Bulk-modified modified screen-printing carbon electrodes with both lactate oxidase (LOD) and horseradish peroxide (HRP) for the determination of \( \text{L-lactate} \) in flow injection analysis mode

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Abstract

A screen-printed carbon electrode modified with both HRP and LOD (SPCE–HRP/LOD) has been developed for the determination of L-lactate concentration in real samples. The resulting SPCE–HRP/LOD was prepared in a one-step procedure, and was then optimised as an amperometric biosensor operating at \([0, -100]\) mV versus Ag/AgCl for L-lactate determination in flow injection mode. A significant improvement in the reproducibility (coefficient variation of about 10%) of the preparation of the biosensors was obtained when graphite powder was modified with LOD in the presence of HRP previously oxidised by periodate ion (\(\text{IO}_4^-\)). Optimisation studies were performed by examining the effects of LOD loading, periodation step and rate of the binder on analytical performances of SPCE–HRP/LOD. The sensitivity of the optimised SPCE–HRP/LOD to L-lactate was 0.84 nA L\(^{-1}\) at a detection range between 10 and 180 \(\mu\)Mol. The possibility of using the developed biosensor to determine L-lactate concentrations in various dairy products was also evaluated.

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Keywords: Biosensor; Screen-printed carbon electrode SPCE; HRP; Direct electron transfer; L-Lactate

1. Introduction

The electrochemical determination of L-lactate using electrodes modified with enzyme has received much attention due to its importance in food fields [1,2]. Various enzyme electrodes for L-lactate determination have already been described in literature and different configurations have been proposed based on lactate oxidase (LOD) [3–5] or lactate dehydrogenase (LDH) [6–9].

Recently, major attention has been focused on miniaturisation of these biosensors [10]. Screen-printing technology continues until today to be one of the most promising technologies allowing simple, rapid and inexpensive biosensors mass production. Different configurations were used in the aim of obtaining disposable sensors mostly based on successive layers placed on the surface of screen-printed transducers [11]. However only few publications were found about L-lactate screen-printed sensors based on biocomposite carbon ink modified in its bulk with both HRP and LOD [12,13]. In order to reduce the operating potential, platinised carbon had been used as catalyst [10]. It was also found that, regarding interferences, a potential range between −200 and 0 mV versus Ag/AgCl seems more suitable for biosensors based on the measurement of hydrogen peroxide and, as a consequence most of these systems are based on the reduction of enzymatically produced hydrogen peroxide [14–16]. For this purpose, horseradish peroxidase (HRP) was employed to detect \(\text{H}_2\text{O}_2\) produced by oxidase reactions [17–19]. On the other hand, several works have shown the efficiency of direct electron transfer between adsorbed HRP and graphite or carbon electrode [21–23]. Based on a work previously undertaken on the preparation of a screen-printed electrode (SPCE) modified in its bulk with only HRP [24], we describe in this work, a disposable screen-printed biosensor based on a biocatalytic system (HRP/LOD) operating on direct electron transfer. Both LOD and HRP were incorporated in the graphite ink (SPCE–HRP/LOD). This investigation comprises two main parts. First we examined the effect of the ink composition on the analytical performances of SPCE–HRP/LOD using factorial design. Flow Injection Analysis (FIA) technique was used.
to characterise the amperometric response of the biosensor in terms of sensitivity, repeatability and linear range. Hydrodynamic voltammetry technique was also used to examine the electrochemical behaviour of the resulting SPCE–HRP/LOD towards both hydrogen peroxide and \(\text{l-lactate}\). In the second part of this work, we investigated the possibility of using the proposed biosensor in flow injection analysis mode to determine the \(\text{l-lactate}\) concentration in various real samples such as dairy products. The obtained results were compared with those obtained with UV enzyme method.

2. Experimental

2.1. Reagents

Lactate oxidase (LOD) (EC. 1.1.3.4) was a Boehringer-Mannheim (Germany) product. Horseradish peroxidase (HRP) (EC: 1.11.1.7) was from Roche (France). Hydrogen peroxide (30% (w/w)) (No. H-1009) and cellulose acetate (No. C-3782) were obtained from Sigma Chemical. Cyclohexanone (No. C10, 218-0) was purchased from Aldrich and graphite powder (No. 50870) from Fluka. Alumina ceramic substrates for screen-printed electrodes were from The Laser Cutting Company Ltd. (Sheffield, UK). Dielectric Ink and Silver-based ink were from GEM-Gwent (Pontypool, UK). \(\text{l-lactate}\) aqueous solutions were prepared using 0.1 M phosphate buffer (pH 7.2) containing 0.1 M KCl as support electrolyte. Diluted samples were prepared by suitable dilution with the phosphate buffer solution.

2.2. Screen-printed electrodes preparation

DEK Albany model 245 screen printer machine and stainless screens with a 200 mesh and variable thickness (13, 23 or 36 \(\mu\)m) are used to prepare the three electrodes system in four printing steps: (1) printing of Ag/AgCl electrode (13 \(\mu\)m) using the commercially available ink Ag/AgCl (GEM-Gwent), the resulting printed Ag/AgCl electrode presents a stable half-cell potential (0.276 V versus NHE), (2) printing of both the counter electrode and the conducting tracks of working electrode using graphite-CA ink, (3) printing of the activated surface using the graphite-binder (CA) ink modified with HRP/LOD (the diameter of the activated surface is 2 mm) (4) printing of non-conductive dielectric layer to define the working surface area. For each printing step a group of four electrodes was simultaneously printed on alumina ceramic substrate (1.5 cm \(\times\) 1.5 cm). All printed layers were cured at room temperature overnight.

2.3. \(\text{l-lactate}\) biosensor: preparation and analytical performances

The main reactions involved in the \(\text{l-lactate}\) detection are summarised in Fig. 1. Hydrogen peroxide produced enzymatically by LOD in the presence of \(\text{l-lactate}\) and molecular oxygen is reduced at the electrode surface via direct electron transfer between HRP and carbon surface at low operating potential [19].

SPCE–HRP/LOD was prepared by using a graphite powder already modified with both HRP and LOD. This latter was prepared following the procedure used for building the LOD/HRP/FcH carbon paste biosensor [25]. Briefly, HRP was oxidized by an aqueous solution of sodium periodate (NaIO\(_4\)). The oxidised enzyme was ultrafiltrated, using Amicon Cell with a membrane of 30 kDa, and dissolved in 0.05 M phosphate buffer (pH 8). The graphite powder was then added and the mixture stirred for 20 min. LOD was dissolved in the same buffer and the resulting solution was added to HRP/graphite mixture. After half an hour of mixing, the final mixture was freeze-dried in order to obtain the modified graphite powder. HRP and LOD loadings in dry carbon ink were respectively 5.4 and 2.4 U mg\(^{-1}\). Modified graphite powder was obtained as described below and mixed with polymeric binder (cellulose acetate in cyclohexanone). The resulting ink was deposited and allowed to dry overnight at room temperature. The proportion of polymer in the dry ink was 14% w/w. This general procedure was applied using the variable experimental conditions showed in Table 1. High and low levels of each factor were chosen according to data from previous experiments.

### Table 1

<table>
<thead>
<tr>
<th>No</th>
<th>Variable</th>
<th>Low (−)</th>
<th>High (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LOD charging in dry ink (U mg(^{-1}))</td>
<td>2.4</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>% of CA in dry ink</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>HRP Oxidation</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>

2.4. Apparatus and procedures

- FIA measurements were performed using a three-electrode flow-through amperometric homemade cell of the wall-jet type adapted to the screen-printed configuration. The cell was connected to a potentiostat (BAS model Petit Ampère CV-1B). A strip chart recorder (Linseis model L200E) was used to follow the electrode response. The flow-injection system consisted of a flow carrier and sample pump (Ismatec) and an electrical six way-valve (Rheodyne) for injections of the sam-

![Fig. 1. Principle of electroenzymatic detection of \(\text{l-lactate}\).](image-url)
ples by means of a 100 μL injection loop. The flow carrier, 0.1 M phosphate buffer (pH 7.2) added with 0.1 M KCl, was pumped at a flow rate of 0.7 mL min⁻¹. All presented results were the mean of at least three similar electrodes.

- UV–vis adsorption spectra of HRP between 300 and 700 nm were determined with 0.1 mg/mL enzyme in aqueous buffer (phosphate 0.1 M, pH 7.2) solution. Measurements were carried out at 25 °C on a Spectronic Genesis (Milton Roy) spectrophotometer.

3. Results and discussion

3.1. Optimisation of LOD/HRP bulk-modified carbon ink

Fig. 2 shows the calibration curves for l-lactate at the SPCE–HRP/LOD prepared with (a) and without (b) the periodation step. All measurements were carried out in FIA mode at 0 V versus Ag/AgClSPCE. The biosensors prepared with HRP previously oxidised with periodate ions gave a larger linear range towards l-lactate than those prepared with native HRP (without periodation reaction). It was reported that the treatment of HRP by periodate ions leads to the oxidation of HRP sugar residues forming aldehyde groups [24,26] which could then react with amino-groups of various oxidases such as glucose oxidase [20] and lactate oxidase [17,23,25]. The same authors postulated that the oxidation of HRP using IO₄⁻ led to the formation of a bi-enzyme complex (HRP-oxidase) therefore increasing the trapping efficiency of the H₂O₂ production by the oxidase. In additional experiments we compared the electrochemical response towards hydrogen peroxide of the same biosensors prepared with and without periodated HRP. The biosensors prepared with periodated HRP have been found to be twice as sensitive indicating that the IO₄⁻ treatment of HRP also leads to a significant increase in the sensitivity of the resulting biosensor towards hydrogen peroxide (Fig. 3). This result is in agreement with our previous work [24] in which we studied the effect of the periodation treatment on the amperometric response of SPCE–HRP, and demonstrated that the gain in sensitivity observed towards H₂O₂ with the biosensor modified with only periodated HRP was more likely related to an increase of the amount of HRP immobilised at the electrode surface, rather than to an increase of the rate of direct electron transfer between HRP and graphite.

In the additional experiments, we examined the effect of the periodation step on the native structure of HRP. Fig. 4 compares UV–vis spectra of HRP before and after its treatment with IO₄⁻ solution. Both the native and periodated HRP displayed the Soret band at 403 nm indicating that no significant conformational change occurred around heme region. Based on these results one can conclude that the heme group in HRP was not

![Fig. 2. FIA calibration curves for l-lactate using SPCE–HRP/LOD (HRP 5.4 and LOD 3.5 U mg⁻¹; AC 14% in dried film) modified screen-printed electrodes prepared with (a, ×) and without (b, +) oxidation of the enzyme. Phosphate buffer (0.1 mol L⁻¹, pH 7.2). Operating potential 0 mV vs. Ag/AgClSPCE. Flow rate: 0.7 mL min⁻¹.](image)

![Fig. 3. FIA calibration curves for H₂O₂ using SPCE–HRP/LOD (HRP 5.4 and LOD 3.5 U mg⁻¹; AC 14% in dried film) modified screen-printed electrodes prepared with (a, ⃝) and without (b, /) oxidation of the enzyme. Phosphate buffer (0.1 mol L⁻¹, pH 7.2). Operating potential 0 mV vs. Ag/AgClSPCE. Flow rate: 0.7 mL min⁻¹.](image)

![Fig. 4. UV–vis adsorption spectra of native HRP (broken line) and periodated HRP (unbroken line) 0.1 mg/mL of HRP in aqueous buffer (phosphate 0.1 M, pH 7.2).](image)
and No. 5 shows values of 1.30.

Table 2

Design matrix and responses for biosensor optimisation

<table>
<thead>
<tr>
<th>Runs</th>
<th>LOD</th>
<th>AC</th>
<th>Oxidation</th>
<th>1–2</th>
<th>1–3</th>
<th>2–3</th>
<th>1–2–3</th>
<th>Sensitivity (nA mol L⁻¹)</th>
<th>Linear range (µmol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>1.30 (±0.46)</td>
<td>8.76</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>0.70 (±0.24)</td>
<td>5.40</td>
</tr>
<tr>
<td>3</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0.37 (±0.21)</td>
<td>5.73</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0.62 (±0.21)</td>
<td>7.147</td>
</tr>
<tr>
<td>5</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0.87 (±0.07)</td>
<td>10.147</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0.88 (±0.01)</td>
<td>17.147</td>
</tr>
<tr>
<td>7</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>0.27 (±0.01)</td>
<td>30–195</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>0.37 (±0.01)</td>
<td>20–250</td>
</tr>
</tbody>
</table>

Effect E₁ E₂ E₃ E₁₂ E₁₃ E₂₃ E₁₂₃

The composition of each SPCE modified with both HRP and LOD related to their analytical performances in terms of sensitivity and linear range are displayed in Table 2. From these data, the effects of each parameter and their respective interactions were estimated by examining the electro-chemical response to l-lactate. The sensitivity was estimated by the slope of the linear range of the calibration curve. It is interesting to note that all SPCE–HRP/LOD prepared with previously periodated HRP exhibited higher reproducibility in their preparation. For instance, the comparison between the sensitivities of runs No. 1 and No. 5 shows values of 1.30 ± 0.46 and 0.87 ± 0.37, respectively for SPCE–HRP/LOD (−−−−) and (−−−−), which at the 95% confidence level can statistically be considered the same due to the high variability obtained in the measurements. On the whole, the reproducibility of the assessment of sensitivities is found to vary by only ±2–10% in the case of various SPCE–HRP/LOD prepared with oxidised HRP (runs 5–8) while those of the SPCE prepared with non-periodated HRP is found to vary by ±33–35% (values estimated form standard deviation shown in parenthesis in Table 2). These observations show some interesting changes related to the use of periodated HRP, which permits the preparation of biosensors in a homogeneous way. These results could be due to the leakage of LOD and HRP from SPCE surface in the buffer solution in the case of non-periodated HRP. Also, it could be seen (Table 2) that the linear range is found extended for all SPCE–HRP/LOD prepared with oxidised HRP (runs 5–8). In order to assess the reliability of the obtained results (Table 2), the significance of the obtained effects was evaluated using Student-test in which t-values are calculated by dividing the effect values Eᵢ by the standard error on the effect σₑ which was evaluated using σₓ, the standard error on the response obtained for each run (k ∈ [1,8]) according to the following equation:

σₑ = σₓ / √(N−1)

where N is the number of observations.

At the 95% confidence level, only effects with t-values exceeding the tabulated t₀.₀₅,₀₈ = 2.306 could statistically be significant. Table 3 displays the effect of each parameter, as well as their significance. A slight or non-significant effect of LOD loading (E₁) was neither statistically observed on the sensitivity nor on the linear range. This could be explained by the fact that the amperometric response would not be limited by the enzymatic oxidation reaction of l-lactate. Beyond a loading LOD of 2 U mg⁻¹ in dry
ink, LOD could be considered to be in a large excess on the SPCE–HRP/LOD surface according to the l-lactate concentration range used in this study. From Table 3, one can show that the effect of CA rate ($E_1$) in the ink is significant on the sensitivity, as an increase in the linear range was also observed with the increase of CA rate in the ink. A higher binder rate in the bulk of the ink could significantly limit the diffusion rate of l-lactate or H$_2$O$_2$ due to the possible formation of a thicker CA film on the SPCE–HRP/LOD surface [9, 26–28]. However, the effect of binder rate ($E_2$) on the amperometric response of the biosensors should be more complex and other points such as the effect of CA rate on the electrochemical process or on the enzyme activity (HRP and LOD) have to be taken into consideration. The presence of binder in SPCE could partially block the transfer of electrons between the SPCE surface and active species in solution. Amatore et al. [29] reported a model explaining the electrochemical behaviour on electrode surfaces partially blocked towards electron transfers. In this model it was assumed that only a fraction of electrochemical active sites were available for electron transfer. The increase of CA rate could also cause a similar behaviour by limiting the rate of the direct electron transfer between HRP and graphite. Further results on the effect of binder rate on both electrochemical behaviour and enzyme stability will be reported when completed.

The effect of the periodation of HRP ($E_3$) on both the sensitivity and linear range was also assessed. No significant effect of the periodation step on the sensitivity (estimated on the linear range) was observed but, according to the values displayed in Table 3, the linear range was found extended to higher l-lactate concentrations when HRP was previously periodated. A similar effect was previously observed for hydrogen peroxide [24]. These results could easily be explained by the fact that the periodation step led to the formation of a bi-enzyme complex (HRP–LOD) increasing the efficiency of the trapping of species in solution. Amatore et al. [29] reported a model explaining the transfer of electrons between the SPCE surface and active species in solution. Amatore et al. [29] reported a model explaining the electrochemical behaviour on electrode surfaces partially blocked towards electron transfers. In this model it was assumed that only a fraction of electrochemical active sites were available for electron transfer. The increase of CA rate could also cause a similar behaviour by limiting the rate of the direct electron transfer between HRP and graphite. Further results on the effect of binder rate on both electrochemical behaviour and enzyme stability will be reported when completed.

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The periodation step in the preparation of the resulting biosensor has been found to be significantly beneficial for extending the linear range and for preparing biosensors in a reproducible way. In this study, the best reproducibility and larger linear range were obtained with the SPCE–HRP/LOD corresponding to LOD (+), CA (−) with periodation step (run No 6). This composition was retained for further experiments.

3.2. Electro-catalytical behaviour of the biosensor versus working potential

Hydrodynamic voltammograms were performed to examine successively the amperometric response of the SPCE–HRP/LOD for hydrogen peroxide and l-lactate solution. As can be seen in Fig. 5, the current response towards hydrogen peroxide increases as applied potential decreases. Such results were expected, but at more negative potential values (lower than −100 mV versus Ag/AgCl|Ag| there is now well known that HRP could directly exchange its electrons with various electrochemical transducers without using any redox mediator, and it was demonstrated by several authors that HRP adsorbed on graphite [21–23], carbon nanotube [32], gold [33] or platinum [34] electrodes could exhibit catalytic response towards hydrogen peroxide. Moreover, HRP–Fe(III) could electrochemically be reduced at the electrode surface to HRP–Fe(II) [21, 33, 35] at the more negative potential. In this case, HRP–Fe(II) could catalyse the electrochemical reduction of O$_2$ producing H$_2$O$_2$ as it was suggested by several authors [36, 37].

So the electrochemical behaviour observed towards H$_2$O$_2$ at more negative potentials on the electrode modified with HRP could be due to the involvement of O$_2$ generated by HRP–Fe(II)/HRP–Fe(III) couple [38, 39]. Now if we examine the amperometric response of the SPCE–HRP/LOD towards l-lactate instead of hydrogen peroxide under the same experimental conditions, the curve versus $E$ also displays two regions: in the potential range of [−50; 250] mV versus Ag/AgCl|Ag| the observed catalytic behaviour could be attributed to the electrochemical regeneration of HRP–Fe(III) via Cpd-I and Cpd-II producing a catalytic reduction of hydrogen peroxide [30, 31]. It is now well known that HRP could directly exchange its electrons with various electrochemical transducers without using any redox mediator, and it was demonstrated by several authors that HRP adsorbed on graphite [21–23], carbon nanotube [32], gold [33] or platinum [34] electrodes could exhibit catalytic response towards hydrogen peroxide. Moreover, HRP–Fe(III) could electrochemically be reduced at the electrode surface to HRP–Fe(II) [21, 33, 35] at the more negative potential. In this case, HRP–Fe(II) could catalyse the electrochemical reduction of O$_2$ producing H$_2$O$_2$ as it was suggested by several authors [36, 37].

\[
\begin{align*}
\text{HRP–Fe(III)} + e^- & \rightarrow \text{HRP–Fe(II)} \quad (2) \\
\text{HRP–Fe(II)} + O_2 & \rightarrow \text{HRP–Fe(II)–O}_2 \quad (3) \\
\text{HRP–Fe(II)–O}_2 + 2e^- + 2H^+ & \rightarrow \text{HRP–Fe(II)} + H_2O_2 \quad (4)
\end{align*}
\]
the increase of the amperometric signal with the potential was attributed to the catalytic reduction of hydrogen peroxide produced enzymatically by LOD in the presence of both l-lactate and oxygen. The resulting catalytic current recorded in this region of potential [-50; 250] mV versus Ag/AgCl|SPCE and in the presence of H2O2 produced by l-lactate could be consistent with the classical electrochemical regeneration of HRP–Fe(III) via compound-I and compound-II. For more negative working potential values, a significant decrease of the catalytic current towards l-lactate solution was observed. Since the O2 could be electro-catalytically reduced on the electrode surface via HRP, the decrease in the peak intensities could plausibly be attributed to a partial depletion of dissolved oxygen in the vicinity of the SPCE–HRP/LOD. In this case, the amount of oxygen became insufficient to supply LOD. However, the inactivation of LOD or HRP at more negative potential values could also be considered in this case, but in this study no significant decrease in the sensitivity was observed at a working potential value of 0 V versus Ag/AgCl|SPCE for SPCE–HRP/LOD previously treated at −200 mV versus Ag/AgCl|SPCE for 15 min.

3.3. Analytical features of the resulting electrode

The average sensitivity of the resulting and optimised biosensor HRP/LOD (−, +) was 0.87 nA L mol⁻¹ for l-lactate with a reproducibility error of 10% for three similar electrodes. The detection limit was 10 μM of l-lactate and a good linear range was observed between 10 and 200 μM after which the signal became non-linear. The operational stability of the biosensor was also examined by recording the dependency of amperometric responses on time for successive injections of a l-lactate solution (Fig. 6). Notice that the biosensor prepared with periodated HRP showed higher operational stability compared to that prepared with native HRP. The coefficient of variation to assess the operational stability was found about CV = 3% (n = 50) for the biosensors containing periodated HRP. When the biosensor was prepared with native HRP, a decrease in the signal intensity was found about 10% after 50 injections of the same l-lactate solution. This indicates that the operational stability of the resulting biosensor was improved when HRP was oxidised by NaIO4. The shelf-life of the resulting biosensor was also evaluated by measuring the slopes of the calibration curves at different times. The activity of the electrode surface remained stable for more than two weeks when it was kept without any other specific care at room temperature. After 2 weeks, a continuous decrease of the activity was observed, more than 90% of the loss in activity was reached after 4 weeks of storage at room temperature.

3.4. Applications to standard and real samples

The l-lactate biosensor has been evaluated on real samples and compared to the standard spectroscopic enzymic method using analysis kits. For both techniques the dilution of samples was required in order to fit the linear range. The samples were diluted with buffer solution for the electrochemical assays and with distilled water for spectrophotometric enzyme analysis. In preliminary studies, the accuracy of measurements with the resulting biosensor was examined by using standard l-lactate solutions prepared in phosphate buffer. Fig. 7 shows a good correlation between the both methods (slope: 1.10 ± 0.05 with R² > 0.95) and indicates that the biosensor gives the accurate and repeatable measurements. The performances of the resulting biosensor were then assessed by determining l-lactate concentrations in real samples. Various dairy products were chosen for this purpose. Table 4 displays the comparison between the results obtained with both the SPCE–HRP/LOD and UV methods and indicates here a good correlation between both methods. It is also important to notice that no significant matrix effect was observed on the electrochemical measurements, this is probably due both to high dilution of all samples and to the good performances of the resulting biosensor for low concentrations detection.

![Fig. 6. Operating stability of SPCE–HRP/LOD prepared with periodated HRP (+) and with native HRP (×) in flow injection mode. l-Lactate (200 μM) in phosphate buffer (0.1 mol L⁻¹, pH 7.2): Operating potential 0 mV vs. Ag/AgCl|SPCE. Flow rate: 0.7 mL min⁻¹.](image1)

![Fig. 7. Correlation curve for l-lactate determination in standard solutions using both amperometric and spectrophotometric methods.](image2)
Table 4

<table>
<thead>
<tr>
<th>Sample</th>
<th>UV (%)</th>
<th>FIA (%)</th>
</tr>
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<tbody>
<tr>
<td>Fruit yoghurt</td>
<td>103±2</td>
<td>92±1</td>
</tr>
<tr>
<td>Collise yoghurt</td>
<td>81±4</td>
<td>77±3</td>
</tr>
<tr>
<td>Rhabdoid yoghurt</td>
<td>103±5</td>
<td>88±4</td>
</tr>
<tr>
<td>Soft white cheese 20%</td>
<td>95±31</td>
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</tr>
<tr>
<td>Yoghurt</td>
<td>106±25</td>
<td>99±24</td>
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<tr>
<td>Creamy fruit yoghurt</td>
<td>100±8</td>
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<td>Nut yoghurt</td>
<td>69±6</td>
<td>65±3</td>
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<tr>
<td>Whole milk</td>
<td>0.11±0.17</td>
<td>0.16±0.02</td>
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<tr>
<td>Milk direct from the cow</td>
<td>0.08±0.12</td>
<td>0.05±0.05</td>
</tr>
</tbody>
</table>

Standard deviations are reported in brackets.

4. Conclusion

This work has demonstrated the feasibility of preparing an l-lactate SPCE based on one-step screen-printing procedure. The printed active layer comprises graphite ink modified in its bulk with both HRP and LOD. Our results showed that the SPCE–HRP/LOD prepared with HRP previously periodated its bulk with both HRP and LOD. Our results showed that the resulting biosensor gives reliable results. However, the shelf-life stability remains poor and needs to be improved by adding a specific stabiliser into the HRP/LOD graphite ink.

Acknowledgments

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