HAND-HELD LUMINOMETER WITH ECL-BASED BIOSENSOR FOR LACTATE DETERMINATION

A. Martinez-Olmos, A. J. Palma
Department of Electronics and Computer Technology, University of Granada, Granada 18071 Spain

J. Ballesta-Claver, M. C. Valencia-Miron and L. F. Capitan-Vallvey
Department of Analytical Chemistry, University of Granada, Granada 18071, Spain

Keywords: Electrochemiluminescence, Portable instrumentation, Potentiostat, Biosensor, Screen-printed device, Lactate determination, Saliva.

Abstract: A new hand-held luminometer for electrochemiluminescence (ECL)-based one-shot biosensor for lactate is described. The lactate recognition system is based on lactate oxidase and the transduction system consists of luminol, all reagents immobilized in a Methocel membrane. The measurement of ECL from a screen-printed electrode by a portable instrument designed and developed by the authors makes it possible to determine lactate concentration. The compositions of the membrane and reaction conditions have been optimized to obtain adequate sensitivity. The one-shot biosensor responds to lactate rapidly, with the typical ECL acquisition time being 3 min, with a linearized dependence whose dynamic range was from $9 \cdot 10^{-6}$ to $2 \cdot 10^{-3}$ M, a detection limit of $2.4 \cdot 10^{-6}$ M and a sensor-to-sensor reproducibility (relative standard deviation RSD) around 10 % at the medium level of the range. The performance of the ECL one-shot biosensor and portable instrument was tested for the non invasive analysis of lactate in saliva, validating the results against a reference procedure.

1 INTRODUCTION

L(+)-Lactate is produced in the anaerobic metabolism of glucose and its determination is of interest in clinical analysis, sports medicine and food analysis. The measurement of lactate is routinely performed with liquid chromatography (Ewaschuk, 2002), spectrophotometry (Benthin, 1991) and amperometry, mainly with enzymatic electrodes (Compagnone, 1998). Lactate analysis is needed in different fields such as food, sports medicine and health. In foodstuffs, lactate is produced by bacterial fermentation and is an essential component related to the manufacture of cheese, yoghurt, milk, etc., thus monitoring lactate being an important quality control parameter.

Rapid evaluation of lactate levels can be performed with one-shot sensors, that mainly are of electrochemical type (Klonoff, 2003). Chemiluminescence measurement could be of interest for one-shot sensor design due to its good sensitivity and selectivity, although the use of electrochemiluminescence (ECL) could offer clear advantages for controlling the chemical system (Richter, 2004).

The use of screen printing technologies, with benefits for low cost and mass production, appears to be interesting to develop ECL one-shot biosensors next to portable instrumentation.

The presented lactate biosensor is based on its enzymatically catalyzed oxidation and back ECL transduction using luminol (L) according to:

$$\text{Lactate} + O_2 \xrightarrow{\text{LOD}} \text{Piruvate} + H_2O_2$$

$$L - e \rightarrow L^* \text{ (electrode)}$$

$$H_2O_2 + L^* \rightarrow \text{3-aminophthalate} + N_2 + H_2 + h\nu$$

2 MATERIALS

The one-shot biosensor is formed by a screen printed electrode where the working electrode contains all

Martínez-Olmos A., J. Palma A., Ballesta-Claver J., C. Valencia-Miron M. and F. Capitan-Vallvey L. (2009).
HAND-HELD LUMINOMETER WITH ECL-BASED BIOSENSOR FOR LACTATE DETERMINATION.
In *Proceedings of the International Conference on Biomedical Electronics and Devices*, pages 343-346
DOI: 10.5220/0001432703430346

needed reagents immobilized in a Methocel membrane. The sensing layer was spotted as solutions of luminol, lactate oxidase (LOD), BSA, sodium chloride and 8.8 pH phosphate buffer in aqueous solution of Methocel. The screen-printed electrode was covered by a thick overlapping plastic layer with a 40-µl volume hole in the electrode area to place the sample.

The characterization of the screen-printed electrodes was investigated through cyclic voltammetry. The ECL measurements were performed measuring the light intensity emitted while triggering the ECL reaction.

3 INSTRUMENTATION

After describing the biosensor, a portable instrument based in ECL detection and designed and fabricated for this sensor will be detailed. The prototype has been applied to lactate concentration determination in saliva.

The system is based on a solid-state photodiode detector, which generates an electric current proportional to the ECL being measured. In Figure 1 the general scheme of the system is presented.

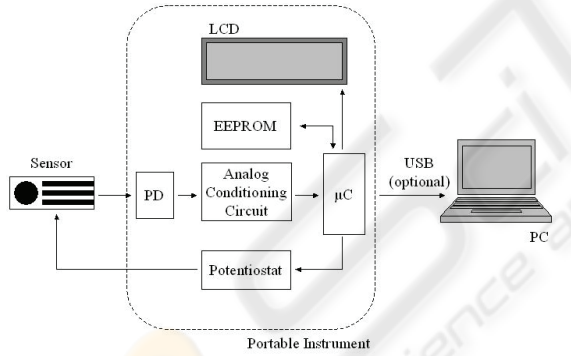

Figure 1: Block diagram of the instrument.

The light resulting from the ECL reaction on the sensor excites the photodiode detector (PD) (S1227-66BR, Hamamatsu Photonics) which generates an electric current in response. The analog circuit for measuring this current is shown in Figure 3. ECL is produced when a voltage difference of 0.5V is applied between the reference and the working electrodes in the biosensor. This polarization of the sensor is carried out using a programmable built-in potentiostat, which is designed to apply variable voltage steps between the sensor electrodes.

A detailed schematic of the potentiostat is presented in Figure 2. In this circuit, a serial digital-to-analog converter (DAC) (DAC8574, from Texas Instruments) generates an analog voltage from a 16-bit digital word sent by the microcontroller, which is the input value to the potentiostat. If the electrochemical cell is full of a conductive liquid, the operational amplifiers A1 and A2 form a non-inverting amplifier stage with gain 2. This establishes a voltage at the working electrode that is double than the input voltage value. The voltage at the reference electrode is forced to virtual ground because of the negative feedback of the operational amplifier A3. Thus, the voltage difference between the working and the reference electrodes is simply twice the analog value generated by the DAC.

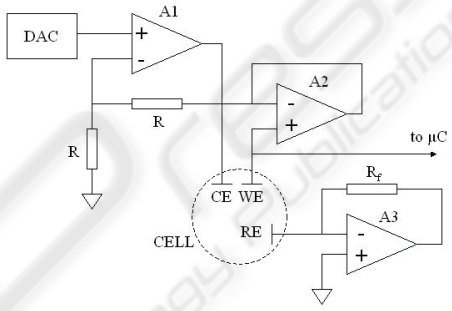

Figure 2: Potentiostat circuit.

The voltage at the working electrode is monitored directly by the microcontroller, since its function is to detect when the test drop is deposited on the biosensor. This event causes the start of a time count, thus allowing a precise determination of the time elapsed between the drop deposition and the beginning of the measurements. Therefore, a perfect timing control of the measurement procedure can be achieved.

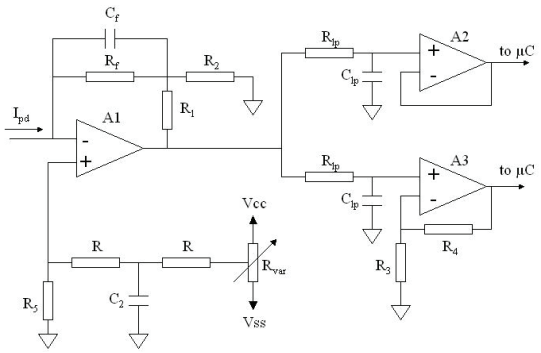

Figure 3: Measurement circuit.

The current is converted into voltage trough the current-to-voltage converter formed by the operational amplifier A1 (TLC277, from Texas Instruments). This device has a feedback network

compounded by the resistors R_f, R_1 and R_2, which results in a high gain conversion, being the output voltage of A1:

$$V_o = \frac{V^+(R_1+R_2) - I_{pd}\left(R_1(R_2+R_f)+R_fR_2\right)}{R_2} \quad (1)$$

where V^+ is the voltage at the non-inverting input of the operational amplifier A1, and I_{pd} is the current generated by the photodiode. The voltage V^+ takes a value of few mV and can be changed through the variable resistor R_{var}. The effect of this parameter is to compensate the input offset voltage of the operational amplifier, which can have a high influence on the output because of the great gain of this stage.

Under ideal conditions (V^+=0) and assuming $R_f >> R_2$, Equation (1) can be rewritten as:

$$V_o = I_{pd}R_f\left(1+\frac{R_1}{R_2}\right) \quad (2)$$

Selecting a high value for R_f and making $R_1>R_2$, a gain factor of $10^{11} - 10^{13}$ V/A can be achieved.

The output voltage of A1 is conditioned using two parallel stages , formed by the operational amplifiers A2 and A3. In each stage, the signal is firstly filtered through a *RC* low-pass filter. The operational amplifier A2 acts as a buffer, whereas A3 amplifies the output voltage of the I/V converter before sending it to the microcontroller. In this way, the μC receives two signals, one corresponding to the filtered output of the first stage, and another that is an amplification of this last one. The purpose of having two different channels for measuring the same signal is to expand the range of lactate that can be analysed.

The outputs of A2 and A3 are connected directly to the microcontroller (μC) (PIC18F2550,from Microchip Inc.), which uses an internal 10-bit analog-to-digital converter to alternatively sample these signals at high frequency. A serial EEPROM module (24LC512, from Microchip Inc.) of 512kbit is used to store the sampled data. Finally, once the calibration function (see next section) programmed in the microcontroller is applied, results are sent to the LCD display (Figure 1). All electronic circuitry is included in an enclosure with optical, magnetic and electrical shielding.

Moreover, control software written in Visual Basic allows the user to optionally communicate the instrument with a computer via an USB port to receive the data for further analysis.

Main advantages of our design lie on portability, low cost because of the use of a photodiode instead of a costly or bulky photomultiplier, and the use of non invasive samples. Most commercial portable lactate meters use blood or serum for lactate analysis (www.lactate.com, Poscia, 2005).

4 BIOSENSOR COMPOSITION AND MEASUREMENT CONDITIONS

Composition of sensing membrane was optimized in terms of type and concentration of membrane polymer, supporting electrolyte, pH and buffer, luminol concentration, enzyme units, and BSA concentration.

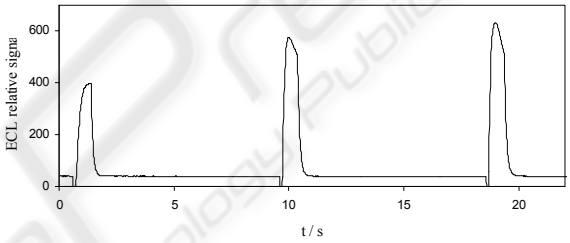

Figure 4: Relative ECL lactate signals.

Different types of ECL analytical signals were studied using the instrument described in the previous section in order to obtain an analytical parameter for lactate concentration. The intensity of the collected light, resulting from the reaction on the sensor, did not show a direct relationship with the lactate concentration, as can be seen in Figure 4, where three steps at fixed potential were applied to the same problem drop. The intensity of the light is increased with successive potential steps. From these current pulses, a kinetic signal derived from the relative increase of the signal was chosen for the measurement of the lactate concentration, since it remains stable for different excitation pulses. The measurement conditions studied were: a) applied potential (0.5 V); b) waiting time before the first pulse (3 min); c) time between pulses, being 10 s for better sensitivity; d) pulse time with 1 s as best for sensitivity and time of analysis.

The sample volume in the screen-printed device was spotted with a micropipette. From the influence of sample volume, studied between 20 and 40 μL. Low volumes have high ECL signals but poor repeatability. The signal and the standard deviation decrease when the volume increases. The reason of this behaviour is that low volumes don't cover the

three electrodes totally, specially the reference electrode, making then oscillating potentials. Therefore a volume of 35 uL for the test drop has been selected, wich provides a good precision (5-8 % RSD).

5 ANALYTICAL CHARACTERIZATION

The dependence of ECL signal with lactate concentration was studied between 10^{-7} and 10^{-3} M obtaining a linear relationship between 10^{-6} M and $2 \cdot 10^{-4}$ M (Figure 6).

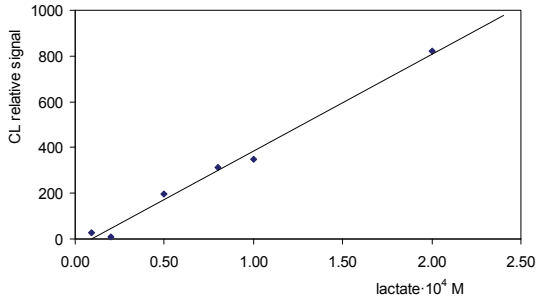

Figure 6: Linear calibration of lactate biosensor.

Table 1 shows some analytical parameters of biosensor for lactate.

Table 1: Analytical characteristics.

Parameter	Value
Linear range (M)	$9 \cdot 10^{-6} - 2 \cdot 10^{-4}$
Intercept	1.87
Slope	786150
r^2	0.9956
Detection limit (M)	$2.4 \cdot 10^{-6}$
RSD blank (%)	8.2 %
RSD lactate (%) $8 \cdot 10^{-4}$ M	10.3 %

This biosensor was applied to lactate determination in saliva obtaining good preliminary results.

6 CONCLUSIONS

A new hand-held luminometer for electrochemiluminescence (ECL)-based one-shot biosensor for lactate is described. Exciting the sample volume with consecutive steps of 0.5V and measuring the light resulting from the reaction on the sensor provides a method for the evaluation of the lactate concentration. A good linear calibration in the range of $9 \cdot 10^{-6}$ to $2 \cdot 10^{-4}$ M has been achieved, what indicates that lactate in saliva, rather than lactate in blood can be measured. This fact results in a better behaviour of the prototype than the existing commercial instruments, because of its minimal invasive requirements for the measurement of lactate in humans. The use of a solid-state photodiode as optical detector, instead of a photomultiplier, which is the usual technique in available commercial ECL systems, as well as the integration of the potentiostat and the measurement electronics in the same design has allowed a low cost and compact instrument.

ACKNOWLEDGEMENTS

This work is supported by the Ministerio de Educación y Ciencia, Dirección General de Investigación (Spain), under projects CTQ2005-09060-C02-01 and CTQ2005-09060-C02-02, and by the Junta de Andalucía under project P06-FQM-01467.

REFERENCES

J. B. Ewaschuk, G. A. Zello, J. M. Naylor, and D. R. Brocks, J. Chromatog. B, 781 (2002) 39-56.

S. Benthin, J. Nielsen, and J. Villadsen, Anal. Chim. Acta, 247 (1991) 45-50.

D. Compagnone, D. Moscone, and G. Palleschi, Recent Res. Devel. in Pure & Applied Anal. Chem., 1 (1998) 73-86.

D. C. Klonoff, Diabetes Tech. Therapeut., 5 (2003) 929-931.

M. M. Richter, Chemical Rev., 104 (2004) 3003-3036.

www.lactate.com

A. Poscia, D. Messeri, D. Moscone, F. Ricci, and F. Valgimigli, Biosensors and Bioelectronics 20 (2005) 2244-2250