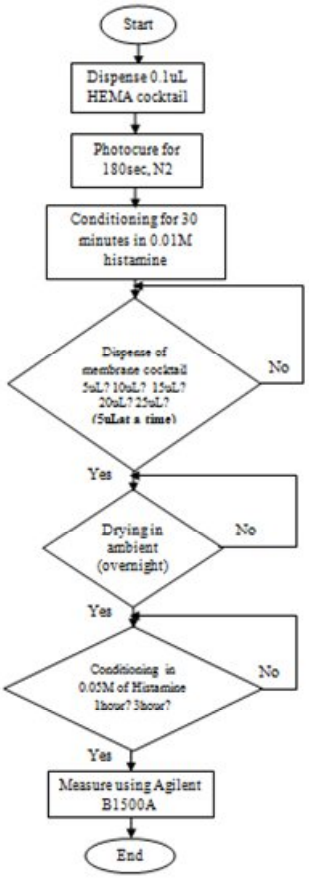

Fig.4 Procedural Flow of Membrane Deposition

D. Histamine Sensitivity Test

Five ISFETs with acceptable Nernstian response to the pH sensitivity test from Section III(A) are deposited with PVC-based membrane as in Section III(C) before they are used for sensitivity test for histamine in response to variation in deposition volumes and conditioning time.

The experiment was performed in the dark box to steer clear of interference from other sources such as temperature, light. All the leads from drain, source and reference junction of ISFET in the dark box were connected to an Agilent Technology semiconductor device analyzer (B1 500A), as in Fig.3.

The reference electrode of ISFET of our design consists of a silver wire coated with silver chloride (Ag/AgCl) in a fill solution of potassium chloride (KCl). The reason for the use of KCl is to maintain a reproducible concentration of silver ions in the fill solution, to engineer a reproducible potential on the Ag/AgCl.

D.1 Effect of Deposition Volume

Each ISFET was deposited with different volumes of membrane cocktail. The gate voltage (VG) was recorded at constant drain current (ID) of 100µA for every sweep, at temperature 25^{0}C. Measurement at each volume of the membrane solution was repeated six times; as the volume was varied from 5µL to 25µL, at an increment of 5µL, while the range of histamine concentration from 10-6 to 10-1 molar. The pH value of the solution is maintained at pH7[19].

D.2 Effect of Conditioning Time

The purpose to condition the membrane is to remove the interfering species, or roughness that can alter catalyze of the selective ion, thereby improving the low detection limit of the ISFET sensor. Here, ISFETs with 20µL of deposition volume was conditioned in 0.05molar of histamine for 1 hour and then 3 hour. Conditioning time of longer interval was not considered because it could cause the thin membrane to leak.

IV. Result and Discussion

It is necessary to check the functionality of raw ISFETs from fabrication before use. Result from pH sensitivity test for this purpose is thus shown first in Section IV(A). After that membrane is deposited on the functional ISFETs, follow by conditioning of the membrane as described in Fig.4. Result from histamine sensitivity test to observe the effect of the deposition volume of membrane and conditioning time are hence shown in Section IV(B) respectively.

A. pH Sensitivity Test

Reaction of ISFET devices towards pH is gauged using Nernstian or quasi-Nernstian response. The Nernst response describes the potential of electrochemical cell, where ion concentration plays a part in the reaction. Theoretically, a good ISFET is said to approach to a Nernstian constant of 59.2mV at temperature 25^{0}C. MIMOS, the national premier applied research and development center in frontier technologies under purview of the Malaysian Ministry of Science, Technology and Innovation, specifies a standard Nernstian constant of 45mV/dec at the same temperature on ISFETs fabricated by them[20].

Fig.7 shows the pH sensitivity of the five blank ISFETs, 41A, 50D, S4H, S4I and S4J chosen for our experiments here. All the blank ISFETs yield linear response from pH4 to pH10, with regression coefficients close to one. They also display good Nerstian slope, 45-48mV per unit change in pH, more than the standard Nernstian sensitivity constant, 45mV/dec, stipulated by MIMOS, indicating they are all functional.

It is interesting to find that blank ISFETs respond with high sensitivity to pH also respond accordingly to histamine, as illustrated in Fig.6-Fig.9 in Section IV(B).

Table 2 Gate voltage at constant drain current of 100µA

pH/ISFET	41A	50D	S4H	S4I	S4J
4	777.5	762.5	692.5	720	780
7	930	902.5	842.5	865	922.5
10	1055	1035	982.5	1002.5	1070

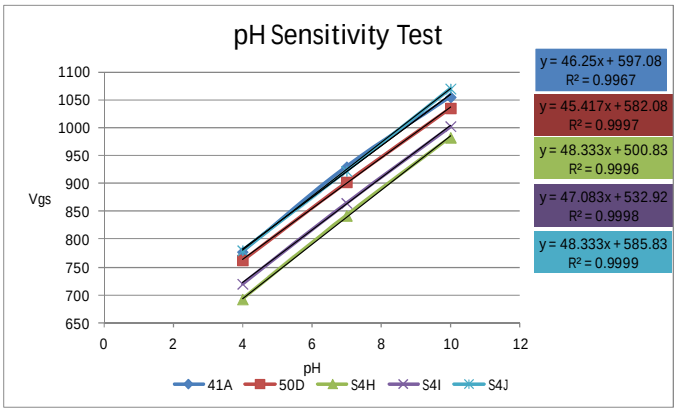

Fig.5. pH sensitivity test for ISFET

B. Histamine Sensitivity Test

B.1 Histamine Sensitivity Test at Different Deposition Volumes

Our novel PVC-based membrane adapts ISFET to be sensitive to histamine ions. Here the effect of deposition volume on Nernstian response of adapted ISFETs is investigated[19].

Fig.6 and Fig.7 show the sensitivity graph of adapted ISFETs in response to the different deposition volumes of membrane cocktail, as concentration is varied from 10^{-6} to 10^{-1} molar of histamine, at conditioning times of 1 hour and 3 hours.

For concentration range between 10^{-2} to 10^{-1} molar of histamine solution, the adapted ISFET S4H-15µL (blue) and S4I-20µL (red) yield the highest Nernstian slope of 72.5mV/dec and 52.5mV/dec respectively with 1-hour conditioning. The sensitivity is increased to 97.5mV/dec and 85mV/dec respectively with 3-hour conditioning. Meanwhile, for concentration range between 10^{-4} to 10^{-2} molar of histamine solution, the adapted ISFET S4H-15µL (blue) and S4I-20µL (red) once again give the highest Nernstian slope of 16.25mV/dec and 2.5mV/dec respectively with 1-hour conditioning. With 3-hour conditioning, sensitivity of ISFET S4I-20µL increases to 13.75mV/dec while ISFET S4H-15µL maintains at 16.25mV/dec. The adapted ISFET S4J-25µL is not responsive to histamine over the entire concentration range. Hence, it can be deduced that a deposition volume of 20µL is optimal for sensitivity to histamine.

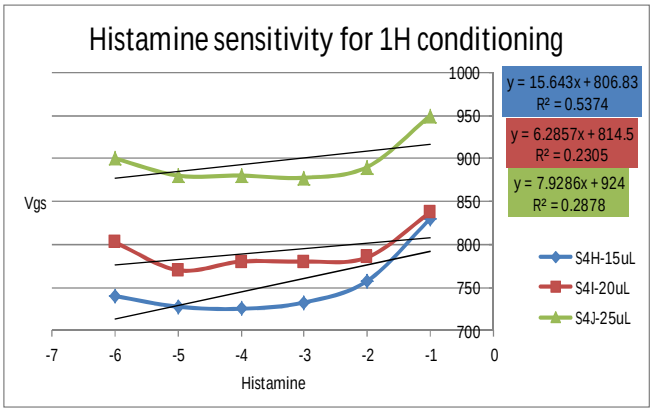

Fig.6. Sensitivity of histamine at different deposition volumes with 1-hour conditioning

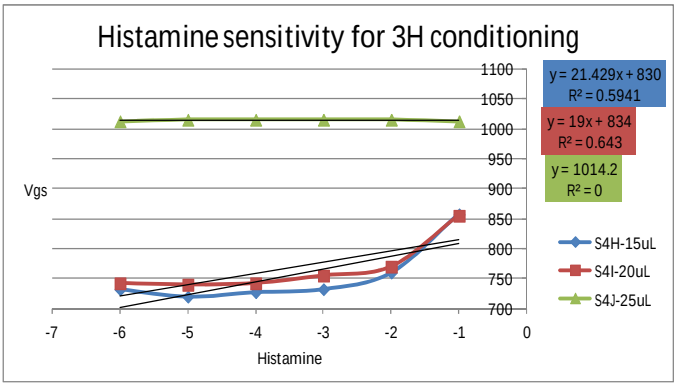

Fig.7. Sensitivity of histamine at different deposition volumes with 3-hours conditioning

B.2 Histamine Sensitivity Test with Different Conditioning Time

Contained in the membrane cocktail of our design is manganese tetraphenylporphyrin (Mn(Tpp)), serving as ionophore. Ionophore functions as an ion carrier for histamine. Conditioning is known to have the effect of reducing the interfering ions, which in turn pushes the low detection limit further lower. Here the effect of conditioning time of adapted ISFETs in histamine solution is investigated.

Fig.10 and Fig.11 shows the sensitivity responses of two adapted ISFETs, 41A and 50D, with conditioning in 0.05molar of histamine for 1 hour and 3 hours. Deposition volume is constant at 20µL.

For concentration between 10^{-2} to 10^{-1}molar of histamine, the sensitivity for ISFET 41A (blue, 30mV/dec) and 50D (red, 10mV/dec) are found higher than those for 3-hours conditioning, where sensitivity of ISFET 41A (blue) is 22.5mV/dec) and ISFET 50D (red) is 2.5mV/dec.

Similarly for the lower range of concentration, 10^{-4} to 10^{-2} molar of histamine, sensitivity of ISFET 41A (blue, 6.25mV/dec) and ISFET 50D (red, 3.75mV/dec) with 1-hour conditioning are found higher than those with 3-hour conditioning, where ISFET 41A reports a sensitivity of 3.75mV/dec while ISFET 50D (red) shows non-reactive.

Outcome of sensitivity responses implies that conditioning time of 1 hour is more appropriate than that for 3 hours, over the entire range of histamine solution concentration.

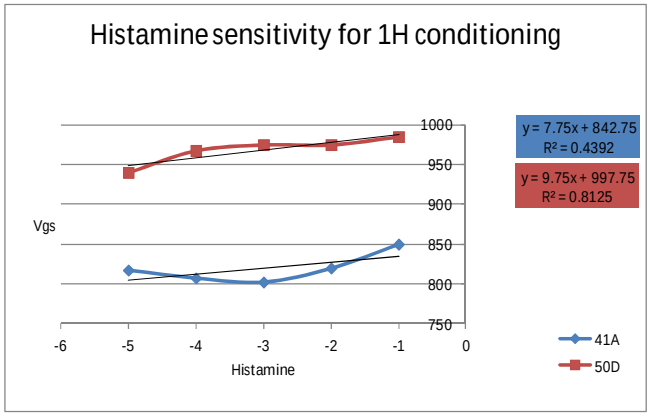

Fig.8. Sensitivity of histamine with 1-hour conditioning

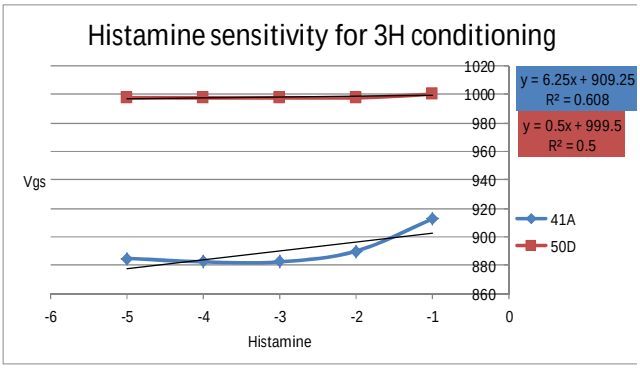

Fig.9. Sensitivity of histamine with 3-hours conditioning

V. Conclusions

Our research here intends to determine the deposition volume and conditioning time for a novel PVC-based membrane with DOP as plasticizer, THF as solvent, polyhema and Mn(TPP)Cl as ionophore that adapts ISFETs to be sensitive to histamine ions. The selection is based on sensitivity of adapted ISFETs to histamine nearest to good Nernstian sensitivity. All the five blank ISFETs, 41A, 50D, S4H, S4I and S4J are verified functional using the pH sensitivity test. They exhibit linear Nernstian slope between 45 to 48mV per unit change in pH, at regression coefficient close to one, as pH varies from pH4 to pH10. This is well above 45mV/dec, the standard Nernstian constant stipulated by MIMOS. In examining the effect of deposition volume on sensitivity of adapted ISFETs, for concentration range between 10-2 and 10-1 molar of histamine, adapted ISFETs S4H-15µL and S4I-20µL of membrane cocktail yield the highest Nernstian slope 72.5mV/dec and 52.5mV/dec respectively, with 1-hour conditioning time. Their sensitivities then rise to 97.5mV/dec and 85mV/dec respectively, with 3-hours conditioning time. For concentration ranges from 10-4 to 10-2 molar of histamine, adapted ISFET S4H-15µL and S4I-20µL again give the highest Nernstian slope of 16.25mV/dec and 2.5mV/dec, with 1-hour conditioning time. As the conditioning time is increased to 3 hours, sensitivity of ISFET S4I-20µL is increased to 13.75mV/dec while ISFET S4H-15µL maintains at 16.25mV/dec. Hence, it can be deduced that a deposition volume of 20µL is optimal for sensitivity to histamine. In examining the effect of conditioning time of membrane on sensitivity of adapted ISFETs, it is found that longer conditioning time produces reaction graph with gentler slope, or lower in sensitivity. Adapted ISFETs with membrane conditioned for 1 hour are found with higher sensitivity than those for 3 hours, over the full range of histamine solution concentration, with 0.05 molar of histamine and a constant 20uL of membrane cocktail.

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