A multianalyte flow electrochemical cell: application to the simultaneous determination of carbohydrates based on bioelectrocatalytic detection

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Abstract

A multianalyte flow electrochemical cell (MAFEC) for bioanalysis is constructed, characterised and used for simultaneous carbohydrate analysis incorporating mediated amperometric enzyme electrodes. Although multidetection schemes can be addressed with microfabricated systems, it is demonstrated that a “meso” analytical device of low cost can give answers to traditional simultaneous multianalysis problems, being robust, and easy to construct and operate. The cell operates as a radial flow thin-layer device and can achieve mass transport controlled response for fast electrochemical reactions. When appropriate enzymatic electrodes are used the response becomes kinetically limited, but still shows a better than 5% R.S.D. for response to different sugars analysed. All the enzymatic sensors are mediated with different osmium compounds appropriate for each enzyme’s mechanism (NAD or PQQ dehydrogenases) in some cases combining multienzyme sensors. All sensors were optimised so that different sugars do not produce interferences to other sensors. Matrix interferences were kept low by operating all sensors at or below 150 mV versus Ag/AgCl. The integrated system was used for the simultaneous detection of fructose, sucrose, glucose, galactose, and lactose, fully characterising the system for these analytes (sensitivity, dynamic range). Cross referenced calibration curves were used for signal treatment and interpretation and it was possible to analyse real juice and milk samples with results agreeing with the standard enzymatic methods for the same analyses with a sampling frequency of more than 100 h⁻¹.

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1. Introduction

Multianalyte determination is receiving increased attention in chemical analysis in search of selective array systems that can rapidly provide simultaneous information in a cost-effective manner. The construction of such systems has become increasingly feasible with the advent of micro-machined sensor arrays although the spatial resolution of selectivity necessitates further sophisticated technologies (Vilkner et al., 2004). Multianalyte information does not, however, always demand micrometric resolution, particularly if the quality of the final product can be assessed by a limited and defined number of chemical parameters.

Simultaneous multidetection of sugars is being traditionally approached by means of chromatographic separation coupled to different techniques of detection (Montero et al., 2004). Amongst these techniques, the refractive index-based measurement remains the most commonly used due to the lack of more selective physical/chemical properties of carbohydrates (Chavez-Servin et al., 2004) and mass spectrometry (Zala, 2004). Irrespective of the sensitivity of the detection technique, the efficient separation of closely related carbohydrates is compromised by their co-elution, thereby necessitating sample derivatisation.
Enzyme carbon paste electrodes based on either oxidases or NAD*/NADH dependent dehydrogenases have been extensively described for the analysis of mono, di and oligosaccharides (Gorton, 1995). Contrary to oxidase-based detection, the response of electrodes based on dehydrogenases is not dependent on the presence of dissolved molecular oxygen. However, the necessary overpotential for direct electrochemical oxidation of NADH has to be reduced by an efficient redox chemistry if the risk of interference threatens to compromise the selectivity of the response (Katakis and Dominguez, 1997). Gorton and Dominguez (2002). In addition, NADH has to be entrapped on the electrode matrix if a reagentless configuration is pursued (Dominguez et al., 1993).

This work focuses on a new design for a multianalyte flow electrochemical cell (MAFEC) used for the simultaneous quantification of several analytes following a single sample injection. The geometry of the cell corresponds to that of a thin-layer detector. The analytical performance of the cell is demonstrated through the simultaneous bioelectrocatalytic detection of five sugars in food samples using dehydrogenase-based carbon paste electrodes. Our previous transducing schemes for the selective detection of mono- (Hedenmo et al., 1996; Paredes et al., 1997) and disaccharides (Maestre et al., 2001) are incorporated into the multianalyte flow electrochemical system. It will be shown that after calibration of the system and following the injection of a single sample, five sugars can be simultaneously quantified in food samples that have not been pre-treated. The accuracy of the results will be compared with those achieved using commercially available enzymatic kits.

2. Materials and methods

2.1. Reagents

α-Glucose dehydrogenase (EC 1.1.1.47) from Thermoplasma acidophilum is a commercially available recombinant enzyme, expressed in E. coli, that shows substantial activity for α-glucose and α-galactose (Smith et al., 1989).

This dehydrogenase was produced from Sigma as a suspension in 2.8 M (NH₄)₂SO₄ solution of 50 mM phosphate buffer (pH 7.0). The suspension was treated with Gluconobacter sp., invertase (EC 3.2.1.26) from baker’s yeast, and β-galactosidase (EC 3.2.1.23) from Aspergillus oryzae were also purchased from Sigma. α-Glucose dehydrogenase (EC 1.1.1.47) from Bacillus megaterium, mutarotase (EC 5.1.3.3) from porcine kidney, were supplied by Merck. β-Nicotinamide adenine dinucleotide phosphate (NADP⁺), succrose, polyethyleneimine (PEI) and acetonitrile were received from Sigma. β-Nicotinamide adenine dinucleotide free acid (NAD⁺) was supplied by Merck. Graphite powder and paraffin oil were purchased from Fliuka. All solutions were prepared with reagent grade chemicals without further purification using ultra-pure Milli-Q water.

The Os (4,4’-dimethyl-2,2’-bipyridine)²⁺ (1,10-phenanthroline-5,6-dione) (osphendione) mediator was synthesized according to published procedures (Greer and Abruña, 1985), precipitated from a PfO₂-saturated aqueous solution and used as the PFO₂ salt. The osphendione-based redox mediator [Os(bpy)₃Cl₂]⁺ used for the PQQ-based enzymatic reoxidation was synthesized according to a procedure reported by Lay et al. (1996).

The F-kit UV method, which enables sucrose, d-fructose and d-glucose to be determined simultaneously, was purchased from Boehringer Mannheim GmbH (Cat. No. 716260). The method is based on the spectrophotometric monitoring of NADPH formation after a multienzymatic reaction on the sample. The catalytic cascade comprises the addition of hexokinase (EC 2.7.1.1), glucose-6-phosphate 1-dehydrogenase (EC 1.1.1.49), phosphoglucoisomerase (EC 5.3.1.9) and invertase (EC 3.2.1.26) in the presence of adenosine-5′-triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADP⁺). The sucrose content is then calculated from the difference of the d-glucose concentration before and after enzymatic hydrolysis. Lactose and d-galactose were also simultaneously determined by a F-kit UV method purchased from Boehringer Mannheim GmbH (Cat. No. 176303). The detection of lactose comprises its catalytic hydrolysis to d-glucose and d-galactose by β-galactosidase. d-Galactose is then oxidised by β-galactose dehydrogenase to d-galactonic acid in the presence of NAD⁺. Similarly, spectrophotometric measurements of NADH and the subtraction of sugar content before and after hydrolysis results in galactose and lactose determination, respectively.

2.2. Preparation of mediated carbon paste electrodes

A stock mixture containing 25 mg of thermally-pre-treated graphite powder (700°C for 60 s) and 2.5 mg of osphendione mediator was prepared. This mixture, 2.5 mg of NAD⁺ for glucose, sucrose and lactose, or 11.4 mg of NADP⁺ for galactose electrodes were added and gently mixed. The glucose sensor was prepared by the addition of 1600 IU of GDH from B. megaterium. The sucrose sensor contained 1120, 6187, and 1600 IU of invertase, mutarotase and GDH from B. megaterium, respectively. The galactose sensor was prepared by the addition of GDH from T. acidophilum. Finally, the lactose sensor included 1120, 6187, and 1600 IU of β-galactosidase, mutarotase, and GDH from B. megaterium, respectively. Galactose modified paste was then dried in a desiccator for 10 min prior to the addition of paraffin oil. Thirty microliters of paraffin oil was added to the different modified pastes and packed in a Teflon tube (inner diameter 0.85 mm and a geometric surface area of 0.0057 cm²) with a 3 mm long cavity containing also unmodified carbon paste (25 mg graphite and 10 µL paraffin oil) and a platinum wire for the electrical contact. The surfaces were gently rubbed on glass to produce flat shining electrode surfaces.
The fructose electrodes were prepared as previously reported in the literature (Paredes et al., 1997). In brief, an enzyme solution was obtained after dissolution of 58 IU of FDH in 1 mL of 0.1 M acetate buffer pH 5 containing 0.1% (w/v) Triton X-100, 0.1% PEI and 0.1 M CaCl₂. The solution is then added to 25 mg of a graphite mixture containing 2.5 mg of Os(bpy)₂Cl₂, gently mixed and dried in a dessicator. Following the addition of paraffin oil, the paste was packed into the Teflon tubes as described above. Blank electrodes were also prepared using unmodified paste.

2.3. Instrumentation

The six working electrodes, the Ag/AgCl (KCl sat) reference electrode and two platinum counter electrodes were connected to a multi-potentiostat (AUTOLAB, Eco Chemie BV, Utrecht, NL). The equipment operates with a multitasking General Purpose Electrochemical System (GPES) windows software package. Amperometric responses were measured at 150 mV (Hedenmo et al., 1996). In the case of fructose sensors, 100 mV were applied (Paredes et al., 1997). The multi-analyte flow cell was mounted on a FIA system consisting of a high-pressure pump (Hewlett Packard, Series 1050) and a manually operated injection valve (Rheodyne, type 7125). The carrier used was a 0.1 M acetate buffer pH 5, unless otherwise stated.

3. Results and discussion

3.1. Description of the multi-analyte flow electrochemical cell

The geometrical characteristics and the design of the MultiAnalyte Flow Electrochemical Cell (MAFEC) are shown in Fig. 1. The upper and bottom parts of the cell were constructed of PMMA, (poly)methyl methacrylate, providing high-level precision for the assembly of both surfaces. The dimensions are 45 mm × 45 mm × 35 mm and the assembled cross-section is shown in Fig. 1. A polyetheretherketone plastic gasket (0.10 mm thickness) between both parts determines a cell dead volume of 70 μL. The six working electrodes (WEs) and the reference electrodes are located in the upper part. The inlet and the six outlets, corresponding to each WE, are placed in the bottom part of the cell. The radial arrangement of the WEs, all of which were equidistant from the inlet, guarantees a laminar and identical hydrodynamic flow regime at the six electrodes. Next to each WE, but at the bottom block, the six outlets are placed to prevent cross-talk from occurring and also to help in contributing to identical hydrodynamic pressure across the electrodes. The location of the reference electrode, as well as the dimensions of the two platinum counter electrodes, prevents inadequate conductance of the cell solution (Myland and Oldham, 2000). The reference electrode is a Ag/AgCl/2 of the double liquid-junction type. The refillable outer electrolyte solution serves as a salt bridge to prevent contamination of the reference element. This reference electrode is placed equidistantly from each working electrode.

3.2. Hydrodynamic characterisation of MAFEC

A variety of experimental assessments were conducted to characterise the hydrodynamic regime of the flow cell. Initially, it was found that the radial flow distribution at the six WEs was almost identical. Measurements of the flow rate at the outlets of each electrode for three different inlet flows resulted in variations below 5% within electrode outlets. The cell is designed as a thin-layer detector as was confirmed.
Table 1: Catalytic and electrochemical reactions occurred for glucose (1), galactose (2), fructose (3), sucrose (4) and lactate (6) biosensors

<table>
<thead>
<tr>
<th>Catalytic detection scheme</th>
<th>EC detection/transducing chemistry</th>
<th>Applied potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-D-Glucose + NAD+</td>
<td>GDH BM</td>
<td>O⁶⁻ + O⁷⁻ + O⁸⁻ + O⁹⁻</td>
</tr>
<tr>
<td>β-Galactose + NAD⁺</td>
<td>GDH TM</td>
<td>O⁶⁻ + O⁷⁻ + O⁸⁻ + O⁹⁻</td>
</tr>
<tr>
<td>D-Fructose + NAD⁺</td>
<td>FDH</td>
<td>O⁶⁻ + O⁷⁻ + O⁸⁻ + O⁹⁻</td>
</tr>
<tr>
<td>Sucrose + D-fructose + α-D-glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-D-Glucose + NAD⁺</td>
<td>GDH</td>
<td>O⁶⁻ + O⁷⁻ + O⁸⁻ + O⁹⁻</td>
</tr>
<tr>
<td>Lactate + β-D-galactose + D-glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-D-Glucose + NAD⁺</td>
<td>GDH</td>
<td>O⁶⁻ + O⁷⁻ + O⁸⁻ + O⁹⁻</td>
</tr>
</tbody>
</table>

The substrates BM and TA in the enzyme GDH denote the different organism source, i.e. B. megaterium and T. acidophilum. [Os(bpy)²Cl²]⁺ is represented by the redox couple Os⁶⁻/Os⁷⁻ and O⁶⁻/O⁷⁻ (dimethyl) 2,2'-bipyridine/1,10-phenanthroline 5,6-dione by OsQ/OsQH₂.

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Fig. 2: Effect of the flow rate on the response of the cell using two different injection loops: (A) 20 µL injection loop and ferrocyanide 16 mM; (B) 100 µL injection loop and ferrocyanide 16 mM. In both cases, carbon paste electrodes were poised at 0 V vs. Ag/AgCl sat.

Fig. 3: Comparison of the FIA peak profile obtained with the MAFEC for injections of 16 mM ferrocyanide using three different injection loops (20, 100, and 500 µL). In all cases, the carrier was 0.1 M acetate buffer pH 5 flowing at 2 mL min⁻¹ and the electrodes were poised at 0 mV vs. Ag/AgCl sat.

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The analytical application of MAFEC was demonstrated through the simultaneous analysis of five sugars in food samples. One of the six electrodes in the multianalyte flow cell.
All the experiments were performed in 0.1 M acetate buffer pH 5. The values of $K_{	ext{m}}$ and $I_{\text{max}}$ were calculated by means of Hanes–Woolf plot. Measurements of repeatability were done with 10 successive injections of the respective analytes and reproducibility was calculated, at least, with three different modified electrodes. Sampling frequency results are calculated assuming a carryover of 2%.

![Dependence of the electrocatalytic response on the flow rate for glucose and sucrose biosensors. Hundred-microliter injections of either 5 mM glucose or 5 mM sucrose were made into the carrier stream of 0.1 M acetate buffer at pH 5. Samples were measured at 0.150 V vs. Ag/AgCl sat.](image)

Fig. 4. Dependence of the electrocatalytic response on the flow rate for glucose and sucrose biosensors. Hundred-microliter injections of either 5 mM glucose or 5 mM sucrose were made into the carrier stream of 0.1 M acetate buffer at pH 5. Samples were measured at 0.150 V vs. Ag/AgCl sat.

2 mL min$^{-1}$ was chosen as a compromise between sensitivity and sample throughput.

The detection of galactose has been traditionally achieved using galactose oxidase electrodes (Yokoyama et al., 1989; Szabó et al., 1996; Sharma et al., 2004). The reasons for not using this enzyme were the loss of activity that galactose oxidase presents at pH 5 and in particular in acetate buffer (Hamilton et al., 1978; Schumacher et al., 1994) as well as its reactivity to other galactose containing disaccharides such as lactose. Instead, glucose dehydrogenase from the thermophilic archaebacterium *T. acidophilum* was chosen for this work for reasons explained below.

This enzyme is a NAD(P)$^+$-NAD(P)H dependent dehydrogenase having catalytic activity not only for glucose but also for galactose and xylose (Smith et al., 1989; Woodward et al., 2003). As seen in Table 2, GDH from *T. acidophilum* was successfully mediated by the osphendione mediator resulting in a $K_{	ext{m}}$ of 68 mM for galactose. This value remains higher than the 20 mM found for glucose under the same conditions presuming an interference when both monosaccharides are present in the sample (see below).

One of the major problems relating to the enzymatic analysis of the disaccharides sucrose or lactose is the content of the corresponding monosaccharides in food samples. In the case of sucrose determination, the interference of glucose can be suppressed by a catalytic route based on sucrose phosphorylase (Kogure et al., 1997; Hamada et al., 1999).

Table 2

<p>| Analytical parameters of the different sensors in steady state and FIA conditions |
|----------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Steady State</th>
<th>FIA</th>
<th>$K_{\text{m}}$ (mM)</th>
<th>$I_{\text{max}}$ (µA)</th>
<th>Linear range (mA cm$^{-2}$)</th>
<th>Detection limit (mM)</th>
<th>Repeatability (%)</th>
<th>Reproducibility (%)</th>
<th>Sampling frequency (samples h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose sensor</td>
<td>47</td>
<td>2.4</td>
<td>0.4</td>
<td>1.0</td>
<td>2-50</td>
<td>0.1</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Sucrose sensor</td>
<td>12</td>
<td>1.0</td>
<td>4.2</td>
<td>0.4</td>
<td>1-12</td>
<td>0.1</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>Fructose sensor</td>
<td>13</td>
<td>4.2</td>
<td>0.7</td>
<td>4.7</td>
<td>3-25</td>
<td>0.1</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Galactose sensor</td>
<td>68</td>
<td>2.5</td>
<td>0.4</td>
<td>1.7</td>
<td>1-3</td>
<td>0.2</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Lactose sensor</td>
<td>46</td>
<td>2.6</td>
<td>0.3</td>
<td>0.3</td>
<td>6-50</td>
<td>0.8</td>
<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>

All the experiments were performed in 0.1 M acetate buffer pH 5. The values of $K_{\text{m}}$ and $I_{\text{max}}$ were calculated by means of Hanes–Woolf plot. Measurements of repeatability were done with 10 successive injections of the respective analytes and reproducibility was calculated, at least, with three different modified electrodes. Sampling frequency results are calculated assuming a carryover of 2%.
Maestre et al., 2001). The pH profile, however, of this enzyme shows low activity at pH 5 (Maestre et al., 2001). Consequently and in search of simultaneous analysis including both the mono and the disaccharide, other alternatives had to be investigated. In this context, many approaches have been described in the literature (Olsson et al., 1986; Mizutani and Yabuki, 1997; Thavarungkul et al., 1999; Aoki et al., 2002; Curey et al., 2002; Held et al., 2002). Surareungchai et al. (1999) have reported a dual-electrode signal-subtracted biosensor for the simultaneous determination of sucrose and glucose based on glucose oxidase, mutarotase and invertase. The system is based on signal subtraction of two identical electrodes where in one electrode the hydrolase was inactivated in order to obtain similar enzyme thick films on the platinum surface (i.e. similar diffusion profiles within the film) and thus, identical sensitivities for glucose in both electrodes irrespective of the presence of sucrose in the sample (Gulce et al., 1995).

In contrast, in this work, the possibility of obtaining a sensitivity for glucose within the sucrose, lactose and galactose electrodes similar to that of the glucose sensor (GDH from \textit{B. magatuerium}) for signal subtraction between electrodes was not pursued mainly due to the different kinetics of the GDHs used for the glucose and galactose sensors. The glucose sensor, however, could always be used as a reference electrode for the determination of glucose content in the sample and subsequently, interpolation of this value in the corresponding glucose calibration curve in the galactose, sucrose and lactose sensor would permit signal subtraction. This demands that the sensitivity for the target sugars in these sensors is not affected by the presence of glucose in the sample though higher signals are always expected. Initially, this can be envisaged if the catalytic activity of the hydrolase remains lower than the activity of the dehydrogenase, i.e. the newly formed glucose on the electrode surface does not change the mass transport profile of the diffusing glucose from the sample. For this reason and in detriment of making the electrodes kinetically controlled, presumably through the first hydrolytic step, the limits of invertase and \(\beta\)-galactosidase remained lower than the units of GDH. Thus, the enzyme ratio of invertase (or \(\beta\)-galactosidase)/GDH was kept at 0.7:1 (see Experimental).

Contrary to this approach, Surareungchai et al. (1999) found that the sensitivity for sucrose was significantly altered by the presence of glucose when the enzymatic ratio was 16:1. Consequently and in search of simultaneous analysis including other alternatives had not been pursued mainly due to the different kinetics of the GDHs used for the glucose and galactose sensors. The glucose sensor, however, could always be used as a reference electrode for the determination of glucose content in the sample and subsequently, interpolation of this value in the corresponding glucose calibration curve in the galactose, sucrose and lactose sensor would permit signal subtraction. This demands that the sensitivity for the target sugars in these sensors is not affected by the presence of glucose in the sample though higher signals are always expected. Initially, this can be envisaged if the catalytic activity of the hydrolase remains lower than the activity of the dehydrogenase, i.e. the newly formed glucose on the electrode surface does not change the mass transport profile of the diffusing glucose from the sample. For this reason and in detriment of making the electrodes kinetically controlled, presumably through the first hydrolytic step, the limits of invertase and \(\beta\)-galactosidase remained lower than the units of GDH. Thus, the enzyme ratio of invertase (or \(\beta\)-galactosidase)/GDH was kept at 0.7:1 (see Experimental).

Contrary to this approach, Surareungchai et al. (1999) found that the sensitivity for sucrose was significantly altered by the presence of glucose when the enzymatic ratio was 16:1 in the electrode film.

**Table 3**

Comparison of glucose, sucrose, fructose, galactose and lactose content in fruit juices and milk derivatives as determined by the corresponding mediated oxidation signals in a FIA system with the MAFEC and commercial enzymatic kits (\(n=3\)).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analyte (g/100 g)</th>
<th>Sensor</th>
<th>F-kit method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>JUICES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pineapple</td>
<td>Glucose</td>
<td>45.71±0.61</td>
<td>43.13±3.74</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>9.23±0.74</td>
<td>10.01±1.28</td>
</tr>
<tr>
<td>Peach</td>
<td>Glucose</td>
<td>43.63±1.96</td>
<td>48.79±2.54</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>15.70±1.65</td>
<td>15.65±3.41</td>
</tr>
<tr>
<td>Orange</td>
<td>Glucose</td>
<td>38.01±1.71</td>
<td>38.47±4.62</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>23.21±1.61</td>
<td>19.00±3.36</td>
</tr>
<tr>
<td><strong>Milk derivatives</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLESA BIO</td>
<td>Glucose</td>
<td>0.05±0.00</td>
<td>0.06±0.00</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>0.42±0.02</td>
<td>0.67±0.99</td>
</tr>
<tr>
<td>PULEVA MERENGADA</td>
<td>Glucose</td>
<td>0.53±0.02</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>0.65±0.00</td>
<td>0.00±0.02</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>2.54±0.10</td>
<td>2.54±0.10</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>3.74±0.30</td>
<td>2.87±0.05</td>
</tr>
</tbody>
</table>

Contrary to this approach, Surareungchai et al. (1999) found that the sensitivity for sucrose was significantly altered by the presence of glucose when the enzymatic ratio was 16:1 in the electrode film.
Consequently, standard mixtures of the target analytes in the presence of glucose were analysed. It was observed that the shape of the FIA peaks in the presence of glucose was changed, as shown in Fig. 5, and signals were thus obtained by integration of the peak area. As expected, no signal was measured in the blank electrode and the response of the fructose sensor was not affected by the presence of glucose due to the strict selectivity of this enzyme to the ketopentose. Systematic variation of glucose concentration did not significantly alter the response of the galactose sensor. Similar results were obtained for sucrose and lactose sensors as far as the concentrations remained below the apparent $K_{m}$ (data not shown). This is to say that irrespective of the sensitivity for glucose in each electrode, the content of the disaccharides and galactose could be obtained from the difference between the total signal of the sample and that corresponding to glucose. Albeit not directly subtracting the signals but instead interpolating in the corresponding calibration curves.

Different food samples were then analysed with the previously described enzyme electrodes in the MAFEC. Hundred microlitres of appropriately diluted fruit juice and milk samples were injected into the FIA system at a flow rate of 2 mL min$^{-1}$. The samples were previously filtered in order to avoid solid particles from entering the system. Independent comparative measurements were undertaken using commercially available enzymatic kits and the results were compared as shown in Table 3. Standard deviation values were obtained from three measurements. It is noticeable that a good agreement was achieved for all the sugars found in these samples.

4. Conclusions

A multianalyte electrochemical cell has been developed and integrated into a monochannel FIA system for simultaneous multicomponent analysis. The cell behaves as a three-layer detector. When appropriate enzyme electrodes are incorporated, the system allows the determination of five carbohydrates commonly found in food industry and fermentation processes, namely, glucose, sucrose, fructose, galactose and lactose. The selectivity of the cell relies on commercially available dehydrogenases that are efficiently mediated by osmium derivatives thereby enabling the amperometric detection at 150 mV versus Ag/AgCl and thus reducing the risk of non-selective electrochemical reactions. Mediated enzyme carbon paste electrodes were conveniently employed enabling the analysis of disaccharides with no interference of glucose.

The presented cell design is believed to contribute to the resolution of multianalyte detection in cases where further miniaturisation is not required. The system is reagentless and simple to use when compared to other strategies based on multichannel configurations and, thus, can be easily implemented as a selective detection unit in flow injection systems.

References


