



# Amperometric lactate biosensor for flow injection analysis based on a screen-printed carbon electrode containing Meldola's Blue-Reinecke salt, coated with lactate dehydrogenase and NAD<sup>+</sup>

M. Piano<sup>a</sup>, S. Serban<sup>b</sup>, R. Pittson<sup>b</sup>, G.A. Drago<sup>a</sup>, J.P. Hart<sup>c,\*</sup>

<sup>a</sup> Applied Enzyme Technology Ltd., Monmouth House, Mamhilad Park, Pontypool, NP46HZ, UK

<sup>b</sup> Gwent Electronic Materials Ltd., Monmouth House, Mamhilad Park, Pontypool, NP46HZ, UK

<sup>c</sup> Centre for Research in Analysis, Materials, and Sensor Sciences, Faculty of Applied Sciences, University of the West of England, Bristol, Frenchay Campus, Coldharbour Lane, BS16 1QY, UK

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## ABSTRACT

A biosensor for the measurement of lactate in serum has been developed, which is based on a screen-printed carbon electrode, modified with Meldola's Blue-Reinecke Salt (MBRS-SPCE), coated with the enzyme lactate dehydrogenase NAD<sup>+</sup> dependent (from Porcine heart), and NAD<sup>+</sup>. A cellulose acetate layer was deposited on the top of the device to act as a permselective membrane. The biosensor was incorporated into a commercially available, thin-layer, amperometric flow cell operated at a potential of only +0.05 V vs. Ag/AgCl. The mobile phase consisted of 0.2 M phosphate buffer pH 10 containing 0.1 M potassium chloride solution; a flow rate of 0.8 ml min<sup>-1</sup> was used throughout the investigation. The biosensor response was linear over the range 0.55–10 mM lactate; the former represents the detection limit. The precision of the system was determined by carrying out 10 repeat injections of 10 mM L(+)lactic acid standard; the calculated coefficient of variation was 4.28%. It was demonstrated that this biosensor system could be applied to the direct measurement of lactate in serum without pre-treatment; therefore, this would allow high throughput-analysis, at low cost, for this clinically important analyte.

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## 1. Introduction

Lactic acid is an important analyte in clinical analysis where it is an important biomarker in the detection of heart failure [1]. Its measurement is also important in the food industry, for example during fermentation of beer, wine and cider [2].

For such determinations, simple, inexpensive, and reliable methods are highly desirable; we considered that amperometric biosensors offer these features.

This is certainly the case when screen-printed carbon electrodes form the base transducers upon which a suitable enzyme/cofactor system is immobilised [3–5]. We considered that a suitable enzyme for this measurement would be lactate dehydrogenase, which converts NAD<sup>+</sup> to NADH in the presence of lactate; the NADH produced is then detected amperometrically and forms the analytical response. In order to reduce the overpotential for NADH oxidation, several mediators may be used to modify the SPCEs surface.

Phenazine ethosulphate and methosulphate [6], Prussian Blue [7], Meldola's Blue [8] are some examples of the mediators used in

the past for the fabrication of lactate biosensors. Our goal was to employ Meldola's Blue-Reinecke Salt Screen-Printed Carbon Electrodes (MBRS-SPCEs) as these were previously shown by us to be suitable in flow injection analysis, both for NADH detection and glucose determinations [9].

The main benefits of MBRS-SPCEs for incorporation into a dehydrogenase based biosensor are that the devices do not readily dissolve in the FIA mobile phase and the operating potential is close to zero volts. FIA systems are attractive, as it is possible to perform analyses of clinical samples with minimal operator effort, and they can be easily automated.

In the present paper we describe the development of an FIA/amperometric biosensor system for lactate, based on these principles. The application of the new assay to serum analysis is also discussed.

## 2. Experimental

### 2.1. Chemicals and reagents

Lactate dehydrogenase (LDH from *Porcine heart* EC 1.1.1.27) was purchased from BBI (Bleanavon South Wales UK) with a specific activity of 257 U/mg material, newborn calf serum from bovine

\* Corresponding author. Tel.: +44 0117 328 2469; fax: +44 0117 328 2904.  
E-mail address: [john.hart@uwe.ac.uk](mailto:john.hart@uwe.ac.uk) (J.P. Hart).

calves (N-4637), BSA fraction V (A 3059) and  $\text{NAD}^+$  were obtained from Sigma. All other chemicals were of analytical reagent grade and also purchased from Sigma–Aldrich (UK).

$\text{NaH}_2\text{PO}_4$  (0.2 M), and  $\text{Na}_2\text{HPO}_4$  (0.2 M) containing KCl (0.1 M) were mixed together in order to prepare the buffer solutions for all electrochemical investigations. The L(+) lactic acid standard stock solutions were prepared fresh daily at 1 M concentrations in phosphate buffer at the pH required. The pHs were adjusted with sodium hydroxide if necessary.

The Meldola's Blue-Reinecke salt complex was prepared using the method described in our previous paper which was concerned with the design of a glucose biosensor [9]. This was then used in the preparation of the appropriate modified screen-printed carbon electrodes and for the fabrication of the lactate biosensors.

## 2.2. Apparatus and biosensors

Cyclic voltammetric measurements, for characterisation of the screen-printed electrodes, were performed using an  $\mu$ Autolab Type II computer controlled Potentiostat (Windsor Scientific, UK).

Flow injection analysis was performed with a peristaltic pump, connected to a Rheodyne (AnaChem, UK) 4-way injection valve fitted with a 65  $\mu\text{l}$  loop; this was connected to a thin-layer flow cell (Uniscan Ltd, UK), containing a screen-printed lactate biosensor.

The screen-printing of the MBRS mediated SPCEs was carried out at Gwent group using a DEK 1202 semiautomatic printer (DEK Weymouth, UK). The MBRS-SPCEs (GEM Ltd base electrodes code: BE2060227D2), were printed on to an alumina substrate (from Coorstek, Fife, UK, laser cut at Laser Cutting, Ceramics Ltd, Sheffield UK) using a specially laser cut substrate from which the sensor were snap to fit into the flow cell. SPCEs for use in a flow cell were fabricated by screen-printing carbon inks containing 2% MBRS (GEM Ltd code: C2070418D4), this was prepared by modification of a base carbon ink (Gem Ltd code: C2030319D4); an Ag/AgCl reference/counter electrode (GEM Ltd ink code: C61003P7), was screen-printed alongside the working electrode. These MBRS-SPCEs electrode was investigated for their cyclic voltammetric behaviour with NADH in carrier buffer.

Biosensors were manufactured by depositing 3  $\mu\text{l}$  of a biococktail containing the following: 10 U of LDH, 225  $\mu\text{g}$   $\text{NAD}^+$ , 294  $\mu\text{g}$  of BSA, and 15  $\mu\text{g}$  of glutaraldehyde onto the working electrode area. The biosensors were stored at +4 °C desiccated.

A membrane was deposited on the top of the dried biosensor on the day of testing by depositing 15  $\mu\text{L}$  of 2% cellulose acetate in acetone and allowed to dry for 30 min at room temperature. When dry the biosensor was ready to be incorporated in the FIA system for serum lactate determinations.

## 2.3. Procedures

### 2.3.1. Cyclic voltammetry

Cyclic voltammetry was performed with NADH prepared at a concentration of 1 mM and dissolved in 0.2 M phosphate buffer pH 10.0–0.1 M KCl.

The cyclic voltammetric behaviour of this solution was examined with Meldola's Blue-Reinecke Salt modified screen-printed electrodes (MBRS-SPCEs), with no added enzyme. The voltammetric conditions were: initial potential  $-0.80\text{ V}$ , final potential  $+0.80\text{ V}$  (vs Ag/AgCl); scan rate  $50\text{ mV s}^{-1}$ .

### 2.3.2. Hydrodynamic voltammetry

The current/voltage behaviour of our complete lactate biosensor, housed in a commercial thin-layer cell [9] was examined over the potential range  $-0.5\text{ V}$  to  $+0.1\text{ V}$ ; the potential was changed in 100 or 50 mV steps. Triplicate injections of 3 mM lactate were made through a 65  $\mu\text{L}$  loop at each potential step.

### 2.3.3. pH study

Calibration studies at different pH values were performed using a biosensor in conjunction with flow injection analysis. A separate biosensor was used to perform each calibration study. The effect of pH was investigated over the pH range 7.0–10.0, with constant phosphate buffer strength (0.2–0.1 M KCl).

These studies were performed in triplicate with a 65  $\mu\text{L}$  loop for each lactate concentration examined. The operating potential was +50 mV and the lactate concentration investigated in the range 0–25 mM.

### 2.3.4. Analytical application

The proposed biosensor, housed in a commercial thin-layer cell, was incorporated into a FIA system and evaluated for the direct measurement of lactate in calf serum.

Undiluted serum was injected directly into the FIA system through a 65  $\mu\text{L}$  loop; this procedure was repeated a total of 6 times on a single biosensor. The unknown lactate concentration was determined by comparing the resulting FIA peaks to the calibration plot obtained using carrier buffer pH 10.

## 3. Results and discussion

### 3.1. Principle of biosensor operation

The cascade of reactions occurring during operation of the lactate biosensor is shown in Fig. 1. Initially, lactate undergoes an enzymatic oxidation in the presence of lactate dehydrogenase and  $\text{NAD}^+$  to produce pyruvate,  $\text{H}^+$  and NADH. Next, the reduced form of the cofactor (NADH) reacts with the oxidised form of Meldola's Blue to produce the reduced form of the mediator. The latter form then undergoes electrochemical oxidation at the surface of the screen-printed carbon electrode. These last two reactions characterise the electrocatalytic oxidation of NADH, which generates the analytical response. In this format, higher concentrations of lactate will generate higher amperometric responses.

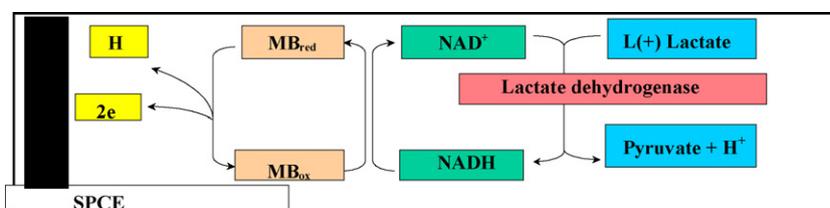


Fig. 1. Sequence of reactions involved in the operation of the lactate biosensor.

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