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Investigation of Ag/AgCl Reference Electrode Prepared at Low Heat Treatment Temperature for Electrochemical EGFET pH Sensor

Nurbaya Zainal[1, 2], Sukreen Hana Herman[1, 2, 3] *, Nur Syahirah Kamarozaman[1, 2], Shaiful Bakhtiar Hashim[1, 2], Zurita Zulkifli[1, 2]

[1]*Nano-Electronic Center (NET), School of Electrical Engineering, College of Engineering,*

[2]*Integrated Sensors Research Lab, School of Electrical Engineering, College of Engineering,*

[3]*Microwave Research Institute, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*

Abstract

In this study, the influence of heat treatment on a thin film Ag/AgCl reference electrode was investigated. The thermal evaporation method was used to deposit a 300 nm thick Ag layer on indium-doped tin oxide (ITO) coated glass. The surface properties and electrochemical behavior of Ag/AgCl electrodes prepared at different heat temperatures were examined. Surface analysis of the AgCl layer was performed using scanning electron microscopy (SEM). Measurements of the extended gate field-effect transistor (EGFET) pH sensor were conducted in various pH buffer solutions using Ag/AgCl thin films as the reference electrode and TiO_2 thin films as the sensing electrode. The electrochemical performance of the fabricated Ag/AgCl electrode was evaluated by comparing it with a commercial Ag/AgCl electrode using the same sensing electrode. The comparison revealed that the Ag/AgCl electrode prepared at 100°C exhibited optimum sensitivity (66.18 mV/pH) and linearity (0.9569). The electrode demonstrated good reproducibility and stability, with an average sensitivity value and deviation of 63.7 ± 1.87 mV/pH as confirmed by conducting sets of repeatability measurements. The results indicate that the prepared thin film Ag/AgCl electrodes can be obtained through a low heat treatment temperature procedure and can serve as reference electrodes for EGFET pH sensing measurements.

Keywords: Electrochemical, EGFET, Sensitivity, Repeatability, Reference electrode, pH Sensor.

Full length article **Corresponding Author*, e-mail: hana1617@uitm.edu.my

1. Introduction

In recent years electrochemical pH sensors are commonly used for scientific research, food and environmental monitoring as well as in agriculture fields, due to their advantages such as fast detection, high sensitivity and reliable. Among them, extended gate field effect transistor (EGFET) is one of the promising devices that can offer simplicity fabrication process involves device miniaturization with low-cost factor. EGFET pH sensor is mainly constructed with two electrodes, reference electrode (RE) and sensing electrode (SE). SE is acted as an electrode to sense the presence of ions in a substance, while RE provides constant potential reading during the measurement. The importance of RE has been highlighted by previous studies that focused on the electrode stability to provide accurate measurement output [1-3]. This is attributed to the different type of materials, for instance, standard hydrogen electrode (SHE), saturated calomel (Hg/Hg_2Cl_2) electrode (SCE) or silver/silver chloride (Ag/AgCl) in saturated potassium chloride (KCl) solution. Ag/AgCl type of RE is commonly used for electrochemical based sensor due to its convenient features such as easy maintenance, inexpensive, miniaturization and non-toxicity [1,4-6]. However, the conventional RE are built in bulky size near to micro-scale and has complicated structure which unfit to be applied in sub-micron scale compact sensor. This causes many researchers focused in minimizing the size of RE thus lead to the expansion of its usage towards nanoscale sensor device integration on a chip for biomedical and environmental applications. Tae *et. al.* highlighted in his study that they managed to produce high pH sensitivity of 55 mV/pH and good linearity of 0.99 with the minimization of solid-state thin-film Ag/AgCl reference electrode (SSRE) coated with a layer of graphene oxide [4]. This showed minimization of reference electrode is needed to improve the sensing measurement stability.

Besides the electrode size, the microstructural properties of the AgCl surface also play important role in the same contribution of electrochemical sensor. The influence of microstructural properties towards electrochemical behaviour was presented in previous study [5]. The study proposed application of high current density during electrochemical deposition in order to produced crystal plane (111) of AgCl and the formation of spherical AgCl grains growth that leads to porous microstructure of AgCl film which is essential for fast ion diffusion to be occurred. Several studies also confirmed that the improvement in pH sensitivity derived by the presence of pores which is attributed to nanocrystalline structure of electrode materials [7-8]. Moreover, physical and chemical vapour deposition method has been highlighted in previous work to give significant improvement of the AgCl films microstructural properties [3,4,9]. A granular structure with high pores of AgCl thin films was attained from previous study used electron beam evaporation method with varied electrical current density and resulted good reproducibility (low deviation value of 1 mV) and high pH sensitivity and linearity (48 mV/pH and 0.99) of AgCl thin films [3]. In other work, the Ag layer was deposited by sputter-coating technique with post heat-treated at 320°C in order to improve the mechanical strength of Ag layer [4]. However, the AgCl films were not experienced any heat treated after the chlorination process, yet at the same time graphene oxide was coated on the AgCl films functions as protective layer to avoid the release of chloride ions from electrode. The diffusion of the released chloride ions could create unstable potential reading and further dipping time would lead to the mechanical failure of the AgCl films, i.e., films will be stripped off. Therefore, post heat treatment is necessary to be applied after the chlorination process done as one of the solutions to solve the matter. In this study the thin film Ag/AgCl electrodes were simply fabricated via the thermal evaporation on the conductive indium doped tin oxide (ITO) coated glass substrate. Post heat treatment on AgCl films were performed at low temperature. This study only highlighted low temperature for the heat treatment procedure as claimed by other work, low temperature is commonly suggested to be applied in Ag/AgCl RE fabrication which also suitable for RE flexible substrate [10].The relation of the heat treated and the microstructural and stability behaviour of Ag/AgCl reference electrode in EGFET pH sensitivity measurement were also investigated.

2. Materials and methods

In this study, the Ag/AgCl electrode was fabricated using the combination of two methods: (i) thermal evaporation and (ii) chlorination process. The process is presented in the illustration of **Figure 1**. The first step is the cleaning process of 1cm x 2cm indium doped tin oxide (ITO) coated glass substrate with methanol and Deionized water in ultrasonic bath for 10 min each. A 300-nm-thick layer of Ag were deposited on a glass substrate using thermal evaporator (step 2) and followed by a chlorination process using $FeCl_3$ to form AgCl surface layer (step 3). In last step, the Ag/AgCl RE were dried at low heat temperature of 100°C and 200°C with ambient pressure in an electric furnace. This step was conducted to promote the mechanical strength by implying heat on the as-deposited AgCl layer. Then, the electrode was allowed to cool to room temperature. The final product of RE is ready whenever Ag wire were placed on top of the originated Ag surface and the connectivity was secured with copper tape. Then, the area of the connectivity was covered with Kapton tape to strengthen the contact and avoid the exposure to the substance during the electrochemical measurement process.

Figure 2 shows the configuration setup of EGFET pH sensor measurement using a semiconductor device analyser (SDA) (Keysight B1500A). The fabricated Ag/AgCl and the commercialized RE were connected to SMU 1. Meanwhile, the SE is connected to the gate of commercialized CD40007UBE MOSFET device, which is representing the extended gate field effect transistor (EGFET) device. Other pins like drain and source of FET were connected to SMU 2 and SMU 3 respectively. Both sensing and RE were immersed in the pH buffer solution for more than 1 minute during the measurement takes place. Variations of pH buffer solutions (2, 4, 7, 10, and 12) were used in this study. Graphs of transfer characteristic (I_{DS} vs. V_{REF}) were representing the typical semiconductor device characteristic. The voltage reference value was recorded at I_{DS}=100 µA for each measurement. Furthermore, the sensitivity of the produced sensor was determined with regard to different pH values. In this study, the sensing performance of fabricated sensor with Ag/AgCl RE was evaluated by comparing with the commercial RE. The surface morphology and microstructure of the prepared Ag and Ag/AgCl electrode were observed by scanning electron microscopy (SEM). The SEM images were obtained at different magnification of x5,000 and x10,000 using accelerating voltage at 10 kV.

3. Results and Discussions

The surface morphology of Ag films and AgCl films prepared with different post heat treatment are shown in **Figure 3**. Compared to the smooth surface of Ag substrate (**Figure 3 (a)**), rough surface with whitish grains were observed for Ag/AgCl electrode surface. Also, homogenous distribution of this type of grain were seen on the deposited samples prepared with different heat treatment (**Figure 3 (b), (c), and (d)).** The whitish elements might be due to the chemical oxidation effect mostly when applying aqueous $FeCl_3$ on the metal Ag surface. At x10, 000 magnification, pores can be seen clearly on each sample. AgCl films prepared at 100ºC reveals highly porous structure which may extend to micro size channel embedded in AgCl grains. The size of the micro-channels is estimated between 300 to 800 nm which reveals the size are larger than pores. The size of the microchannel may aid in efficient ion diffusion [5]. **Figure 4** shows the transfer characteristics (I_{DS}–V_{REF}) plot in the linear region presents commercial, as-deposited, and heat-treated Ag/AgCl RE (100ºC and 200ºC). The reference voltage (V_{REF}) ranged from 0 to 3 V and the drain voltage (V_{DS}) was kept constant at 0.1 V. Each measurement was made in accordance with the pH range of 2 to 12. The commercial RE shows the increased voltage values when the pH value increased, leading to linear relationship. This behaviour showed that the voltage is shifted from left to the right is caused by the changes made at the interface between the SE and the electrolyte solution (different ion concentration). The device sensitivity was determined using the linear fit expression derived from the figure as shown in **Figure 4**.

The EGFET pH sensor's sensitivity of 59.29 mV/pH and linearity of 0.9981 was obtained using the commercial RE, which is closest to the Nernstian equation. Most of EGFET pH sensor-based metal oxide SE material paired commercial Ag/AgCl RE obtained optimum sensitivity value near Nernstian value [11-15]. With the same SE material (TiO_2), similar transfer characteristic response was observed for all REs. From the Figure 4, high sensitivity value of 66.18 mV/pH was gained by the Ag/AgCl RE prepared with 200°C post-heat treatment temperature. Meanwhile, RE prepared as-deposited and with 100°C post heat treatment managed to gain 52.01 mV/pH and 64.34 mV/pH respectively. Both heat-treated RE samples possessed super Nernstian sensitivity values (≥ 60 mV/pH), and this proves that the post-heat treatment gives a slight improvement in pH sensitivity compared to the as-deposited and commercial RE. **Figure 5** shows the output characteristics (I_{DS}-V_{DS}) plot in the saturation region for commercial and all Ag/AgCl RE samples prepared in this study. This plot represents a channel that has been formed to allow current to flow between the drain and source of the EGFET device. As shown in the figure, the drain current approaching constant current value for all drain voltage generated at fixed gate voltage. The saturated current sensitivities for all measurements were obtained at VDS = 2.0 V, as shown in the figure. The pH current sensitivity and the linearity for Ag/AgCl RE prepared as-deposited at 100°C and 200°C were 1.0035, 1.3128, and 1.0915 $(\mu A)^{1/2}$/pH respectively. Meanwhile, the linearity that represents the linear regression, R^2 was 0.9538, 0.9455, and 0.9452 respectively. As for comparison, the commercial RE was used and obtained a current sensitivity of 1.2659 $(\mu A)^{1/2}$/pH and a linearity of 0.9471.

Among the samples, the Ag/AgCl RE prepared at 100°C can be shown to obtain the highest pH current sensitivity. This result is reflected in the transfer characteristic (**Figure 4**), as the highest voltage sensitivity was produced by the same sample. Due to this reason, this sample was selected to be used as RE for the TiO_2-based EGFET pH sensor. But before actual conclusion can be drawn, the stability and performance of the RE must be determined first. Most of the previous works have also focused on the stability of the EGFET pH sensor [7, 15,16]. Repeatability measurement is one of the methods involved in the stability performance of the EGFET pH sensor. By repeating measurements across sets of pH ranges, this analysis can help reveal the precision response of the sensor, which may lead to accurate data. The repeatability measurement for the sample of Ag/AgCl RE prepared at 100°C was analyzed by repeating three sets of pH sensing measurements that produced similar results of transfer characteristic (I_{DS}-V_{REF}) graph. The voltage reference (taken at I_{DS}= 100 µA) for each corresponded pH value was plotted in a similar way as before (Figure 4). **Figure 6** shows the plot of reference voltage for each set, and the data was analyzed in **Table 1**. The data covers the reference voltage, average, and standard deviation among the three reading sets. The sensitivity and linearity values were also obtained for each set. From the data, it is proven that the Ag/AgCl RE (100°C) sample has good repeatability that corresponds to each pH value and manages to obtain a small standard deviation of less than 0.025. Moreover, this study reveals that the 2nd and 3rd set of pH sensing measurements produced almost similar sensitivity values, indicating that this sample possessed good stability as RE.

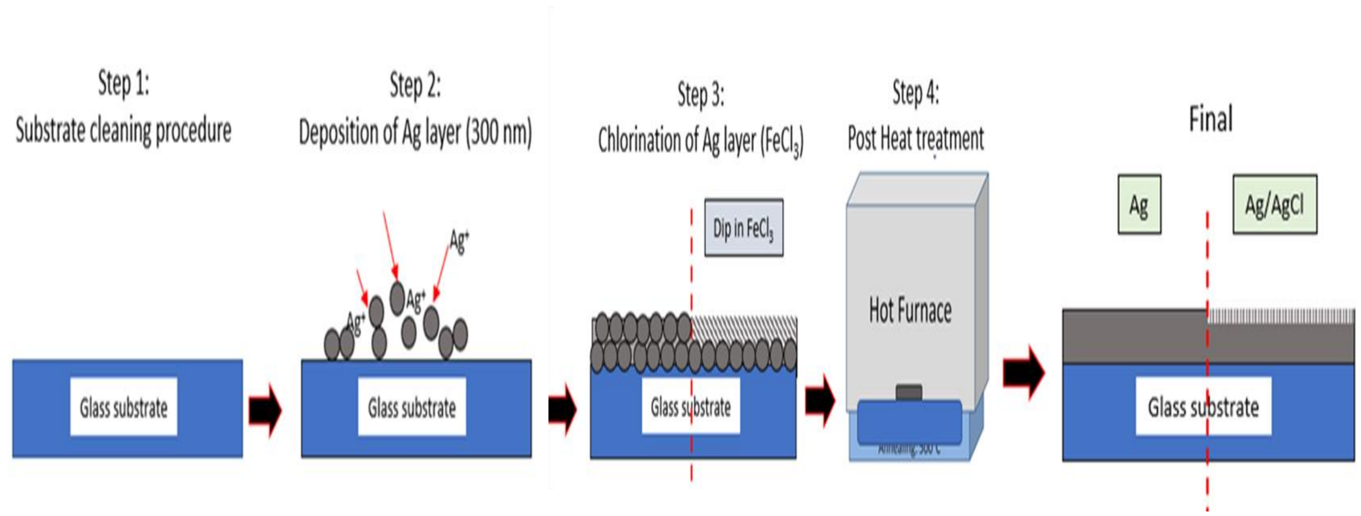


Figure 1: Fabrication process of Ag/AgCl RE, step1: substrate cleaning procedure, step 2: Deposition of Ag layer, step 3: chlorination of Ag layer ($FeCl_3$), and step 4: post heat treatment.

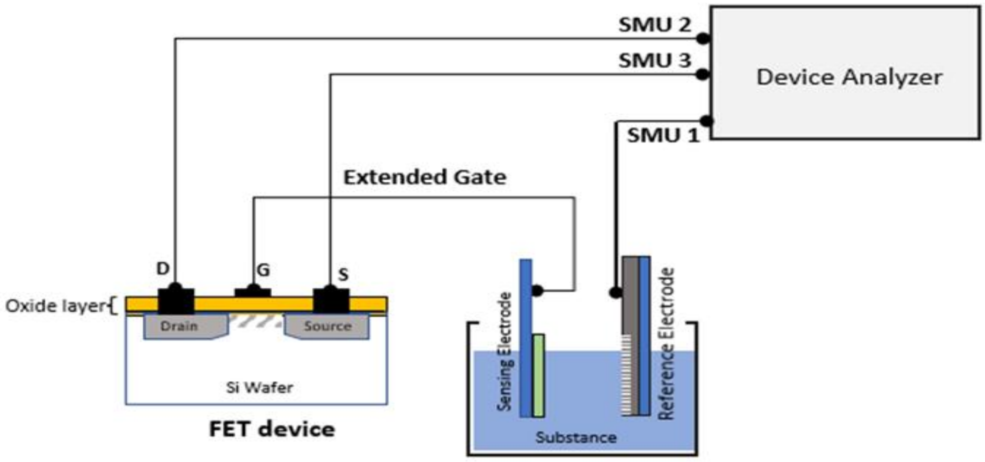


Figure 2: Typical configuration of EGFET pH sensor measurement setup, connection SMU 1 → RE, SMU 2 → pin drain and SMU 3→ pin source of FET device. SE → pin gate of FET device.

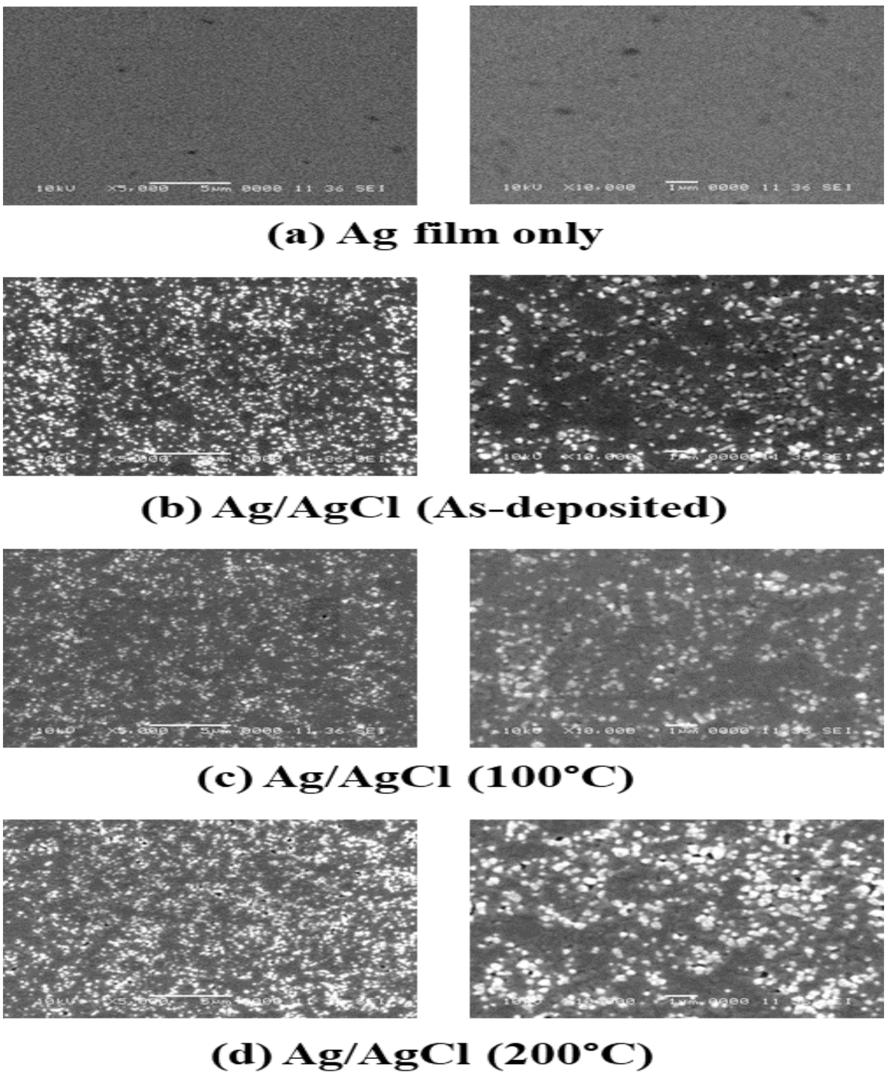
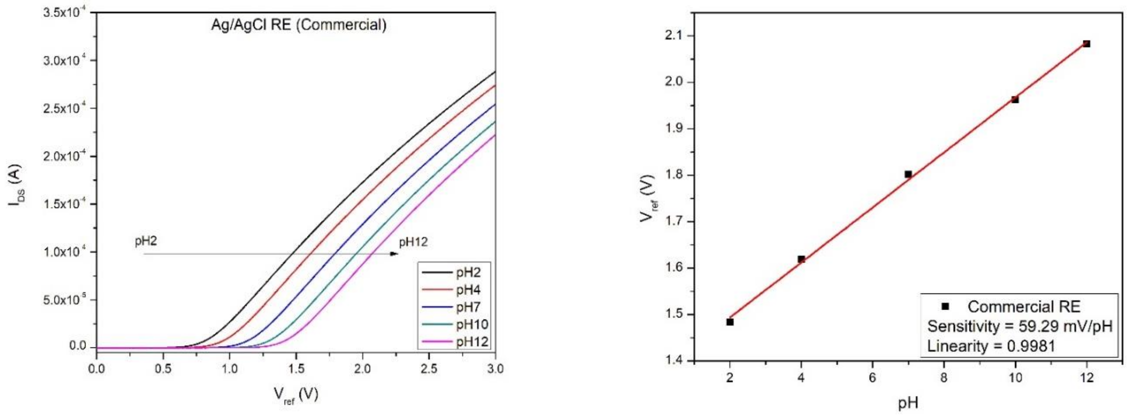
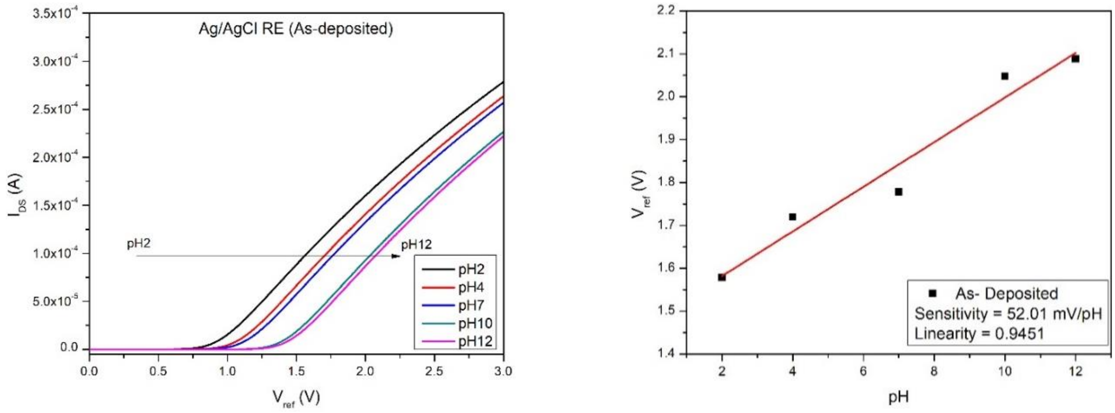


Figure 3: SEM images of (a) Ag films and Ag/AgCl film prepared at (b) as-deposited, (c) 100°C and (d) 200°C. Magnification at x5,000 (left) and x10, 000 (right).

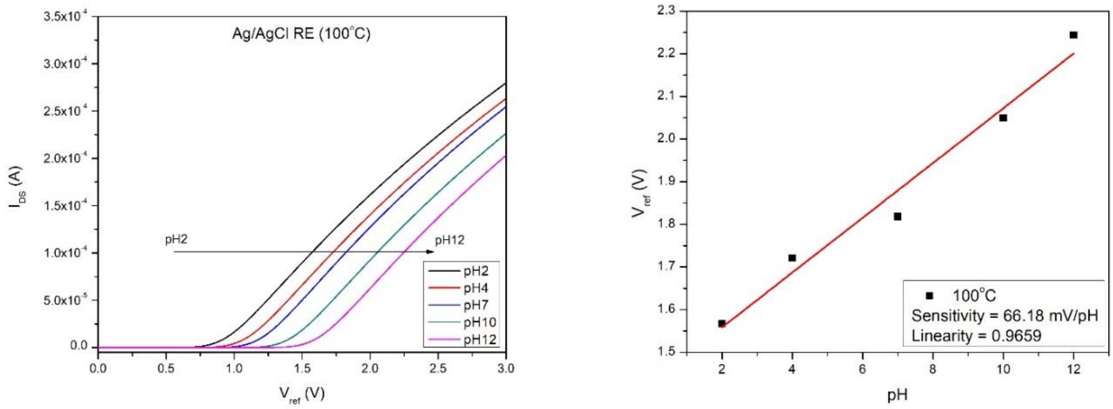
Transfer characteristic | Voltage Sensitivity



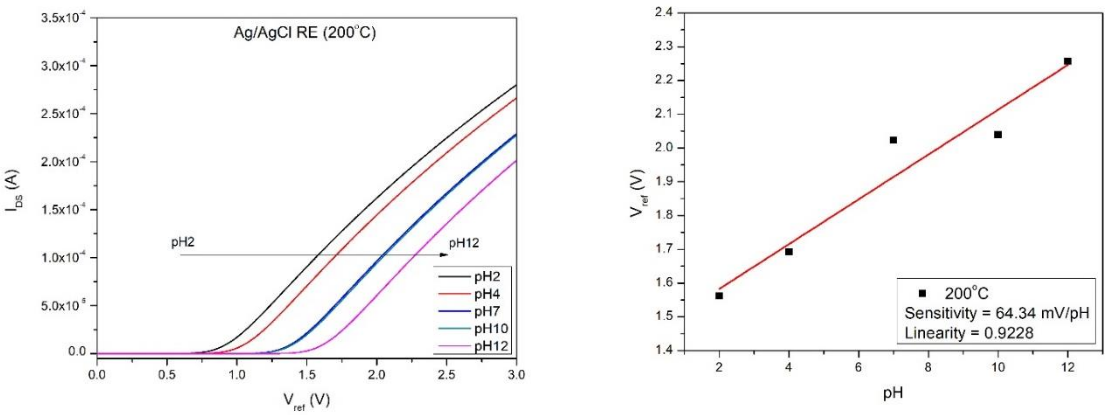
(a) Commercial Ag/AgCl RE



(b) Ag/AgCl RE (As-deposited)



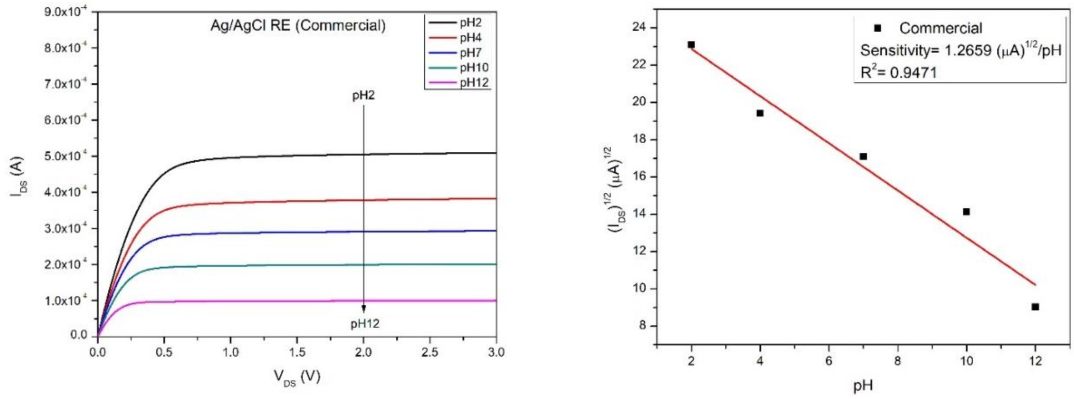
(c) Ag/AgCl RE (100°C)



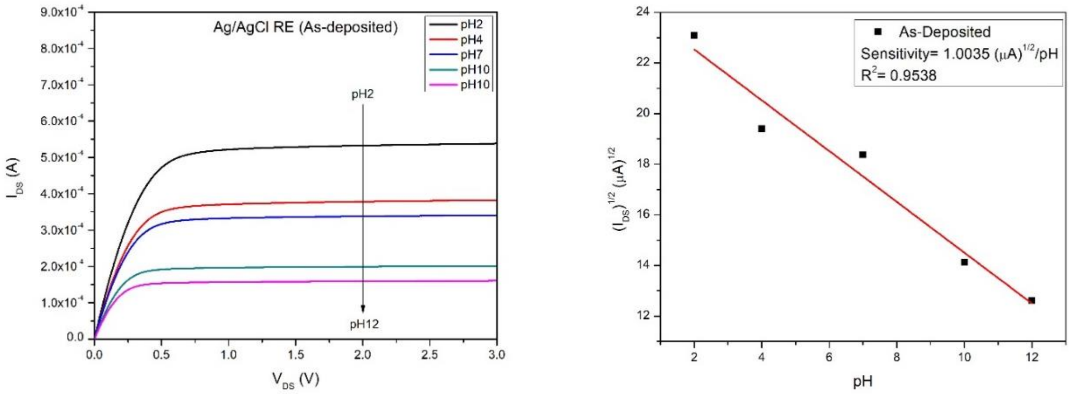
(d) Ag/AgCl RE (200°C)

Figure 4: I-V transfer characteristic in linear region measured in variation of pH value (pH2 to pH12) and pH sensitivity plot for (a) commercial AgCl RE and Ag/AgCl film prepared at (b) as-deposited, (c) 100°C and (d) 200°C.

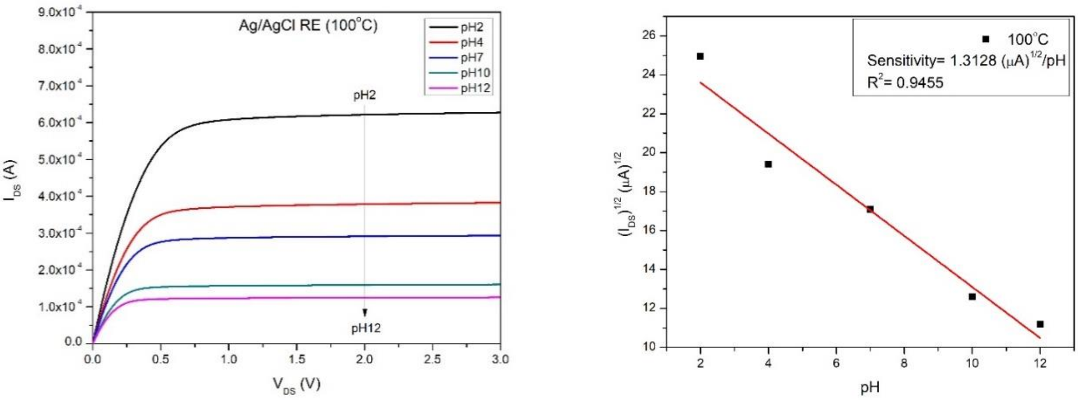
Output Characteristic | Current Sensitivity



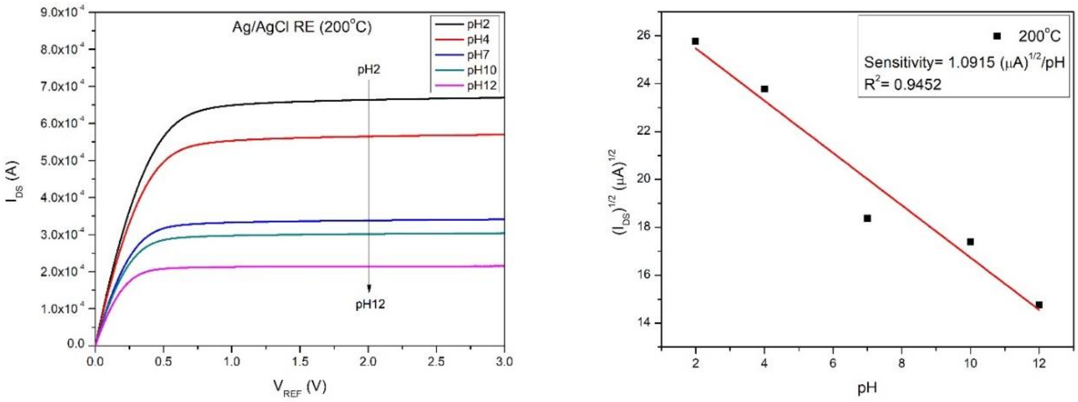
(a) Commercial Ag/AgCl RE



(b) Ag/AgCl RE (As-deposited)



(c) Ag/AgCl RE (100°C)



(d) Ag/AgCl RE (200°)

Figure 5: Output characteristics (I_{DS}–V_{DS}) and sensitivity in saturation region measured in variation of pH value (pH2 to pH12) for (a) commercial AgCl RE and Ag/AgCl film prepared at (b) as-deposited, (c) 100°C and (d) 200°C.

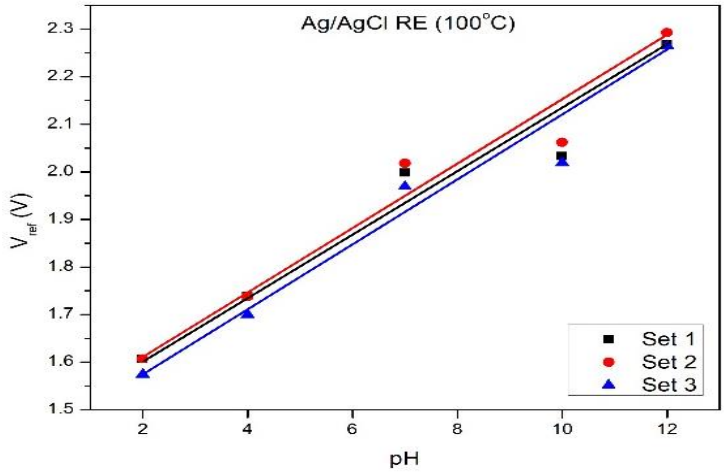


Figure 6: Three sets pH sensing measurement of Ag/AgCl RE prepared at 100°C.

Table 1: Repeatability measurement data for Ag/AgCl RE prepared at 100°C with average value and standard deviation.

pH	**Sets of pH Sensing Measurement**				
	1st Set	**2nd Set**	**3rd Set**	**Average**	**Standard Deviation**
2	1.606 V	1.607 V	1.574 V	1.596 V	0.019 V
4	1.739 V	1.738 V	1.700 V	1.726 V	0.022 V
7	1.998 V	2.018 V	1.969 V	1.995 V	0.025 V
10	2.033 V	2.062 V	2.019 V	2.038 V	0.022 V
12	2.268 V	2.293 V	2.265 V	2.275 V	0.015 V
Sensitivity	61.6 mV/pH	64.7 mV/pH	64.9 mV/pH	63.7 mV/pH	1.850 mV/pH
Linearity	0.9539	0.9588	0.9597	0.957	0.003

4. Conclusions

In conclusion, we have successfully prepared Ag/AgCl RE to be used with TiO_2 SE for pH sensing-based EGFET measurement. The Ag/AgCl layer was fabricated using thermal evaporation and a simple chlorination process with a low post-heat treatment of 100°C and 200°C. The prepared RE was tested for pH sensing performance in different pH buffer solutions ranging from pH 2 to 12, and for sensing performance comparison, commercial RE was also used in this study. The optimum RE, which possessed good sensitivity properties with the highest sensitivity and linearity values of 66.18 mV/pH and 0.9659, respectively, was performed by Ag/AgCl RE prepared at 100°C. The sample also possessed high stability, which is proven by repeatability measurements. Thus, the preparation of RE needs to be controlled at very low temperatures, and further analysis that covers different parameters such as current density adjustment will be highlighted in the future.

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