

# **Development of lactate sensor based on an extended gate FET with a ferrocenyl-alkanethiol modified gold electrode**

## **Objectives**

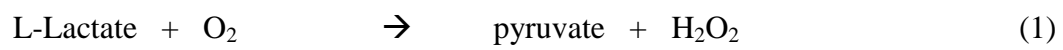
1. To develop a lactate sensor based on an extended gate field effect transistor sensors with a ferrocenyl-alkanethiol modified gold electrode
2. To evaluate the efficiency performance of the purposed sensor device

## **1. Introduction of L-lactate determination**

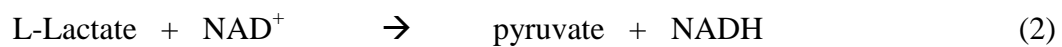
L-Lactate is an important parameter in several areas such as clinical diagnosis, sport medicine and food analysis [1]. It is a metabolite formed from the anaerobic metabolism of glucose in muscles. Generally, the levels of L-lactate in blood range from 0.5 – 1.5 mM in normal people at rest and 12 mM during exercise [2]. However, it may further increase in extreme conditions and extensive physical exercise to 25 mM. Therefore, in sport medicine requires monitoring of L-lactate for indicating the effectiveness of the training and fitness program of athletes [2, 3]. In clinical analysis, the plasma lactate levels indicate the balance between lactate production and lactate clearance that may influence by several disorders (i.e. hemorrhagic shock or pulmonary embolism) and also pathological conditions (i.e. cardiogenic or endotoxic, shock, respiration failure and tissue hypoxia). In addition, the lactate levels are alarm signals for the diagnosis of patient conditions in the intensive care units and operating rooms, whereas the labor room used them as an indicator of hypoxia/acidotic distress in fetuses during labor [4]. The lactate levels are important parameters not only in fitness and clinical diagnosis but also in food analysis. The lactate concentrations are used for the control of the fermentation by indicating freshness, stability, and quality products of cheese, yoghurt, butter, pickles, sauerkraut, wine, cider and other products in food industry [2, 5]. There are many analytical procedures have been used to measure L-lactate concentration such as amperometry, potentiometry, high performance liquid chromatography (HPLC), fluorometry, chemiluminescence, and UV-Vis spectrophotometry [6]. Although they are valuable methods for L-lactate determination, they are arduous, time-consuming and laborious, and some of them also need some sample pre-treatment and reagent preparation. As a result, biosensors represent an alternative methods of analysis with potential advantages; i.e. a rapid, simple and direct

measurement besides their low cost production [1]. The measurement of L-lactate in biosensor is usually performed by employing immobilized enzymes such as lactate dehydrogenase (LDH) or lactate oxidase (LOx) because of their simple enzymatic reaction and relatively easy sensor design configuration [1, 2].

Lactate oxidase catalyzes the oxidation of L-lactate to pyruvate and formed hydrogen peroxide that can be either reduced or oxidized to give a current proportional to the L-lactate concentration. However, this reaction is limited by the oxygen concentration (oxygen solubility in water; 1.4 mM at 1 atm, 20°C) [7]. The reaction of lactate oxidase can be summarized as follows:



To overcome the limitation of the solubility of oxygen, lactate dehydrogenase (LDH) is used instead of L-lactate oxidase. LDH works with a cofactors (NAD(P)<sup>+</sup> or NAD(P)H) according to the following reaction:



The NAD<sup>+</sup>/NADH cofactors act as mediators to shuttle the electrons between the enzyme and the electrode. The NAD<sup>+</sup>/NADH cofactors operate by a diffusional mechanism. Therefore, this coenzyme must be added to the analytical system due to its cost. However, the NAD(P)<sup>+</sup>/NAD(P)H cofactors exhibit poor electrochemical properties and their direct electrochemical reduction or oxidation is kinetically unflavored [8]. There are several redox mediators were utilized such as quinones, phenazine, phenoxazine, phenothiazine, ferrocene and Os-complexes were reported to catalyze the oxidation of NAD(P)H [9].

## 2. Introduction of Ion-sensitive field effect transistor (ISFET)

Ion-sensitive field-effect transistor (ISFET) has been developed for various kinds of biological molecules (e.g., enzymes, antibodies, DNA and cells) due to its attractive features such as small size, low cost, high sensitivity and rapid response [10, 11]. It can instantly transform intrinsic charge of molecules trapped on the gate surface into electrical signals [12]. The first ISFET was reported in 1970 by Bergveld who intended to use it as a tool for electrophysiological measurements of ion

composition in the area around of nerve tissues [13]. The first concept of ISFET was originated from a MOS (metal oxide semiconductor) transistor in which the gate of MOSFET was substituted by the electrolyte solution tested. There are many membranes have been used as a gate insulator such as  $\text{SiO}_2$ ,  $\text{Si}_3\text{N}_4$ ,  $\text{Al}_2\text{O}_3$  and  $\text{Ta}_2\text{O}_5$ . In particular, ISFET with silicon nitride ( $\text{Si}_3\text{N}_4$ ) membranes have been extensively used as a gate insulator for pH measurement and biomolecule detections. In 1980, Janata and Caras introduced ISFET based enzyme sensor for penicillin determination by immobilizing an enzyme on the gate insulator of the ISFET [14]. When a target substrate is present in a solution, the immobilized-enzyme catalyzes the substrate that results in the production of proton and then a local pH change around the gate insulator. This reaction is registered by the ISFET. Since ISFET-based enzyme sensors are pH-sensitive, it is strongly affected by buffer conditions, such as pH and salt concentration [11]. To overcome the limitation, an extended gate FET has been purposed as an alternative type of biosensor in which an organic layer is deposited on the gate of conventional FET sensor. Therefore, it is less sensitivity to surrounding temperature and light [12]. It was initially develop for DNA detection and also applied for immunosensor based on potentiometric measurement of molecular adsorption [15]. Principally, the extended-gate FET sensor detects a chemical reaction on its gold electrode as a change in its interfacial potential. Therefore, Ishige et al. applied an extended-gate FET sensor as an enzyme sensor with a ferrocene-modified gold electrode has been reported [10] for measuring the change of redox potential arising from the redox reaction [10]. This sensor detects electrons by using mediators that transfer the produced electrons to the ferrocene molecules on the gold electrode of the FET sensor. Thus, the sensitivity of the FET-based biosensor with a ferrocene-modified gold electrode is rarely affected by the change of pH or buffer capacity [5, 16].

In this study, we develop a lactate sensor based on an extended gate field effect transistor with a ferrocenyl-alkanethiol modified gold electrode. The hexacyanoferrate (II) and (III) ions are used as redox reagent. This sensor measure the interface potential on the ferrocene immobilized gold electrode instead of a local pH change.

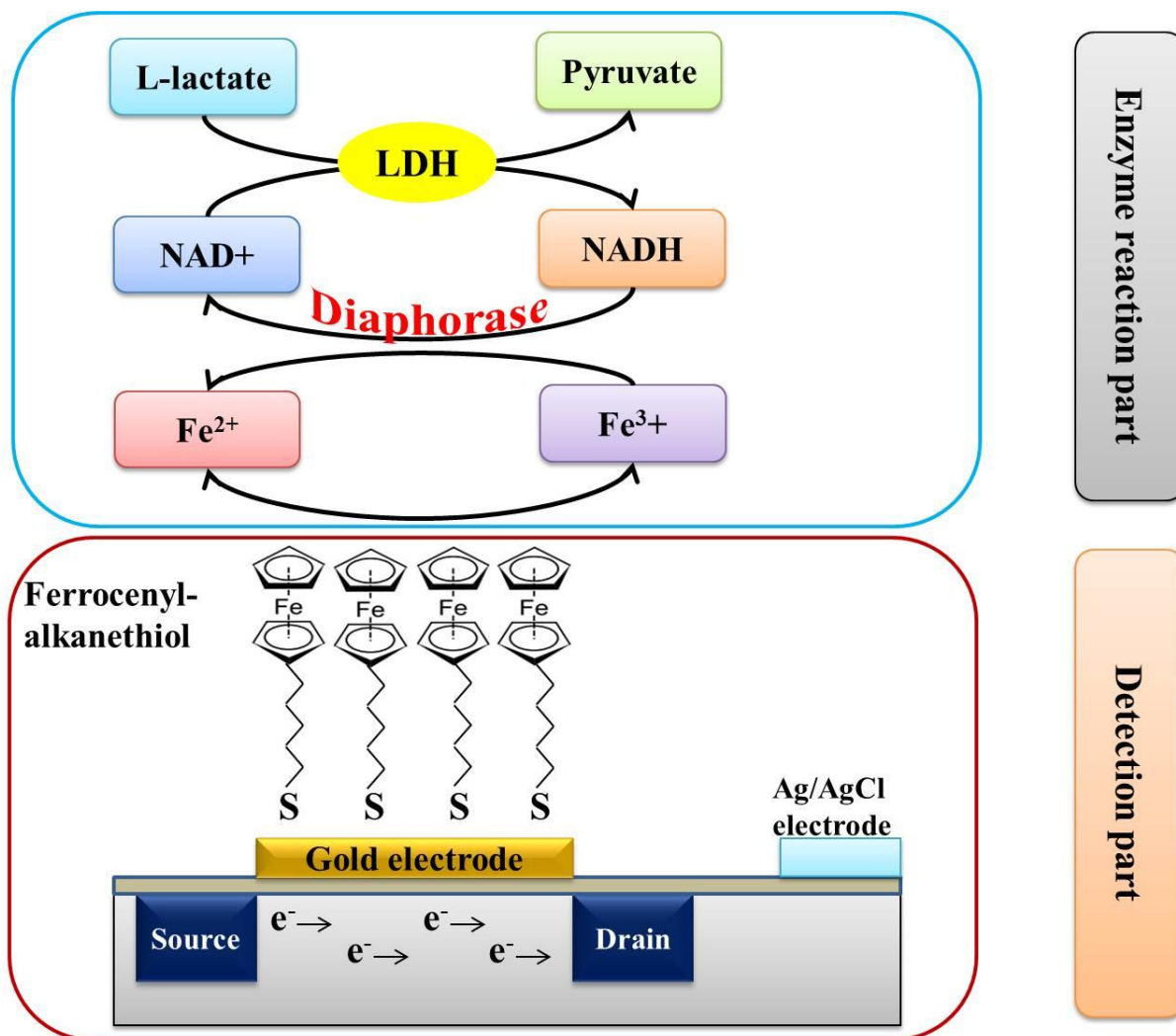


Figure 1 Structure of lactate sensor based on an extended gate FET with a ferrocenyl-alkanethiol modified gold electrode (modified from [10]). It consists of two parts between enzyme reaction and detection parts. L-lactate in the working solution is catalyzed by LDH with concomitant interconversion of NAD<sup>+</sup> in the enzyme reaction part leading to NADH and pyruvate formation. During reaction, diaphorase also catalyzes NADH to NAD<sup>+</sup> and the hexacyanoferrate (III) (a redox compound) is converted to hexacyanoferrate (II) leading to the changing of the ratio between redox compound in the oxidation state and the reduction state. This phenomenon is registered by the extended-gate FET sensor as the change in the interfacial potential of the ferrocenyl-alkanethiol modified gold electrode.

### **3. Materials and Methods**

#### **3.1 Materials**

- 3.1.1 Extended gate field effect transistor sensors
- 3.1.2 Lactate dehydrogenase (LDH)
- 3.1.3 Lactate
- 3.1.4  $\beta$ -Nicotinamide adenine dinucleotide reduced dipotassium salt hydrate (NADH)
- 3.1.5  $\beta$ -Nicotinamide adenine dinucleotide ( $\text{NAD}^+$ )
- 3.1.6 Potassium hexacyanoferrate (II) and Potassium hexacyanoferrate (III)
- 3.1.7 11-(ferrocenyl) undecanethiol
- 3.1.8 Diaphorase
- 3.1.9 1X Phosphate buffered saline (PBS) solution

#### **3.2 Methods**

##### **3.2.1 Functionalization of gold electrode**

The ferrocenylalkanethiol modification of the gold electrode is performed according to [17]. First, 11-(ferrocenyl)undecanethiol was dissolved in ethanol to form an alkanethiol solution. Second, the gold electrode chips were dipped into 1 M nitric acid for 15 s and rinsed with deionized water to remove unreacted alkanethiol molecules. The washed gold electrode chips were immersed and kept in the alkanethiol at room temperature for 24 h to fully functionalization and then rinsed with ethanol and deionized water. Finally, they were stored in the 100 mM sodium sulfate solution at room temperature until use [10, 17].

##### **3.2.2 Optimization of the ratio between hexacyanoferrate (II) to hexacyanoferrate (III) concentration**

The hexacyanoferrate, ( $\text{Fe}^{2+}/\text{Fe}^{3+}$ ), solution is prepared in 1x phosphate buffered saline (PBS buffer) solution at a ratio ranging from  $10^{-4}$  to  $10^4$  [10, 17]. Then, an extended gate FET with a ferrocenyl-alkanethiol modified gold electrode and the Ag/AgCl reference electrode with a saturated KCl are immersed in each of the mixed solution  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ . The interfacial potential of an extended gate

FET with a ferrocenyl-alkanethiol modified gold electrode is measured and established a calibration curve between the interfacial potential (mV) and the ratio of hexacyanoferrate (II) to hexacyanoferrate (III) concentration. This ratio concentration is employed as the working concentration for all the following tests.

### **3.2.3 Responsibility of the ferrocenyl-alkanethiol modified FET sensor device to different pH value and salt concentration**

The optimal working concentration from section 3.2.2 is used to evaluate the device response to different pH value. The various pH solutions are prepared in 1x PBS buffer and adjusted to pH 4 to 12 [10, 17]. Then, the extended gate FET with a ferrocenyl-alkanethiol modified gold electrode and the Ag/AgCl reference electrode with a saturated KCl are immersed in each of the different pH solution. The interfacial potential of an extended gate FET with a ferrocenyl-alkanethiol modified gold electrode is measured and established a calibration curve between the interfacial potential (mV) and the different pH value.

In addition, I also tested the device response to different salt concentration (added KCl concentration). The various salt concentrations, ranging from 10 mM to 1 M, are prepared in 1x PBS buffer [4, 14]. Then, the extended gate FET with a ferrocenyl-alkanethiol modified gold electrode and the Ag/AgCl reference electrode with a saturated KCl are immersed in each of the different salt concentration solution. The interfacial potential of an extended gate FET with a ferrocenyl-alkanethiol modified gold electrode is measured and established a calibration curve between the interfacial potential (mV) and the different salt concentration.

### **3.2.4 Optimization of lactate based extended-gate FET-based biosensor with a ferrocenyl-alkanethiol modified gold electrode**

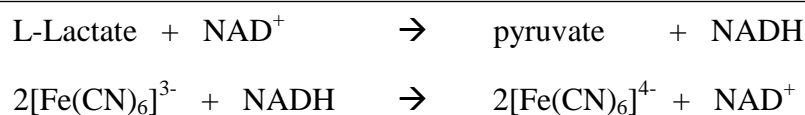
#### **3.2.4.1 Optimization of NADH concentration**

The various NADH concentrations are prepared in 1x PBS buffer (pH 7.4), ranging from 1 mM to 10 mM. The working solutions are prepared in 1x PBS (pH 8.8) containing diaphorase, potassium hexacyanoferrate (III) with different NADH concentration. Then, the extended gate FET with a ferrocenyl-alkanethiol modified gold electrode and the Ag/AgCl reference electrode with a saturated KCl are separately immersed in each of the different

NADH concentration. The interfacial potential of an extended gate FET with a ferrocenyl-alkanethiol modified gold electrode is measured and established a calibration curve for the interfacial potential (mV) responding to different concentrations of NADH.

#### 3.2.4.2 Optimization of LDH concentration

The various LDH concentrations are prepared in 1x PBS buffer (pH 7.4), ranging from 1 mM to 10 mM. The working solution is prepared as same as section 3.2.4.1 except LDH concentration. The reaction of the purposed sensor device for lactate determination is summarized as:



Then, the extended gate FET with a ferrocenyl-alkanethiol modified gold electrode and the Ag/AgCl reference electrode with a saturated KCl are separately immersed in each of the different LDH concentration with excess L-lactate and  $\text{NAD}^+$  concentrations. The interfacial potential is measured and established a calibration curve for the interfacial potential (mV) responding to different concentrations of LDH.

#### 3.2.4.3 Optimization of $\text{NAD}^+$ concentration

The various  $\text{NAD}^+$  concentrations are prepared in 1x PBS buffer (pH 7.4), ranging from 1 mM to 10 mM. The working solution is prepared as same as section 3.2.4.1, except  $\text{NAD}^+$  concentration. Then, the extended gate FET with a ferrocenyl-alkanethiol modified gold electrode and the Ag/AgCl reference electrode with a saturated KCl are separately immersed in each of the variable concentration of  $\text{NAD}^+$  with optimal LDH concentration (from section 3.2.4.2) and excess L-lactate concentration. The interfacial potential is measured and established a calibration curve for the interfacial potential (mV) responding to different concentrations of  $\text{NAD}^+$ .

#### 3.2.4.4 Optimization of L-lactate concentration

The various L-lactate concentrations are prepared in 1x PBS buffer (pH 7.4), ranging from 1 mM to 100 mM. The working solution is prepared as

same as section 3.2.4.1, except the lactate concentration. Then, the extended gate FET with a ferrocenyl-alkanethiol modified gold electrode and the Ag/AgCl reference electrode with a saturated KCl are separately immersed in each of the variable concentration of L-lactate with optimal  $\text{NAD}^+$  concentration (from section 3.2.4.3). The interfacial potential is measured and established a calibration curve for the interfacial potential (mV) responding to different concentrations of L-lactate. The sensitivity, linearity and reportable range are calculated from the calibration curve.

### **3.2.5 Method validation**

The proposed sensor device is validated for sensitivity, linearity, precision, accuracy, limit of detection, and long-term stability.

#### **3.2.5.1 Precision**

Precision of the method is determined by repeatability (intra-day precision) and intermediate precision (inter-day precision). The working solution is prepared as same as section 3.2.4.1. Three L-lactate concentration levels, including low, middle and high, are prepared in 1x PBS, pH 7.4.

In Intra-day precision evaluation, each of lactate concentration level is prepared at least 20 samples and separately measured by replicate analysis ( $n=5$ ) under the same condition in single day. Mean, standard deviation and coefficient of variation (CV) are calculated.

In addition, inter-day precision is also evaluated by determining three replication of each L-lactate concentration level on the same day. Mean, standard deviation and coefficient of variation (CV) are calculated.

#### **3.2.5.2 Accuracy**

The accuracy is performed by a comparative method between an extended gate FET with a ferrocenyl-alkanethiol modified gold electrode (new method) and reference method. Twenty samples within tested range are employed for both new and reference methods by three replications. Mean and standard deviation are calculated and expressed by percentage relative standard deviation (%RSD) of three replicates.



### 3.2.5.3 Limit of detection (LoD)

There are two steps involved in limit of detection evaluation between determination of values obtained with sample blank and values obtained with low level positive sample. The sample blank for assay, as called limit of blank (LoB), is prepared by working solution without L-lactate. It is measured replicates of a blank sample and calculated mean and standard deviation (SD) according to:

$$\text{LoB} = \text{mean}_{\text{blank}} + 1.645 (\text{SD}_{\text{blank}})$$

The lowest L-lactate concentration is prepared and measured replicates. It is determined by utilizing both the measured LoB and test replicates of a sample known to contain a low concentration of analyte [18]. Mean and standard deviation (SD) of the low concentration sample is then calculated according to:

$$\text{LoD} = \text{LoB} + 1.645 (\text{SD}_{\text{low concentration sample}})$$

### 3.2.5.4 Storage stability

The storage stability of the devices is tested by keeping them at room temperature for 0, 10, 20, 30, 60, 90, 120, 150 and 180 days. During these time intervals, the interfacial potential (mV) is measured and compared to the newly prepared sensor devices.

#### 4. References

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