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Amperometric L-lactate biosensor based on screen-printed carbon electrode containing cobalt phthalocyanine, coated with lactate oxidase-mesoporous silica conjugate layer

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ABSTRACT

A novel amperometric biosensor for the measurement of L-lactate has been developed. The device comprises a screen-printed carbon electrode containing cobalt phthalocyanine (CoPC-SPCE), coated with lactate oxidase (LOD) that is immobilized in mesoporous silica (FSM8.0) using a polymer matrix of denatured polyvinyl alcohol; a Nafion layer on the electrode surface acts as a barrier to interferents. The sampling unit attached to the SPCE requires only a small sample volume of 100 μL for each measurement. The measurement of L-lactate is based on the signal produced by hydrogen peroxide, the product of the enzymatic reaction. The behavior of the biosensor, LOD-FSM8.0/Naf/CoPC-SPCE, was examined in terms of pH, applied potential, sensitivity and operational range, selectivity, and storage stability. The sensor showed an optimum response at a pH of 7.4 and an applied potential of +450 mV. The determination range and the response time for L-lactate were 18.3 μM to 1.5 mM and approximately 90 s, respectively. In addition, the sensor exhibited high selectivity for L-lactate and was quite stable in storage, showing no noticeable change in its initial response after being stored for over 9 months. These results indicate that our method provides a simple, cost-effective, high-performance biosensor for L-lactate.

1. Introduction

The development of methods for L-lactate quantification has drawn considerable attention due to its importance in clinical chemistry, dairy and food industries, biotechnology, and sports medicine [1–5]. The determination of L-lactate concentration in the blood is essential for the diagnosis of various severe conditions such as shock, metabolic disorders, respiratory insufficiency, and heart failure that occur during intensive care and surgery. It is also useful in sports medicine, particularly for determining the physical conditions of athletes. In the food industry, the L-lactate level is an indicator of fermentation and is related to the freshness, stability, and storage quality of several products such as tomato sauces, fruits, juices, wine, and milk. In particular, it is the principal byproduct of milk fermentation by lactic acid bacteria and is responsible for the flavor of sour milk.

Traditionally, the determination of L-lactate concentration is based on spectrophotometry [6], but this method involves complicated procedures such as sample pre-treatment and/or reagent preparation. Thus, the development of L-lactate biosensors is of considerable interest because they are inexpensive while being capable of making simple, rapid, and direct measurements with high selectivity; in addition, sample preparation is not required. Among the various types of biosensors, special attention has been paid to the development of amperometric biosensors based on the use of immobilized lactate oxidase (LOD); in these biosensors, LOD catalyzes the conversion of L-lactate to pyruvate and hydrogen peroxide (H_2O_2), which can be subsequently oxidized at the electrode surface [7–22]. However, the determination of L-lactate in real samples by the direct amperometric measurement of H_2O_2 can be affected by interfering species that are oxidized at the high potential required for H_2O_2 detection. In order to minimize the contribution of interfering substances to the biosensor response, the use of a permselective membrane [13–18] and electrocatalytic mediators has been reported [16–18,22–25]. Regarding the former, it has been reported that a Nafion layer has high anti-interfering ability and can eliminate interference from electroactive species [13–15]. Regarding the latter, cobalt phthalocyanine (CoPC) has been shown to be an effective electrocatalyst for the electrochemical oxidation of H_2O_2 and low operating potentials could be used during biosensor operation [22–25]. Moreover, CoPC has the advantage that it can simply be added to carbon ink, allowing the simple production of mediated H_2O_2 sensors by screen-printing technology. This fabrication method is gaining great popularity in

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biosensor construction because it makes possible the mass production of biosensors at low cost in a wide variety of geometries [19–22,26–29].

In spite of the fact that several articles have been published on L-lactate biosensors using screen-printed electrodes (SPEs), there is still considerable interest in improving the sensitivity and stability of such devices. Stability is of great importance for the success of these devices as analytical instruments, and it is mainly dependent on the lifetime, or the rate of denaturation or inactivation, of the enzyme employed [5,10–12,30]. Novel immobilization methods [31–34] and/or media [35–38] are usually employed to enhance enzyme stability by optimizing the surrounding microenvironment. One of the best immobilization methods to stabilize enzymes with good catalytic activity is to encapsulate the enzyme in mesoporous silica (MPS) materials. MPS materials inherently have high surface areas and pore volumes, tunable pore sizes to accommodate enzymes of different dimensions, and excellent mechanical stabilities [39,40]. Our previous studies using an enzyme-immobilized electrode in a stirred-batch-cell showed that high-performance biosensors could be constructed by the use of immobilized enzymes within MPS materials [41–44].

In this article, we describe the preparation and characterization of an amperometric L-lactate biosensor based on a screen-printed carbon electrode containing cobalt phthalocyanine (CoPC-SPCE), coated with a Nafion layer and immobilized enzyme in MPS using a polymer matrix of denatured polyvinyl alcohol. In our method, MPS powder with a pore diameter of 8.0 nm (FSM8.0) is employed as a host matrix for LOD (molecular size of 7–8 nm, assuming the LOD is spherical [45,46]) to encapsulate it within the MPS material. In brief, a Nafion layer was formed on the surface of the working electrode as a barrier to interferents. Next, LOD encapsulated within MPS was entrapped within a polymer matrix of modified polyvinyl alcohol, which was then cross-linked and deposited onto the underlying Nafion layer. On the top of SPCE, a sampling unit consisting of a screen-printed electrochemical cell with a circular container was attached; the sampling unit defines the electrode area and requires only a small sample volume of 100 μL for the measurement. The electrochemical performance of the resulting biosensor, LOD-FSM8.0/Naf/CoPC-SPCE, was then investigated by cyclic voltammetry (CV) and amperometric detection. Its response time, sensitivity, and operating range were investigated. In addition, the effects of pH, applied potential, selectivity, and storage stability on its amperometric response were evaluated.

2. Experimental

2.1. Materials

Lactate oxidase (LOD, E.C. 1.1.3.2, from *Microorganism*, 80 units mg^{-1}) was purchased from Toyobo Co., Ltd. (Osaka, Japan). Dococyltrimethylammonium chloride [$C_{22}H_{45}N(CH_3)_3Cl$] (C_{22}TMA) was kindly donated by the Lion Corporation (Tokyo, Japan). Sodium silicate ($SiO_2/Na_2O = 2.07$), tetraethyl orthosilicate (TEOS), 10% Nafion® dispersion solution, and L-lactate were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 1,3,5-Triisopropylbenzene (TIPB) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Several inks for the fabrication of SPCEs, namely C2030519P4 (carbon/graphite ink), C2030408P3 (carbon mediate ink containing cobalt phthalocyanine), C61003P7 (Ag/AgCl ink), and D2081009D6 (dielectric ink) were purchased from Gwent Electronic Materials Ltd. (Torfaen, UK). Denatured polyvinyl alcohol (D-polymer, DF-20) and adipoyl dihydrazide (ADH) were obtained from Shin-Etsu Chemical Co., Ltd (Tokyo, Japan). Glass epoxy sheets (0.3 mm thickness) were supplied by Sumitomo Bakelite Co., Ltd (Tokyo, Japan). All other reagents were of analytical grade and were obtained from commercial sources. Hydrogen peroxide solutions and L-lactate solutions were prepared from 30% and 98% stock solutions, respectively.

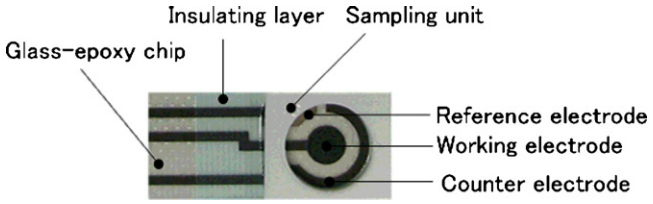

Fig. 1. The manufactured CoPC-SPCE electrode on a glass-epoxy substrate.

2.2. Apparatus and electrochemical measurements

Cyclic voltammetric (CV) and amperometric measurements were performed with a PalmSens handheld potentiostat (Palm Instruments BV, Netherlands) at room temperature (25 °C). All electrochemical experiments were carried out with a sampling unit attached to the manufactured screen-printed carbon electrodes (SPCEs) containing 90 μL of 0.1 M phosphate buffer (pH 7.4). A picture of the manufactured electrode is presented in Fig. 1. CV experiments were performed with and without L-lactate solution; the potential range used was −0.4/+0.6 V, at a scan rate of 10 mV s^{-1}. The amperometric experiments were carried out by applying a constant potential to the working electrode of the biosensor. After the transient current had settled (t = 150 s), 10 μL aliquots of known concentrations of L-lactate standard solutions were injected into the sampling unit by micropipette and the sensor response was measured until the resulting current reached a steady state value. The experimental setup is depicted in Fig. 2.

2.3. Synthesis of mesoporous silica powder (FSM8.0)

Mesoporous silica (MPS) powder, FSM8.0, with a pore diameter of 8.0 nm, was prepared from sodium silicate using dococyltrimethylammonium chloride and 1,3,5-triisopropylbenzene (TIPB) as we previously reported [41]. 16 g of C_{22}TMA was added to 200 mL of water at 70 °C, and to this mixture was added 60 g of TIPB; the resultant mixture was vigorously stirred for 30 min at 70 °C. It was then added to 200 mL of water at 80 °C in the presence of 54.31 g of sodium silicate; the pH of this mixture was adjusted to 8.5 by slowly adding aqueous 2 M HCl. After the suspension was stirred for 3 h at 70 °C, the solid product was filtered out, washed three times with 300 mL distilled water at 70 °C, and dried. The

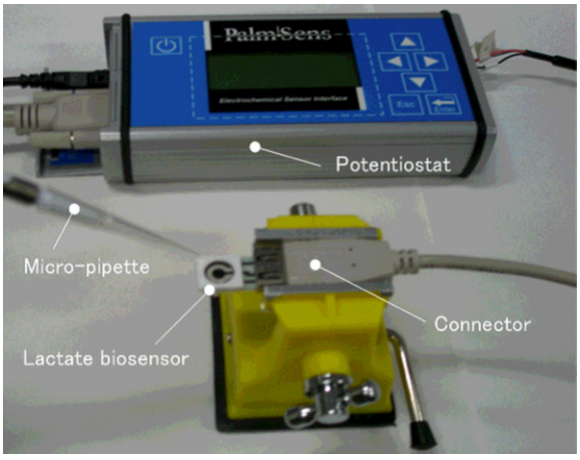

Fig. 2. Experimental setup for the measurement of L-lactate with our biosensor.

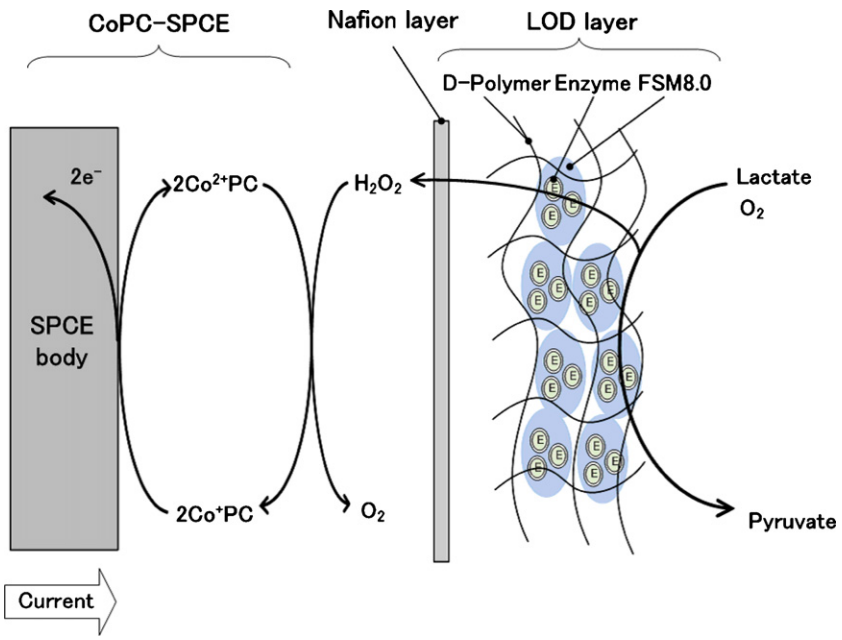

Fig. 3. The principle of operation of our biosensor, where enzymatic generation of H_2O_2 is oxidized on the CoPC-SPCE surface resulting in a flow of electrons to the working electrode.

sample was calcined at 150 °C for 2 h in air, and the calcined product (300 g) was dissolved in a solution mixture of ethanol (35.4 mL) and HCl (1.7 mL). The suspension was then stirred for 5 h at 70 °C to remove the organic fraction. The solid product was filtered out, washed with ethanol three times, and dried; in this manner, we obtained FSM8.0.

2.4. Preparation of screen-printed carbon electrodes (SPCEs)

An LS-56TVA semi-automatic screen-printing machine (Newlong Seimitsu Kogyo Co., Ltd, Japan), equipped with a 250 threads per in. polyester screen, a polyurethane squeegee, and stainless steel flood blade was used to prepare the SPCEs. The SPCEs were printed in groups of 80 (reference, working, and auxiliary electrodes) onto glass epoxy sheets. Fabrication took place in four printing steps: (1) carbon ink was printed to form a conducting layer that also acted as the counter electrodes, (2) Ag/AgCl ink was printed in the positions for the reference electrodes, (3) carbon ink containing CoPC was printed on the working electrode to form an activated surface, (4) insulating ink was printed to cover the non-working and non-conducting region of the SPCE to define the working area. After each deposition, the electrodes were dried at 60 °C for 30 min. The individual CoPC-SPCE was cut from the sheets into size 12 mm × 25 mm rectangles and the sampling unit composed of white PET (⌀ 9.0 mm, 12 mm × 12 mm × 1.6 mm), which defined the working area and sample volume of 100 μL, was bonded on top of each CoPC-SPCE with an epoxy adhesive. In a batch process, inexpensive CoPC-SPCEs with consistent quality were prepared (Fig. 1). As can be seen from Fig. 1, the working electrode was a 4 mm-diameter disk, and the auxiliary electrode and Ag/AgCl reference electrode were large and small portions of a ⌀ 7.8 mm × 1.0 mm curved line, respectively.

2.5. Fabrication of the L-lactate biosensor (LOD-FSM8.0/Naf/CoPC-SPCEs)

The LOD-immobilized FSM8.0, LOD-FSM8.0 conjugate, was prepared as follows [41]: An FSM8.0 powder (100 mg) was added to 4.0 mL of 10 mg mL^{-1} LOD solution (citrate buffer, pH 4.3). The suspension was then shaken for 24 h at 4 °C to establish an adsorption equilibrium. The LOD-FSM8.0 conjugate was collected by centrifugation, and then washed with distilled water. The amount of LOD adsorbed into the pores of the FSM8.0 was estimated to be 232.5 mg g^{-1} of LOD-FSM8.0, as determined by spectrophotometric analysis [41].

The sensing layer of the L-lactate biosensor was constructed by the following procedure: (1) the surface of the working electrode was covered with a layer of Nafion by applying 2 μL of 2.5% Nafion (commercial 10% Nafion® was diluted with ethanol and neutralized with 25% ammonia), followed by evaporation of the solvent and then rinsing with distilled water [20–22] to give Naf/CoPC-SPCEs. (2) A 32.5 mg (1000 μL) of LOD-FSM8.0 and 500 μL of 20% DF-20 solution (2 g in 10 mL of water) was mixed together and stirred for 30 min. Then, 50 μL of 10% ADH solution (1 g in 10 mL of water) was added and stirred for a further 5 min. The resulting mixture was deposited in 2 μL aliquots on the working electrode surface of the Naf/CoPC-SPCEs and allowed to dry for 3 h at room temperature. The obtained LOD-FSM8.0/Naf/CoPC-SPCEs were stored in air at 4 °C when not in use. The final amount of immobilized LOD was estimated to be c.a. 11.6 μg (0.93 U) per electrode.

3. Results and discussion

3.1. Principle of operation

The mechanism of our device's amperometric detection of L-lactate is described by the following reactions [23–25]:

$$\text{L-lactate} + O_2 \xrightarrow{\text{LOD}} \text{pyruvate} + H_2O_2, \quad (1)$$

$$2Co^{2+}PC + H_2O_2 \rightarrow 2Co^{+}PC + O_2 + 2H^{+}, \quad (2)$$

$$2Co^{+}PC \xrightarrow{\text{electrode}} 2Co^{2+}PC + 2e^{-}. \quad (3)$$

Immobilized LOD catalyzes the oxidation of L-lactate to pyruvate with the production of hydrogen peroxide (H_2O_2) in the presence of molecular oxygen. This H_2O_2 is then oxidized via the catalytic cycle of the CoPC, resulting in a flow of electrons to the working electrode and thus a measurable current, the magnitude of which is directly related to the L-lactate concentration. The biosensor operates on

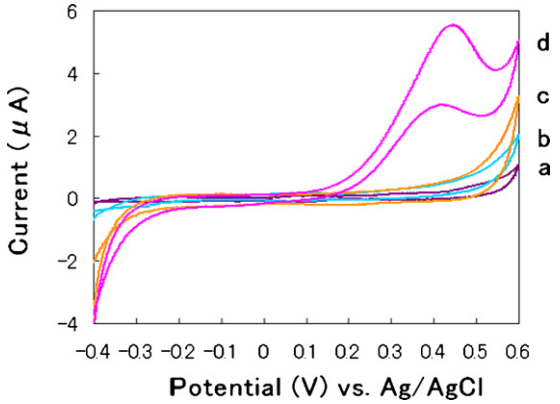

Fig. 4. Cyclic voltammograms obtained with: (a) plain SPCE in phosphate buffer solution (pH 7.4); (b) SPCE with 1.8 mM H_2O_2 added; (c) CoPC-SPCE in phosphate buffer solution (pH 7.4); (d) CoPC-SPCE with 1.8 mM H_2O_2 added. Potential range: −0.4/+0.6 V; Scan rate: 10 mV s^{-1}.

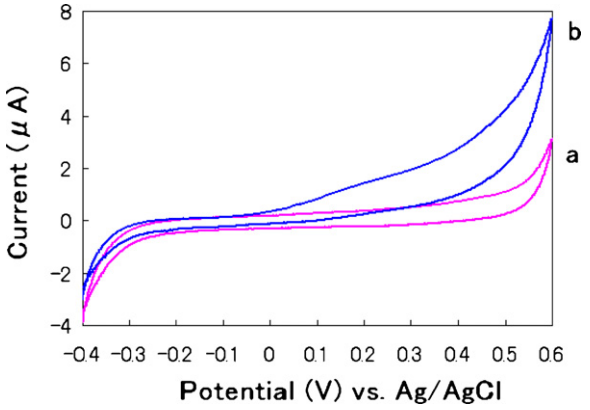

Fig. 5. Cyclic voltammograms obtained with the LOD-FSM8.0/Naf/CoPC-SPCE biosensor: (a) in the absence of L-lactate; (b) in the presence of L-lactate (1.4 mM) in phosphate buffer solution (pH 7.4). Potential range: −0.4/+0.6 V; Scan rate: 10 mV s^{-1}.

the principles illustrated in Fig. 3, where H_2O_2 is generated enzymatically within the reaction layer (LOD-FSM8.0) on the CoPC-SPCE surface.

Each layer of the biosensor plays an important role in the detection process. The top layer containing the LOD-FSM8.0 conjugate efficiently catalyzes and detects the oxidation of L-lactate with densely immobilized LOD within the FSM8.0. Encapsulation of LOD into the pores of FSM8.0 is thought to retard or prevent denaturation and/or aggregation of the enzyme. This layer is designed to ensure the high sensitivity and good storage stability of our sensor. The underlying Nafion layer is an H_2O_2-permselective membrane that improves the selectivity of the sensor by blocking other electroactive species' access to the surface of the working electrode. The formation order of these two layers is important to obtain a high-performance sensor; if the Nafion layer is formed over the LOD-FSM8.0 conjugate, the diffusion of lactate to LOD might be blocked by Nafion layer and would cause serious degradation of sensitivity.

3.2. Electrochemical characterization of the LOD-FSM8.0/Naf/CoPC-SPCEs

First, the electrochemical behavior of the CoPC-SPCEs and plain SPCEs was evaluated through cyclic voltammetry. Fig. 4a–d shows CV traces of the plain SPCEs and CoPC-SPCEs in the absence and presence of 1.8 mM H_2O_2. The scans were initiated at −0.4 V to a switching potential of +0.6 V at a scan rate of 10 mV s^{-1}. These figures show that a well-defined peak for H_2O_2 appears for the CoPC-SPCE at a potential of +0.45 V versus Ag/AgCl, while no well-defined peaks occur for plain SPCEs. This oxidation peak around +0.45 V in the presence of H_2O_2 (Fig. 4d) proves the activity of the catalyst on the electrode is consistent with reactions (2) and (3) [24].

Second, cyclic voltammograms were obtained with the LOD-FSM8.0/Naf/CoPC-SPCE biosensor in the absence (Fig. 5a) and presence (Fig. 5b) of a 1.4 mM L-lactate solution (pH 7.4), respectively. This behavior clearly demonstrates that enzymatically generated H_2O_2 can diffuse to the underlying Naf/CoPC-SPCE and undergo efficient electrocatalytic oxidation. Such behavior is a prerequisite for the successful operation of an amperometric L-lactate biosensor, as discussed in previous sections of this paper.

3.3. Effect of applied potential to the sensor

The effect of the applied potentials on LOD-FSM8.0/Naf/CoPC-SPCE biosensor response was studied in order to identify the most suitable potential for amperometric detection. For this purpose, a 10 μL aliquot of 400 μM L-lactate solution was added to the sampling unit containing 90 μL of phosphate buffer at pH 7.4 and the current was monitored. The current responses were plotted against the applied potentials, as shown in Fig. 6. It is evident that very little current response was observed below +300 mV, but a large increase was observed from +300 to +500 mV. However, the time taken for the sensor to reach equilibrium when switched to +500 mV was much longer than for +450 mV. Thus, we chose +450 mV vs. Ag/AgCl as an operating potential in order to obtain a signal with good intensity and short settling time while avoiding interference from several other compounds.

3.4. Effect of solution pH on the sensor

The effect of the pH on the LOD-FSM8.0/Naf/CoPC-SPCE sensor's response to L-lactate was studied in the pH range of 5.8 and 9.0. The results are shown in Fig. 7, in which the output current of the sensor is expressed as the percentage of the maximum response attained at an appropriate optimal pH. We can see that the response decreases for pH values higher than 8.0 or lower than 7.0, and a maximum response is obtained at about pH 7.4. Hence, we selected a pH of 7.4 for our further studies.

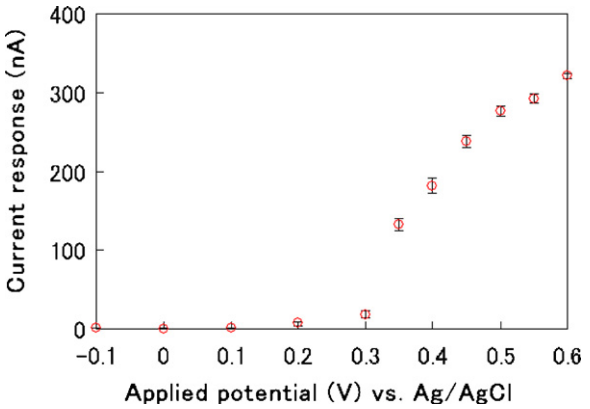

Fig. 6. Response of the LOD-FSM8.0/Naf/CoPC-SPCE sensor in phosphate buffer (pH 7.4) at several applied potentials containing 400 μM of L-lactate solution. The error bars show deviations within six measurements.

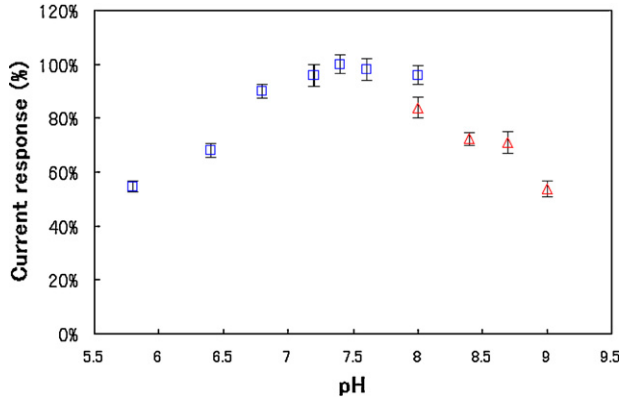

Fig. 7. Responses of the LOD-FSM8.0/Naf/CoPC-SPCE biosensor in different buffer solutions at several pH values (pH 5.8–8.0, phosphate buffer (□); pH 8.0–9.0, borate buffer (△)), containing 400 μM of L-lactate solution. The Applied potential was +450 mV vs. Ag/AgCl. The error bars show deviations within six measurements.

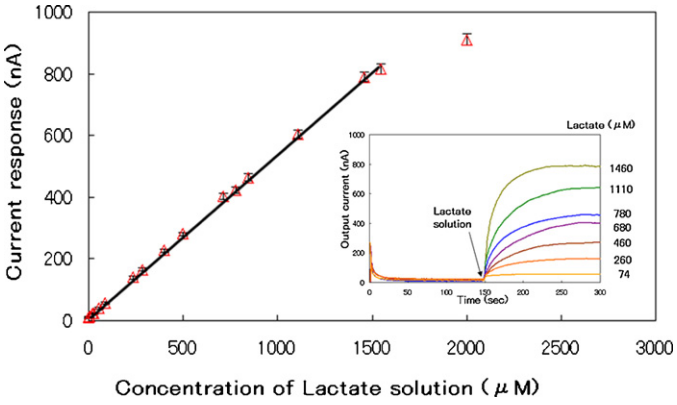

Fig. 8. Typical responses (inset) and calibration curve of the LOD-FSM8.0/Naf/CoPC-SPCE biosensor to injections of aqueous L-lactate solutions, with application of a potential of +450 mV vs. Ag/AgCl in phosphate buffer (pH 7.4). The error bars show deviations within six measurements.

3.5. Amperometric response and calibration curve of the sensor

Next, the sensitivity and operating range of the sensor were studied. Fig. 8 shows the calibration curve and typical amperometric responses (inset) of the sensor to injections of different concentrations of the L-lactate solution (10 μL aliquots), ranging from 1.0 μM to 2.0 mM, into the sampling unit at an applied potential of +450 mV vs. Ag/AgCl in phosphate buffer at pH 7.4. The LOD-FSM8.0/Naf/CoPC-SPCE biosensor output had a good linear relation ($y = 0.57x + 8.9$) in the range of 18.3 μM to 1.5 mM with a correlation coefficient of 0.999 and a sensitivity of 4.54 μA cm^{-2} mM^{-1}. This sensitivity was higher than that reported for lactate biosensors with food or blood (1.18 μA cm^{-2} mM^{-1} [8], 3.98 μA cm^{-2} mM^{-1} [9]). The lower detection limit was 18.3 μM at a signal-to-noise ratio of 3, and the response time to reach a steady output current after applying an L-lactate solution was approximately 90 s. Thus, the LOD-FSM8.0/Naf/CoPC-SPCE biosensor displayed a wide operational range, sufficient sensitivity, and rapid response that could be successfully applied to the detection of L-lactate. The apparent Michaelis–Menten constant of the LOD-FSM8.0/Naf/CoPC-SPCE sensor evaluated from electrochemical version of the Lineweaver–Burk equation was calculated to be 0.86 mM, which is comparable to or lower than corresponding values observed in some papers (0.61 mM [8], 2.25 mM [9]). This indicates a high affinity between the L-lactate and LOD immobilized within the LOD-FSM8.0/Naf/CoPC-SPCE sensor, mainly due to its biocompatibility and large surface area.

Table 1
The relative responses of possible interferents to the LOD-FSM8.0/Naf/CoPC-SPCE sensor (200 μM; L-lactate, ascorbic acid, cholesterol, cysteine, glucose, glutamine acid, urea, and uric acid).

Interferent	Relative response (%)	Interferent	Relative response (%)
L-Lactate	100	Glucose	Not detected
Ascorbic acid	6.4	Glutamine acid	Not detected
Cholesterol	Not detected	Urea	Not detected
Cysteine	Not detected	Uric acid	2.3

3.6. Selectivity against interferents

The interference effects due to the most common electrochemically active species (200 μM; ascorbic acid, cholesterol, cysteine, glucose, glutamine acid, urea, and uric acid) were also evaluated. The relative response gives a measure of the sensor selectivity. Results are summarized in Table 1 and show that the relative responses obtained for most of these interferents were weak under the testing conditions. In particular, the responses to ascorbic acid and uric acid were attenuated to only 6.4% and 2.3% of the 200 μM L-lactate response. These results indicate that the underlying Nafion layer can efficiently avoid interference from these electroactive species, and the LOD-FSM8.0/Naf/CoPC-SPCE sensor has accurate responses in the presence of potentially interfering substances.

3.7. Repeatability, reproducibility, and stability of the sensor

Measurement of the repeatability and reproducibility of the biosensor was conducted at an L-lactate concentration of 400 μM. The relative standard deviation for a series of ten successive measurements was 2.5% (repeatability) and the relative standard deviation for five individual sensors was 5.3% (reproducibility). Thus, the response of the sensor was considered to be satisfactorily repeatable and reproducible.

The long-term storage stability of the LOD-FSM8.0/Naf/CoPC-SPCE sensor was investigated by evaluating its response to a 400 μM L-lactate solution. The sensor was stored desiccated at 4 °C when not in use. Under these storage conditions, the LOD-FSM8.0/Naf/CoPC-SPCE sensor exhibited good stability, and no significant changes were observed in its responses after being stored for over 9 months. In fact, it retained 98% of its initial current response over that period, which is more stable than the previously reported L-lactate biosensor based on screen-printed electrodes [14,19]. Such excellent stability is crucial for any commercial biosensor device. The highly stable nature of this sensor is thought to result from the intimate integration of the enzyme with FSM8.0 and shows that FSM8.0 efficiently retains the activity of LOD.

A comparative study of different L-lactate biosensors based on screen-printed electrodes and lactate oxidase is presented in Table 2, showing that our biosensor offers comprehensive electrochemical properties compared with other biosensors reported previously in the literature. Our biosensor also has good storage stability that is longer than other biosensors. Its linearity was better than those of the sensors presented in [14,20], and its limit of detection was comparable to that of the sensor presented in [20] and better than that of the sensor reported in [22].

Furthermore, our proposed biosensor achieves excellent storage stability, high selectivity, and linearity in a wide range with comparable limits of detection when compared with previous studies based on physically and covalently immobilized-LOD layers on non-screen-printed gold electrodes [11,12].

Table 2
Comparisons of different methods to prepare L-lactate biosensors based on screen-printed electrodes and lactate oxidase.

Enzyme	Material of working electrode	Immobilization method	Potential	Linear range	Detection limit	Response time	Stability	Interference protection	Reference
LOD	Graphite ink	Entrapment in polymer matrices	0.35 V vs. Ag/AgCl	0–10 mM	n.a.	n.a.	>200 days (storage at 25 °C)	Differential determination	[19]
LOD + POX	Graphite ink	Chemically covalent binding	0 V vs. Ag/AgCl	10–180 μM	10 μM	n.a.	>2 weeks (storage at R.T.)	Dilution of sample	[20]
LOD	Graphite ink + ferricyanide	Chemically covalent binding	0.4 V vs. Ag/AgCl	1–50 mM	1 mM	50 s	<10 months (storage dry at −30 °C)	n.a.	[22]
LOD	Platinized graphite ink	Entrapment in polymer matrices	0.35 V vs. Ag/AgCl	n.a.	n.a.	n.a.	200 days (storage at 25 °C)	n.a.	[21]
LOD	Pt sputter on SPCEs	Entrapment in hydrogel layer	0.6 V vs. Ag/AgCl	0–0.5 mM to 0–1 mM	0.5 μM	50 s	6 weeks (storage dry at 4 °C)	Nafion layer	[14]
LOD	Carbon ink containing cobalt phthalocyanine	Entrapment in mesoporous silica	0.45 V vs. Ag/AgCl	0.02–1.5 mM	18 μM	90 s	>9 months (storage dry at 4 °C)	Nafion layer	This paper

4. Conclusions

An amperometric L-lactate biosensor based on screen-printed carbon electrodes containing cobalt phthalocyanine, coated with immobilized lactate oxidase in mesoporous silica using a polymer matrix of denatured polyvinyl alcohol (LOD-FSM8.0/Naf/CoPC-SPCE) has been developed; a Nafion layer acts as a barrier to interferents and a sampling unit attached on the SPCE requires only a small sample volume of 100 μL. The response of the biosensor with a LOD-FSM8.0/Naf/CoPC-SPCE was linear between substrate concentrations of 18.3 μM to 1.5 mM with a response time of 90 s. The sensor showed an optimum response at a pH of 7.4 and at an applied potential of +450 mV. In addition, the sensor exhibited high selectivity and good stability, and it retained its initial current response without any notable change after being stored for over 9 months in dry conditions. Our method provides a simple, cheap, high-performance biosensor for L-lactate.

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