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Investigation of ISFETs' polyurethane matrix membrane to suppress drift in saliva

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Abstract: We have studied on the stress measurement by making use of salivary nitrate, which can be a candidate of stress markers, with ion-selective field-effect transistors (ISFETs). ISFETs are suitable for on-site single-drop analysis of salivary nitrate within 10 seconds. However, when ISFETs are used for salivary nitrate, ISFETs have a problem which is called the initial drift. The initial drift makes it difficult for determination of an accurate nitrate monitoring. Thus, the purpose of this study is to suppress an initial drift and to search for new easy polymer to possess more performance of sensor responses than conventional matrix membrane such as PVC. In this research, we investigated ISFETs using specific matrix membrane for example, KP-13, Pellethane® and P7281-PU. The initial drift was evaluated from the fluctuation of the response values generated by ISFETs which are immersed in saliva or aqueous solution. As a result, P7281-PU showed its suppression effect for the initial drift in the whole saliva and various solutions. Furthermore, the cause of drift may be H^+ diffusion, and drift suppression effect of P7281-PU may be affected by urethane bond capturing H^+ in ion-selective membrane. This result suggests a continuous nitrate monitoring and development of wearable sensors.

Keywords: ISFETs; ISE; polyurethane; salivary nitrate; stress; drift; ion-selective membrane

1. Introduction

The cause of depression is stress and genetic vulnerability [1-3]. In addition, there are various diseases and physical disorders associated with stress. Therefore, stress measurement is an important issue and it contributes to the prevention of diseases such as depression. In this study, we are focusing on the biochemical method to evaluate stress from the stress-marker in body fluid. One of the body fluids, blood which contains typical stress-markers (adrenaline and noradrenaline [4,5]) is collected invasively accompanied by stress. In contrast, saliva which is derived from blood is collected non-invasively. Hence saliva is suitable for stress-measurement. Salivary nitrate is secreted by blood nitrate which is a NO metabolite through salivary grand [6]. NO is an endothelium-derived relaxing factor (EDRF) [7] which is produced in response to vascular tone associated with the effect of the autonomic nervous system. Therefore, salivary nitrate is expected to be used as a stress-marker of the autonomic nervous system. The candidates of salivary stress-marker are cortisol, chromogranin A [8], α-amylase [9]. However these candidates are not suitable for the on-site stress measurement, because they are determined with ELISA, it takes time to measure stress hormone. As a measurement device, Ion-Selective Field Effect Transistors (ISFETs) are the most suitable for on-site single-drop analysis of salivary nitrate within 10 seconds. Hence, the ISFETs are adapted as the stress measurement making use of saliva. On the other hand,

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current salivary NO_3-ISFETs have the problem that drift occurs during measurement. Drift is the phenomenon in which the response value of the sensor changes gradually, making difficult to determinate salivary nitrate accurately. The ISEFTs' gate part consists of H^+ sensitive metal oxide, for example, SiO_2, S_3N_4 and Ta_2O_5. Fogt, et al. considered that the cause of drift is the pH change at the ISM/Gate interface's aqueous layer. It's concerned with CO_2-gas diffusion which approaches the ISM/Gate interface's aqueous layer through the ion-selective membrane [10]. The gate part which we use in this paper is Ta_2O_5, hereinafter pH sensitive ISFETs called pH-ISFETs. Other previous researches attempted to insert the inner layer in the ISM/Gate interface. For example, pHEMA-gel saturated buffer [11], Ag/AgCl [12] and H^+ selective membrane [13]. On the other hand, Abramova, et al. considered that the cause of drift is protein adsorption to the ISM surface. Photocurable polyurethane as a matrix membrane is effective in the measurement of blood plasma [14]. However, there is no report that aims to suppress drift in saliva. Therefore, we report that polyurethane is used as the matrix membrane to suppress drift for salivary nitrate. The actual used polyurethanes are Pellethane®, KP-13, and P7281-PU. Pellethane® is widely used in the medical field such as a catheter. Hence, Pellethane® is expected to suppress the adsorption of glycoproteins in saliva. Espadastorre and Meyerhoff reported Pellethane® based ISM showed somewhat less platelet adhesion [15]. KP-13 is the polyurethane urea containing 13 wt% of dimethylsiloxane. Wakida reported KP-13 is the effective matrix membrane to measure blood electrolyte [16] and salivary nitrate [17]. Although P7281-PU has not been used as a matrix membrane, P7281-PU used ISM has adhesiveness and can be closely adhered to the gate to restrict appearance of the water layer.

2. Materials and Methods

2.1. Materials

Original ISFETs for pH measurement (pH-ISFETs) were purchased from ISFETCOM (Saitama, Japan). The pH-ISFETs are integrated a reference electrode. As a nitrate ionophore, Bis(dimethyl-phenanthroline) Copper(I) nitrate ($[Cu(bcp)_2]NO_3$) was synthesized with reference [18]. As a plasticizer, 2-Nitrophenyl dodecyl ether (NPDDE) was purchased from Wako Pure Chemical Industries (Osaka Japan). NPDDE was used for stable ISFET to improve the adhesion to gate material of ISFETs [19]. Polyvinyl chloride (PVC, n=1000) used as a matrix membrane was purchased from Kishida Chemical (Osaka, Japan). KP-13 was received from Kaneka Chemistry (Osaka, Japan). Pellethane® was received from Lubrizol (Ohio, USA). P7281-PU was purchased from Polymer source (Dorval, Quebec, Canada).

NaCl, KCl, KH_2PO_4, Urea, Na_2SO_4, NH_4Cl, $CaCl_2 \cdot 2H_2O$, $NaHCO_3$, KNO_3 were used reagent grade as much as possible. Tetrahydrofuran (THF) which was purchased from Wako. THF was used as a volatile solvent to dissolve the nitrate ionophore, plasticizer, and polymer. Mucin from bovine submaxillary glands was purchased from MP Biomedicals, LLC (California, USA). Real saliva was collected by *salivette* purchased from Sarstedt (North Rhine-Westphalia, Nümbrecht, Germany) and a metal spoon.

All chemicals were used without purification. All standard solutions and artificial saliva were prepared with Milli-Q water (18.2MΩ cm).

2.2. Preparation of NO_3-ISFETs

Nitrate selective ISFETs (NO_3-ISFETs) used to evaluate the sensor response and initial drift were prepared by the following procedure. After forming a THF solution of the nitrate selective membrane containing 5 wt% of nitrate ionophore ($[Cu(bcp)_2]NO_3$), 65 wt % of a plasticizer (NPDDE) and 30 wt% of the matrix membrane polymer, which was completely dissolved in the solution at room temperature. NO_3-ISFETs were prepared by casting THF solution onto the gate part in the pH-ISFETs. After the casting, THF solution onto the gate was completely evaporated in a clean space (Pure Space 01; AS ONE, Osaka, Japan) and the same manner repeated several times. The resulting sensing membrane was allowed to dry overnight. The thickness of the nitrate selective membrane was ca. 0.2 mm.

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2.3. Determinent of calibration curves and selectivity coefficients in NO_3-ISFETs

After the prepared ISFETs was conditioned in 1 mM (mol/L) KNO_3 solution (standard solution) for 3 hours. Its calibration curves and selectivity coefficients were determined using the Nicolsky-Eisenman's equation (1) as follows where E is the potential of the NO_3-ISFETs, E_0 is the standard potential of NO_3-ISFETs, Zi and Zj are the charges of the primary ion i (NO_3) and interfering ions j, and ai and aj are the activities of ions i and j, respectively. R, T, and F have the usual meanings.

$$E = E_0 + 2.303 \cdot RT/(ZiF) \cdot \ln (a_i + K_{ij}^{pot} a_j^{zi/zj}) \quad (1)$$

According to the theoretical response at 25 Celsius, the slope sensitivity of the NO_3-ISFETs is -59.16 mV per decade change of the activity ai. ai was calculated from the Debye-Huckel's equation based on the simple ionic-strength theory. K_{ij}^{pot} is the selectivity coefficient of the NO_3-ISFETs in the presence of interfering ion, *j*. K_{ij}^{pot} was evaluated by the mixed-solution method, and can be considered to be a reliable selectivity parameter. The potential responses were measured in the NO_3 standard solutions in the presence of each interfering ion using 5.0×10^{-1} M NaCl, 5.0×10^{-1} M Na_2SO_4, 5.0×10^{-2} M $NaNO_2$ 5.0×10^{-5} KI and 5.0×10^{-5} KSCN. Furthermore the calibration curve in artificial saliva is also determined. Artificial saliva is an aqueous solution reproducing human's salivary electrolytes. Reagents used for artificial saliva and their concentrations are as follows: 2.15 mM NaCl, 12.9 mM KCl, 4.81 mM KH_2PO_4, 0.33 mM Urea, 2.37 mM Na_2SO_4, 3.33 mM NH_4Cl, 1.55 mM $CaCl_2 \cdot 2H_2O$, 7.51 mM $NaHCO_3$.

2.4. Evaluation of initial drift in NO_3-ISFETs

Evaluation of initial drift is performed by looking at the output value of ISFETs immersed in solution about 8 hours. Main measurement target is human saliva. Two types of human saliva are prepared according to the sampling method. The one is obtained from the saliva collection kit: salivette and another one is obtained from a metal spoon. Four kinds of solutions 10^{-3} M potassium nitrate (standard solution), artificial saliva, the standard solution with 0.5 wt% Mucin (mucin solution), and standard solution with CO_2 bubbling (CO_2 solution), are used for comparison. Artificial saliva used for this section is added 1 mM KNO_3. In experiments, evaluation of initial drift was conducted in a closed system using a cap attached to the ISFETs in order to prevent volatilization of the aqueous solution and contact with air (showed in Fig.1). All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the saliva sample experiments were approved by the Ethics Committee of National Institute of Advanced Industrial Science and Technology (AIST) (Project identification code is human 2015-195) in January 29th 2016.

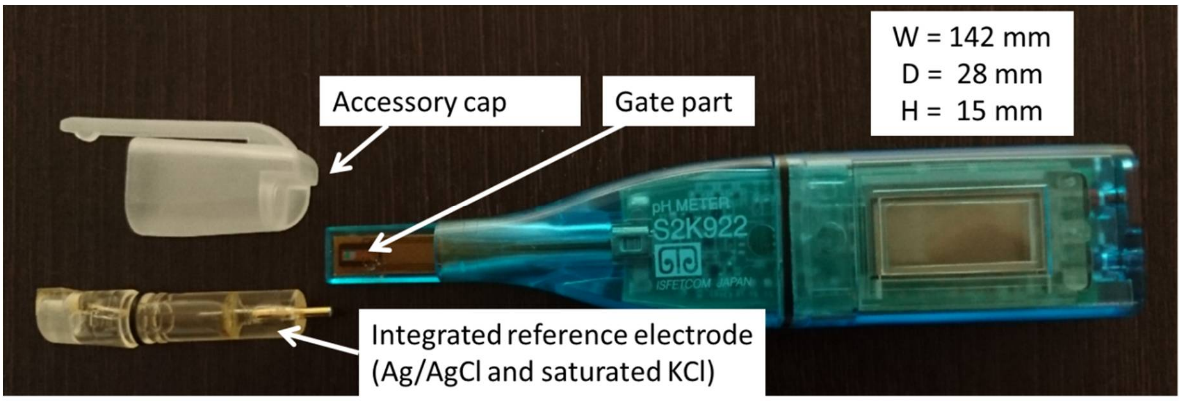

Figure 1. pH-ISFETs and accessory cap

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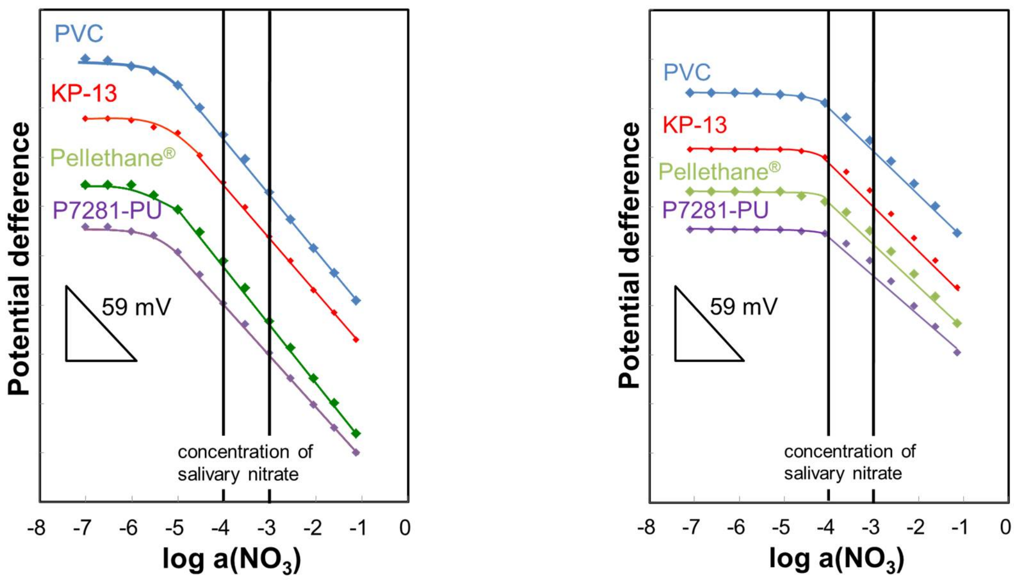
3. Results and Discussion

3.1. Calibration curves and Selectivity coefficients

The calibration curves of NO_3-ISFETs are showed in Figure 2(a). The vertical line shows the potential difference and the horizontal one shows loghrithm of nitrate activity. All NO_3-ISFETs show a linear response to nitrate activity in the range 10^{-5} M to 10^{-1} M. The selectivity of ISFETs is represented by the selectivity coefficients $K_{NO_3}^{pot}$ defined with Nicolsky-Eisenman's equation. Selectivity coefficients for typical anions were measured by the mixed solution method and are summarized in Table 1. The linear response range and the selectivity coefficients of the polyurethane-based ISFETs are coincident those of the PVC based ISFETs. Therefore potential determinants are ionophore and plasticizer but matrix membrane. The calibration curves in artificial saliva are showed in Figure 2(b). All ISFETs show a linear response to nitrate activity in the range of 10^{-4} M to 10^{-1} M. Concentration of nitrate in saliva is approximately 10^{-4} M to 10^{-3} M [19]. Therefore, NO_3-ISFETs are adapted to nitrate measurement in saliva.



(a) (b)

Figure 2. Calibration curves of NO_3-ISFETs. (a) only KNO_3 solution. (b) in artificial saliva

Table 1. *.Selectivity coefficients*

Polymer matrix	Selectivity coefficients, $K_{NO_3^-,j}^{pot}$				
	I	SCN	NO_2	Cl	SO_4
PVC	0.82	0.95	-1.53	-2.55	-4.10
KP-13	0.56	0.95	-1.53	-2.59	-4.03
Pellethane®	0.78	1.09	-1.50	-2.71	-4.11
P7281-PU	0.69	1.07	-1.40	-2.30	-4.13

3.2. Evaluation of initial drift in saliva and various solutions

The drift characteristics in saliva and various solutions are showed in Figure 3. The vertical line shows the potential difference and the horizontal one shows loghrithm of time. In standard solution and CO_2 solution, all NO_3-ISFETs show a comparatively stable response. In mucin solution,

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PVC based ISFETs show a slightly drift but in other polyurethane-based ISFETs show a stable response. It is suggested that CO_2 is not the cause of drift. Furthermore, mucin is the cause of drift for PVC based ISFETs not in other polyurethane-based ISFETs. PVC, KP-13, and Pellethane® based ISFETs show drift in salivary samples (Fig. 3(d), (e) and (f)). However, P7281-PU based ISFETs show a comparatively stable response in salivary samples. Figure 4 shows long-term stability of P7281-PU based ISFETs. The vertical line shows the slope sensitivity and the horizontal one shows the time. P7281-PU based ISFETs maintain the same slope sensitivity for within 27days. It is considered that the stable response of the P7281-PU based ISFETs is due to the strong adhesion of the ion-selective membrane to the Ta_2O_5 gate and the formation of water layer at the gate/ISM interface is suppressed.

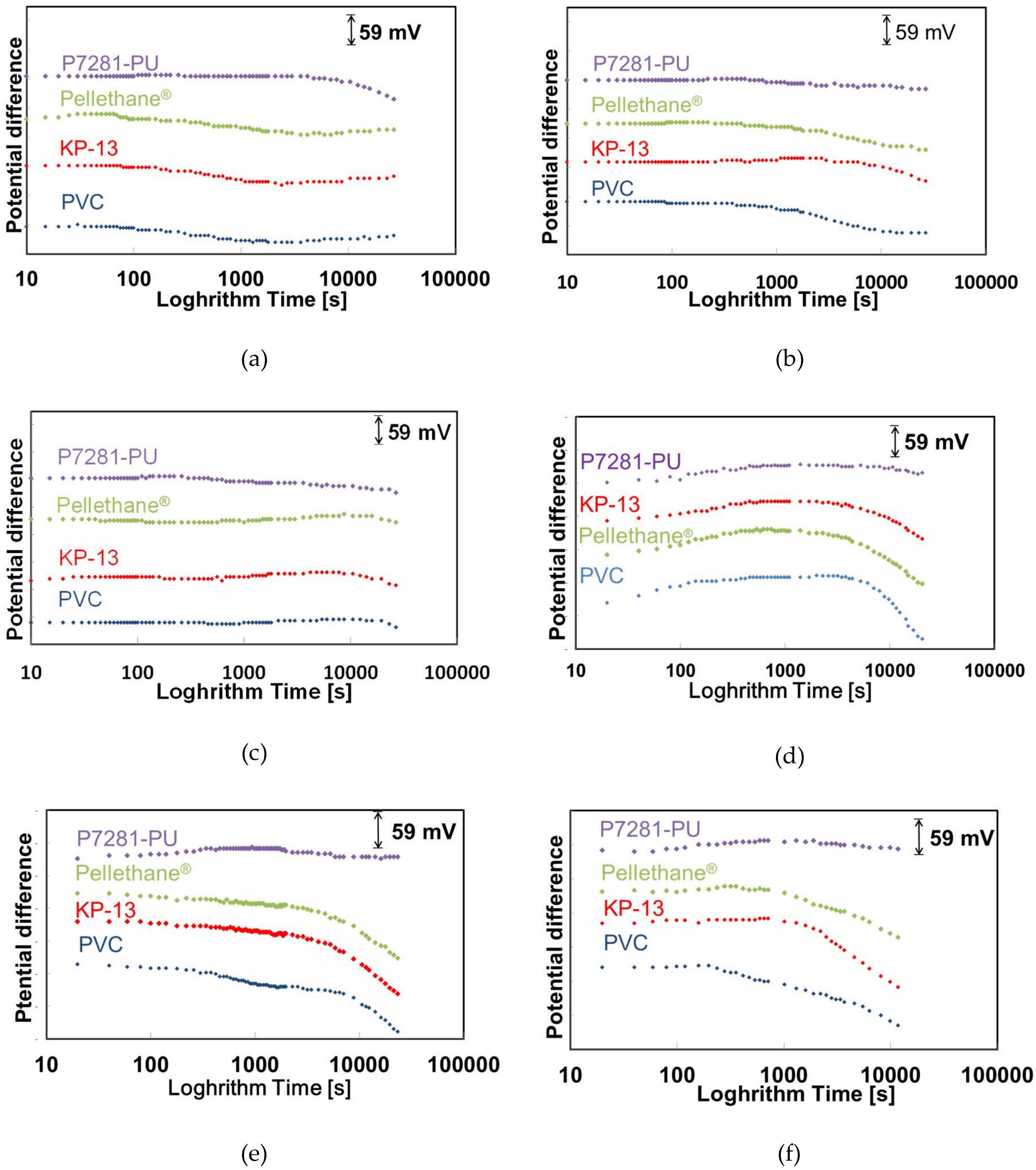

Figure 3. Initial drift of NO_3-ISFETs (a) in standard solution; (b); in standard solution with 0.5 wt% mucin; (c) in standard solution with CO_2 bubbling;(d) in artificial saliva; (e) in saliva via *salivette*; (f) in whole saliva (non-pretreatment)

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Note that the drift characteristic of P7281-PU based ISFETs is superior stable in whole saliva. It is considered that urethane bond in P7281-PU capture H^+, OH^- in the aqueous layer. This hypothesis suggests in Figure 5.

However the cause of drift is not clear, it is currently being investigated using matrix membranes that have the same molecular structure as P7281-PU but only differ in molecular weight. Anyway, P7281-PU is effective matrix membrane for NO_3-ISFETs for measurement of saliva. In the future, it can be expected to achieve continuous nitrate monitoring and deployment to wearable sensors.

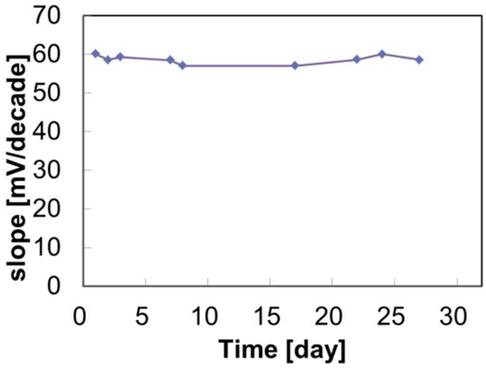

Figure 4. Long-term stability of P7281-PU based ISFETs

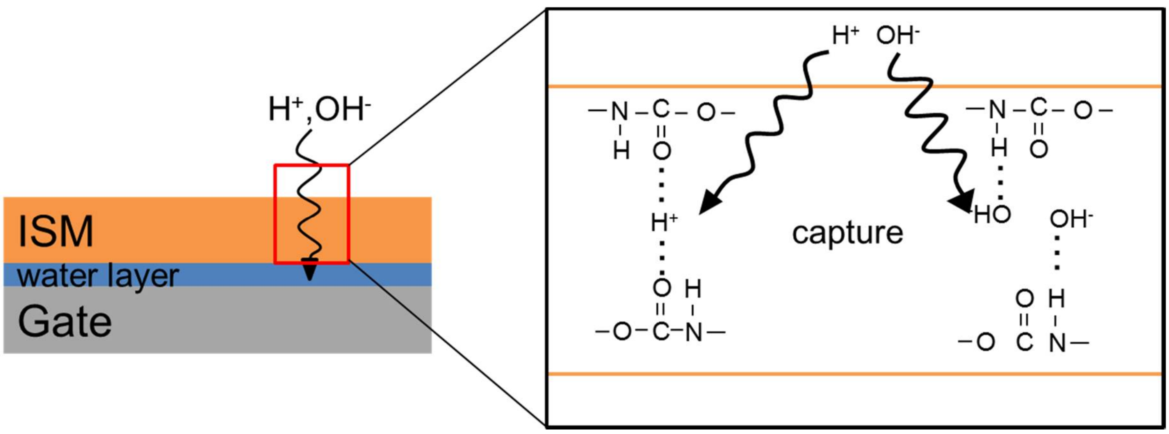

Figure 5. The hypothesis of urethane bond capture H^+ and OH^- by hydrogen bond

Author Contributions: conceptualization, S.W.; methodology, S.O. and S.W.; validation, T.K. and K.K.; formal analysis, S.O. and T.K.; investigation, S.O.; resources, S.O. and S.W.; data curation, S.O., T.K. and S.W..; writing—original draft preparation, S.O.; writing—review and editing, K.K. and S.W.; visualization, S.O.; supervision, T.M. and S.W.; project administration, S.W.; funding acquisition, S.W.

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