A Novel, Low Power Biosensor for Real Time Monitoring of Creatinine and Urea in Peritoneal Dialysis

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ABSTRACT

Novel biosensors, based on immobilized creatininase, creatinase and urease are developed, using ISFETs with weak inversion at pH 6-8 and 37.0°C. The ISFETs with circuitry, demonstrate a linear relationship of urea and creatinine at the range of 0-200 mM and 0-20 mM, respectively. Preliminary results show that biosensors operating in weak inversion mode can eliminate many of the disadvantages of ISFETs operating in the strong inversion region, providing a wide dynamic range output in nanoAmp. Such characteristics fit the analytical requirements for improving real-time monitoring in Peritoneal Dialysis (PD). Further work covers stability of ISFET sensors biased with CMOS circuits in the weak inversion mode working in the room temperature of 15°C to 40°C.

***Keywords**:* Biosensors, ISFET, Weak Inversion, Dialysis

1 INTRODUCTION

1.1 Kidney Failure

Renal replacement therapy in the form of dialysis and transplantation are key elements for sustaining life once a kidney has failed. Adequacy of Peritoneal Dialysis (PD) is still entirely based on empirical correction of mortality with dose parameters, which mainly relies on a pre-programmed timing function of the machine to control the flow of the intake and outtake. An offline adjustable parameter can be inserted after a period of time when test results return from the central laboratory. Adjusting the timing and volume of those intakes during dialysing becomes practically uncommon. As a result, patients may connect unnecessarily with the PD machine longer or shorter than actually required, which may increase or even damage residual renal function.

1.2 Biosensor Technology

The determination of urea concentration in effluent dialysate is widely used as a marker for monitoring the clearance of urea and other toxic molecules during dialysis. Classical spectrophotometric methods although accurate are not applicable for real-time monitoring analyzers. The advance in semiconductor technology in the last few years has allowed the development of potentiometric biosensors. Among these biosensors, enzyme-based field effect transistors (ENFETs) and Ion Sensitive Field Effect Transistors (ISFETs) were developed for urea determination based on the immobilization of urease or creatinase onto the surface of the gate insulator [1].

The FET-based potentiometric biosensors of creatinine have several disadvantages namely, that there is interference due to ammonia and other ionic substances [1]. Another major drawback is that the response is very non-linear, caused by the fact that the induced changes in pH and temperature decrease the enzyme activity and stability drastically [2]. Moreover, the devices generate undesired extra coulomb charges.

2 THEORETICAL CONSIDERATIONS

2.1 Chemical Reactions of Creatinine and Urea

Creatinine and urea are important for diagnosis of renal, thyroid and muscle dysfunctions. During pathophysiological conditions the level of creatinine may rise to > 1000 μM while the normal level of creatinine in urine is 71-276 μM [5-6]. The pathophysiological range of urea covers 30 to 150 mM versus the normal level ranges between 2.5-6.7 mM [7]. Adequacy in Peritoneal Dialysis can be calculated by measuring total weekly creatinine clearance and total weekly Kt/V, which are standardized by the National Kidney Foundation and the Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) [8]. Urease and creatininase are enzymes which catalyses the hydrolysis of urea and creatinine, respectively according to the reactions:

$$\text{Creatinine} + H_2O \xleftrightarrow{creatininase} \text{Creatine} \quad (1)$$

$$\text{Creatine} + H_2O \xrightarrow{creatinase} \text{Urea} + \text{Sarcosine} + \text{Urea} \quad (2)$$

$$\text{Urea} + 2H_2O \xrightarrow{urease} 2NH_3 + CO_2 \quad (3)$$

There are variable factors - enzyme concentration, substrate concentration, pH, ionic strength and temperature which are in consideration to stabilize the chemical reaction process. Of those the theory of enzyme kinetic by Michaelis-Menten [9], the calculation of ionic strength [10] and study of effects of temperature [11] are main novels to refer to.

2.2 Ion Sensitive Field Effect Transistor (ISFET)

The response of an ion-selective electrode following the Nernstian Law is given by;

$$E = E_0 + \frac{RT}{zF}\ln[i] \quad (4)$$

where: E is the measured potential (in volts), E_0 is a characteristic constant for the ion-selective/external electrode system, R is the gas constant, T is the absolute temperature (K), z is the signed ionic charge, F is the Faraday constant, $[i]$ is the molar concentration of the free non complex ionic species.

In a normal working temperature at 25°C potential voltages change is 55mV/pH. Using this basis, ISFET gate/membrane will mirror to ions generated from chemical reaction. Therefore, ISFET output will relate to changes of ions from the chemical reaction.

ISFET is a MOSFET family member and consists of an ion-sensitive membrane and a field-effect-transistor (FET) with a reference electrode inserted in a solution connected to the gate. The different voltage threshold (V_t) of MOSFET is fixed whereby ISFET's V_t is variable to the pH changes of the solution. The drain current of the ISFET in the non-saturated mode is

$$I_{DS} = \mu_n C_{ox} \frac{W}{L}\left[(V_{GS} - V_T)V_{DS} - \frac{1}{2}V_{DS}^2 \right] \quad (5)$$

With C_{ox} is the oxide capacity per unit area, W and L the width and the length of the channel, respectively, and μ is the electron mobility in the channel.

2.3 Weak Inversion Mode of Operation in ISFET [12, 13]

The so-called "weak inversion" mode of operation involves maintaining the gate voltage lower than the threshold voltage (V_T) such that the channel is depleted and only a thin inversion layer exists. In weak inversion, the mobile charge in the thin inversion layer is too low to contribute significantly to any drift current across the horizontal electric field. Drain current in weak inversion is due to the diffusion of electrons across a concentration gradient between source and drain. Since the electron concentrations at source and drain and along the channel are related to the barrier potentials at those points by the Boltzmann distribution, it follows that the drain current is exponentially related to V_S, V_D and V_G relative to V_B, scaled by the thermal voltage $U_T = \frac{kT}{q}$ or $\frac{RT}{F}$. That is:

$$I_D = I_0 \exp\left(\frac{V_G}{nU_T}\right)\left[\exp\left(\frac{-V_s}{U_T}\right) - \exp\left(\frac{-V_D}{U_T}\right)\right] \quad (7)$$

where: I_0 is the pre-exponential multiplier and n is the sub-threshold slope factor.

For this mode of operation, an exponential drain current relates to the gate voltage.

2.4 Advantages of Weak Inversion Region of Operation versus Strong Inversion Region

Firstly, the drain current of one individual ISFET operating in the weak inversion region is in the nanoAmps range whereas in the strong inversion region it is in the microAmps to milliamps region. Thus, the weak inversion mode of operation is more suitable for implantable and wireless applications where a low power consumption device is required. Secondly, since the relationship between pH and hydrogen ion concentration is logarithmic, there is a natural linearization due to the exponential characteristic of the ISFET operating in the weak inversion region. Furthermore, because of change in pH is a logarithmic relationship, then the ISFET has an extended large dynamic range.

3 AREAS OF INNOVATION, NEW DESIGN AND NEW METHODS

3.1 The Sensor System

From the Peritoneal Dialysis machine, waste consisting of creatinine and urea is extracted and pumped into buffer solutions. ISFET immobilized by enzymes connects to the current feedback OpAmp that ensures stability of the readout. With this approach, the immobilized enzyme in the chemical reaction produces ions and mirrors to the sensor, finally passing to ADC for generating digital output. An I/O unit with internet-interface performs changes of system functionality.

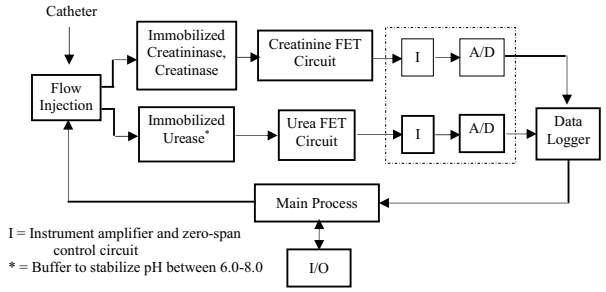

Figure 1: Block diagram of the sensor system

3.2 ISFET Stabilization with Current Feedback System

When operating in the strong inversion region heat is dissipated not only by changes of operating temperature but also from leakage current that can largely influence the pH of the solution even though the current are of the order of picoAmps [14]. To achieve accuracy of the reading, we propose the ISFET operate in the weak inversion region with a circuit that stabilizes the output. In this example, an n-typed ISFET drain terminal connects to the inverting input

of OpAmp with negative feedback to the source terminal of the ISFET.

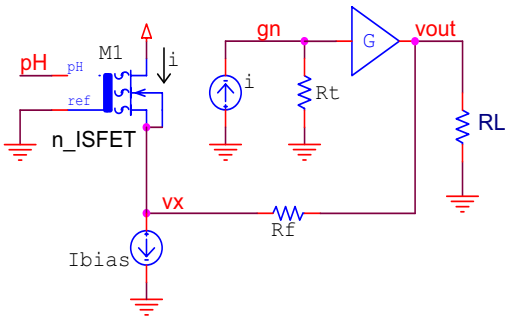

Figure 2: Macromodel of class A pH follower employing current feedback.

4 EXPERIMENT

4.1 Sensor Circuit Design

The ion-sensitive field-effect transistor used is a commercial Al_2O_3-ISFET sensor fabricated by Sentron Europe (Roden, The Netherlands). It was biased close to its isothermal point in constant drain current and constant drain-source voltage mode (I_d=100µA, V_{ds}= 600mV). Observed sensitivity was approximately 55mV/pH with respect to an Ag/AgCl reference electrode.

In order to obtain output stability, a pH follower employing current feedback is proposed. In this configuration, matching between ISFETs and MOSFETs is avoidable in an integrated circuit; Figure 2 shows current feedback to the pH follower. In this system, the ISFET is within the global feedback loop. Performing small signal analysis on Figure 2 yields:

$$\frac{v_{out}}{\Delta pH} = 2.3\alpha V_T \frac{g_{m1}}{g_{m1}+g_{ds1}} \times \frac{R_t}{R_t + GR_f + \left(G/(g_{m1}+g_{ds1})\right)} \quad (9)$$

where: α relates the sensitivity of the ISFET, V_T is the thermal voltage, g_{m1} is the transconductance of the ISFET, g_{ds1} is the output conductance of the ISFET, G is the gain of the voltage buffer, R_f is the feedback resistor, and R_t is the resistance of at the gain node, gn.

In the weak inversion region, the value of R_t can easily be adjusted to several orders of magnitude greater than the feedback resistor R_f and the output resistance of the pH follower $1/(g_m+g_{ds})$. Therefore, Equation 9 can be simplified to Equation 10, which is a linear response, independent of pH or loading condition.

$$\frac{v_{out}}{\Delta pH} = 2.3\alpha V_T \frac{1}{1+\left(nV_T/V_A\right)} \quad (10)$$

where: V_A is the Early Voltage and n is the subthreshold slope factor of the ISFET. Referring to Figure 2 and having carried out simulations of the OpAmp in class using a commercial AMS 0.8 µm CMOS technology, the analogue behaviour of the ISFETs is described based on a simplified macromodel, developed from Maritinoia [15]. We set the sensitivity of the ISFETs to be 55 mV/pH covering the pH range of 0-14. The power supply is ± 1.5V. Figure 3 showed the simulation result having an excellent linearity between output voltage and pH input under different loading conditions.

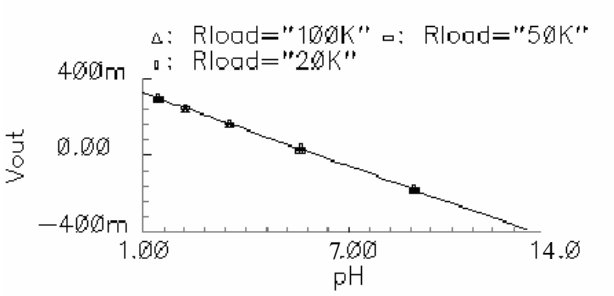

Figure 3: Linearity of output response under different loading conditions.

4.2 Measurements of Creatinine and Urea Concentration

The experiments using the Al_2O_3 ISFET with the solution described in (4.1) at the controlled temperature 37°C and pH range 6-8 confirmed the expected linearity between drain current with urea concentration and creatinine [16]. Linearity is achieved because of compensation of the nonlinear logarithmic Nernstian equation in section (2.2) with the exponential relationship between current and voltage when operating the ISFET in the weak-inversion region. At a constant of 37° C, the urea concentration at the pathological range of 0-120.48 mg/dl, which equals to 0-200 mM and the creatinine concentrations at the range of 0-22.73 mg/dl, which equals to 0-20 mM produce nanoamps of drain current in the range of 10^{-9} Amps.

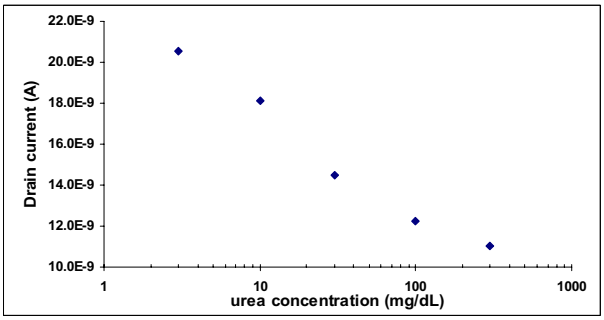

Figure 4: Al_2O_3 ISFET in weak inversion mode measures urea.

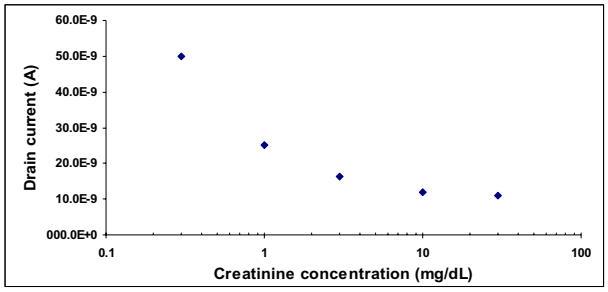

Figure 5: Al_2O_3 ISFET in weak inversion mode measures creatinine.

Therefore, ISFETs operating in the weak inversion region with the circuit in section 4.1 demonstrates linearity of drain current to urea and creatinine concentrations, at output current levels of 10-100 nA in which both pathological ranges are well covered.

5 DISCUSSION AND WAY FORWARD

The results shown in Figure 5 demonstrate the concept of current-mode feedback systems that improve stability of an ISFET sensor in measuring ions in chemical solution.

The next step is to design and fabricate these concepts into integrated circuits. To improve enzyme immobilization, microfluidic systems will replace conventional methods of crosslink immobilization. Figure 4 and Figure 5 show a single sensor with its circuit to detect both urea and creatinine simultaneously without having a separate inflow. In this circumstance, further study will be in the area of quantization techniques to detecting both urea and creatinine under the same microfluidic system, followed by a study of employing a single enzyme, creatinanse, which bypasses a transition stage of using creatinase and urease. Advanced circuit techniques e.g. bandgap and other temperature independent circuits will be incorporated in the final IC design, leading to the potential of wearable and implantable devices.

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