Food Chemistry 140 (2013) 742–747

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Rapid and direct determination of fructose in food: A new osmium-polymer mediated biosensor

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ARTICLE INFO

Article history:
Available online 16 November 2012

Keywords:
Fructose
Food analysis
Biosensor
Fructose dehydrogenase
Osmium redox polymer

ABSTRACT

This paper describes the development and performance of a new rapid amperometric biosensor for fructose monitoring in food analysis. The biosensor is based on the activity of fructose dehydrogenase (FDH) immobilised into a carbon nanotube paste electrode according to two different procedures. The direct wiring of the FDH in a highly original osmium-polymer hydrogel was found to offer a better enzyme entrapment compared to the immobilisation of the enzyme in an albumin hydrogel. The optimised biosensor required only 5 U of FDH and kept the 80% of its initial sensitivity after 4 months. During this time, the biosensor showed a detection limit for fructose of 1 μM, a large linear range between 0.1 and 5 mM, a high sensitivity (1.95 μA cm^{-2} mM), good reproducibility (RSD = 2.1%) and a fast response time (4 s).

Finally, the biosensor was applied for specific determination of fructose in honey, fruit juices, soft and energy drinks. The results indicated a very good agreement with those obtained with a commercial reference kit. No significant interference was observed with the proposed biosensor.

1. Introduction

D-Fructose is an important sugar used as a low-cost sweetener by the food and beverage manufacturers. It is widely distributed in fruit juices, honey, soft and energy drinks and diabetic food as its sweetening ability is greater than that of glucose and sucrose. Excessive uptake of fructose is harmful and therefore it is an analyte of great interest for the food industry and clinical diagnostics (Frattali, 1982). Food quality control is essential both for consumer protection and for food industries. Conventional methods for sugar analysis use techniques such as chromatography, spectrophotometry, electrophoresis and titration (AOAC, 1995; Beutler, 1984) but these methods do not allow an easy and rapid monitoring because they require expensive instrumentation, well trained operators and often elaborate sample pretreatment with an increasing time of analysis. A great deal of research is therefore needed to develop a simple, fast and sensitive method, which could be effectively used by the food industries. Biosensors offer a promising alternative: besides their good selectivity and low cost, they can be used to develop simple and portable equipment allowing fast in situ monitoring of raw materials and food processing steps (Eggins, 2002; Tran & Cahn, 1993; Wagner & Guilbault, 1994).

Fructose dehydrogenase (FDH; E.C. 1.1.99.11), first described by Ameyama and Adachi (1982), catalyses the oxidation of fructose to 5-keto-D-fructose with the concomitant reduction of the bound cofactor flavin adenine dinucleotide (FAD) (Ameyama & Adachi, 1982; Kamitaka, Tsujimura, Setoyama, Kajno, & Kano, 2007), thus representing an ideal enzyme for biosensor construction because no addition of the well-known $NAD(P)^+$/NAD(P)H cofactor is required. Several biosensors, based on carbon paste (Bassi, Lee, & Zhu, 1998; Garcia, Neto de Oliveira, Kubota, & Grandin, 1996; Paredes, Parellada, Fernandez, Katakis, & Domínguez, 1997; Parellada, Domínguez, & Fernandez, 1996), gold (Campuzano, Loaiza, Pedrero, Villena, & Pingarron, 2004; Damar & Demirkol, 2011), Pt (Antiochia & Palleschi, 1997; Moscone, Bernardo, Marconi, Amine, & Palleschi, 1999; Trivedi, Lakshminarayana, Kothari, Patel, & Panchal, 2009), graphite (Piermarini, Volpe, Esti, Simonetti, & Palleschi, 2011) and glassy carbon electrodes (Tkac et al., 2001) have been modified with FDH using different immobilisation techniques. In recent years, nanotechnology and nanomaterials have been revolutionising the area of biosensors. In particular carbon nanotubes have begun to attract enormous interest in electrochemistry for biosensor construction because of their small size and their good electrochemical properties (Antiochia, Lavagnini, & Magno, 2005; Antiochia, Lavagnini, Magno, Valentini, & Palleschi, 2004a; Valentini, Amine, Orlanducci, Terranova, & Palleschi, 2003). Recently, for this reason and for their easy preparation and the possibility of renewal of their surface, carbon nanotube paste (CNTP)

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http://dx.doi.org/10.1016/j.foodchem.2012.11.023

electrodes have started to become popular for electrode modification (Antiochia, Lavagnini, & Magno, 2004b; Gooding, 2005; Wang, 2005).

The objective of the present work is to report a new alternative biosensor for fructose detection based on the modification of a CNTP electrode with an osmium redox polymer. The efficient electron shuttling properties of the osmium redox polymer allowed its utilisation for electrical wiring of cells and enzymes (Timur, Yigzae, & Gorton, 2006b; Timur et al., 2006a) and for biosensor construction (Antiochia & Gorton, 2007; Heller, 1992; Heller & Feldman, 2008).

In our work the osmium redox polymer was used as redox mediator to shuttle the electrons between the immobilised enzyme and the single-walled carbon nanotube paste (SWCNTP) electrode and also as a support for direct wiring of FDH itself into the paste by using poly(ethylene glycol) diglycidyl ether (PEDGE) as a cross-linking agent. As well as optimisation studies, application of the proposed biosensor for fructose analysis in real samples were carried out and the results obtained were in good agreement with those determined with the standard spectrophotometric method.

2. Experimental

2.1. Chemicals

Fructose dehydrogenase (FDH) (E.C. 1.1.1.47) from *Gluconobacter* sp. and D-fructose were purchased from Sigma (St. Louis, MO, USA). Poly(ethylene glycol) (400) diglycidyl ether (PEDGE) was obtained from Polyscience (Warrington, PA, USA). Poly(1-vinylimidazole)$_{12}$-[osmium(4,4′-dimethyl-2,2′-dipyridyl)$_2$Cl$_2$]$^{2+/+}$ (osmium redox polymer) was generously provided as a gift from ThereSense Inc. (Alameda, CA, USA). Single-walled carbon nanotubes Carbolex (diameter 1–2 μm) were obtained from Aldrich (Steinheim, Germany). Mineral oil was obtained from Fluka (Buchs, Switzerland). All other chemicals were from Carlo Erba (Milan, Italy). All solutions were prepared with high purity water produced by a Milli-Q System (Millipore, Bedford; MA, USA).

2.2. Construction of the Os-polymer modified CNTP electrode

The CNTP electrodes were prepared by hand-mixing SWCNTs and graphite powder with mineral oil at a 60:40% ratio (w/w) (Valentini et al., 2003). The paste was mixed in a mortar and packed into a cavity (3 mm diameter, 0.5 mm depth) at the end of a Teflon tube and electrical contact was established via a copper wire connected to the paste. The electrode surface was gently smoothed by rubbing it on a piece of filter paper before use.

The Os-polymer CNTP electrode was prepared by depositing on the CNTP electrode surface 10 μL of a solution (10 mg/mL) of the Os-polymer in Milli-Q water and 1 μL of an aqueous solution (2.5 mg/mL) of the cross-linker agent PEDGE (Antiochia & Gorton, 2007). After the deposition, the electrodes were left to dry overnight at room temperature.

2.3. Construction of the modified CNTP fructose biosensor

The fructose biosensor was assembled according to two different procedures of FDH immobilisation. In the first procedure FDH was immobilised in an albumin hydrogel. A 5 mg aliquot of albumin (BSA) was dissolved in 40 μL of 0.1 mol/L phosphate buffer with 10 μL of a solution of FDH (10 U). A 25 μL aliquot of the FDH-matrix system was successively mixed with 3 μL of glutaraldehyde (0.25% v/v) and entrapped between two polycarbonate membranes and fixed to the surface of the Os-polymer CNTP electrode. A 0.1 M phosphate buffer solution was used to gently rinse the biosensor and remove those of the glutaraldehyde molecules, which did not react with the polymeric matrix.

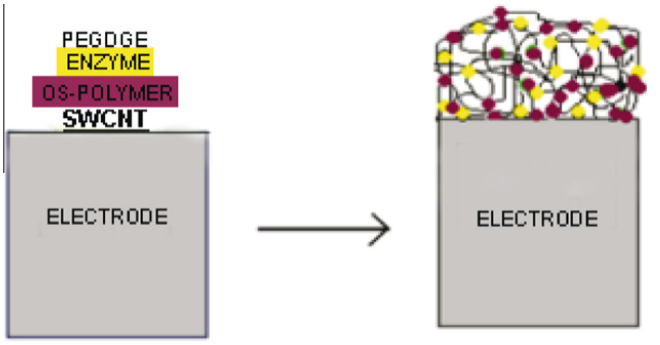

Fig. 1. Schematic representation of the FDH wiring through redox hydrogel of Os-polymer on CNTP electrode.

In the second procedure FDH was directly wired into the Os-polymer hydrogel. This method involved a thorough mixing of 10 μL of a solution of the Os-polymer (10 mg/ml) in Milli-Q water, 1 μL of an aqueous solution of PEDGE (2.5 mg/mL) and 10 μL of a solution of FDH (10 U). Successively, a 10 μL aliquot of this solution was deposited on the CNTP electrode surface and left to dry overnight at room temperature (Antiochia & Gorton, 2007). The modified electrode was rinsed carefully with 0.1 mol/L phosphate buffer at pH 7.0 before use. A schematic representation of the wiring of FDH through the Os-polymer redox hydrogel on the CNTP electrode is shown in Fig. 1.

2.4. Electrochemical characterization

Electrochemical measurements were performed using an Autolab electrochemical system equipped with PGSTS-12 with GPES software (Eco Chemie, Utrecht, The Netherlands). All electrochemical experiments were carried out in a conventional three-electrode cell at room temperature with the modified CNTP electrode (3 mm diameter) as working electrode, an Ag|AgCl/KCl(sat) as the reference and a platinum wire as the counter electrode. The electrochemical cell contained 10 mL of 0.1 M phosphate buffer or 0.1 M acetate buffer at various pHs. A fixed potential, +200 mV versus Ag|AgCl, was used to make the amperometric experiments.

2.5. Biosensor response

For fructose determinations aliquots of a stock solution of D-fructose in 0.1 M acetate buffer at pH 5.0 were successively added in the electrochemical cell and the steady-state current values were recorded. The steady-state current was achieved within 20–25 s.

2.6. Analysis of food samples

The fructose biosensor was tested for the analysis of food samples like honey and some beverages, like fruit juice, soft and energy drinks, purchased from local supermarkets. Fructose concentrations in real samples were determined in five replicates on the basis of the calibration curve obtained for the standard fructose solution. The results obtained were compared with those obtained with the enzymatic spectrophotometric assay kit (Mannheim, Germany, Cat.N. 139106). The determination of fructose is related to the amount of NADPH formed and spectrophotometrically measured at 340 nm. Honey and beverage samples did not require any pretreatment. The honey samples were diluted as follows: 1 g of

each sample was accurately weighed and dissolved in 100 mL of water (1% solution) after heating for 15 min at $t = 60\,^{\circ}C$. Then 50 μL of this solution were added to the cell containing 10 mL of buffer. The beverage samples were analysed by adding 50 μL directly to the electrochemical cell containing 10 mL of buffer.

3. Results and discussion

The principle of operation of the fructose biosensor is based on the oxidation of D-fructose to 5-keto-D-fructose by FDH with the concomitant reduction of the osmium-polymer mediator (Reaction (1)) according to the following reactions:

$$D-\text{fructose} + 2Os^{3+} \xrightarrow{FDH} 5-\text{keto}-D-\text{fructose} + 2H^{+} + 2Os^{2+} \quad (1)$$

$$2Os^{2+} \xrightarrow[+200mV\ vs.Ag|AgCl]{CNTPelectrode} 2Os^{3+} + 2e^{-} \quad (2)$$

The reduced mediator is successively reoxidized at the CNTP electrode at a potential of +200 mV (Reaction (2)). The current values due to the enzymatic reaction are proportional to the concentration of fructose in the reaction medium for fructose concentrations lower than the Michaelis–Menten constant. The simplified reaction sequence of this biosensor given by Reactions (1) and (2) is due to the characteristics of FDH enzyme, which contains the FAD cofactor tightly bound to the enzyme and does not require the adding of NAD^{+} as most other dehydrogenase enzymes thus allowing the construction of oxygen independent reagentless sensors (Davidson & Jones, 1991). The structure of the mediator is shown in Fig. 2. However, FDH also contains 3 hemes connecting the FAD in the catalytic site with the surface of the enzyme (Ameyama & Adachi, 1982; Kamitaka et al., 2007). In previous work with a similar enzyme, cellobiose dehydrogenase, it was shown that an Os-polymer could connect both the FAD in the catalytic dehydrogenase domain as well as the heme in the separate cytochrome domain (Tasca et al., 2009). Thus it can be expected that the Os-polymer accepts electrons directly from the FAD in the active site as well as from the hemes.

3.1. Electrochemical characterization of the FDH/Os-polymer/CNTP electrode

The electrochemical behaviour of a similar Os-polymer CNTP electrode was already investigated in our previous work (Antiochia & Gorton, 2007). Fig. 3 shows a cyclic voltammogram obtained with the Os-polymer CNTP modified electrode at a scan rate of 10 mV/s in the absence (curve a) and in the presence (curves b and c) of

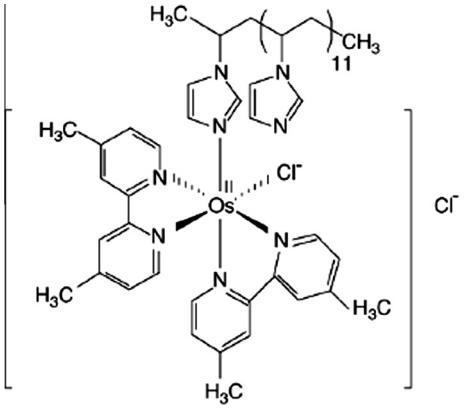

Fig. 2. The chemical structure of poly(1-vinylimidazole)$_{12}$-[osmium(4,4′-dimethyl-2,2′-bipyridyl)$_2$Cl$_2$]$^{2+/+}$ (osmium redox polymer).

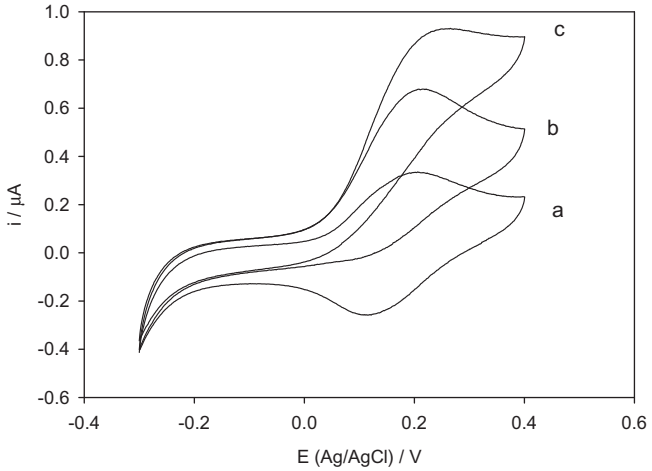

Fig. 3. Cyclic voltammograms obtained with a FDH-Os-polymer modified CNTP electrode in the absence (a) and in the presence of 5×10^{-3} M fructose concentration with FDH immobilised in an albumin hydrogel (b) and directly into the Os-polymer hydrogel (c). Applied potential: 0.2 V (vs. Ag/AgCl). Experimental conditions: Os-polymer: 10 μL of a 10 mg/mL solution; PEDGE: 1 μL of a 2.5 mg/mL solution; BSA: 5 mg in 50 μL; glutaraldehyde: 3 μL of a solution 0.25% v/v; FDH: 5U; v = 10 mV/s; 0.1 M phosphate buffer pH 7.0; electrode diameter: 3 mm.

FDH. As expected, a small peak separation typical of surface-bound species is shown by all voltammograms, showing that FDH does not influence the electrochemical behaviour of the modified electrode. The formal redox potential of the Os-polymer was found to be of about +180 mV vs. Ag|AgCl, in good agreement with that already reported previously (Antiochia & Gorton, 2007; Timur et al., 2006a; Timur et al., 2006b). The proper working potential for the biosensor was therefore choosen at +200 mV. It is interesting to note that when the enzyme is wired to the Os-polymer hydrogel the current observed (curve c) is higher than that registered when the enzyme is entrapped in an albumin hydrogel matrix (curve b). This behaviour can be explained by the fact that the immobilisation of the enzyme with the Os-polymer hydrogel allows a larger number of molecules of mediator in direct contact with the electrode surface and therefore a stronger catalytic effect of FDH is observed with this type of immobilisation.

3.2. Fructose biosensor response

In order to optimise the FDH-Os-polymer CNTP biosensor two different immobilisation methods were tested. Fig. 4 shows the fructose calibration curves derived from the amperometric response and relative to an Os-polymer CNTP electrode with FDH immobilised into an albumin hydrogel matrix. The effect of enzyme loading on the current response of the biosensor was investigated in the range from 2 to 10 U of FDH. All curves showed a linear part at the beginning with a deviation from linearity at higher fructose concentrations. This behaviour is probably due to the saturation of the active site of the enzyme. As for the FDH amount employed, the current gradually increased with increasing FDH values from 2 to 5 U (curves a and b) but it did not show any significant increase for amounts higher than 5 U (curve c). This fact indicates that the amount of enzyme is rate limiting at quite low enzyme amounts employed but then the rate of the reaction becomes limited by other factors. Fig. 5 shows the fructose calibration curves obtained with the Os-polymer CNTP electrode with FDH directly wired to the Os-polymer hydrogel. As can be seen, the calibration curves show plateau current values, which are about 20% higher than those obtained with FDH immobilised into the albumin hydrogel, for all amounts of FDH employed. Once

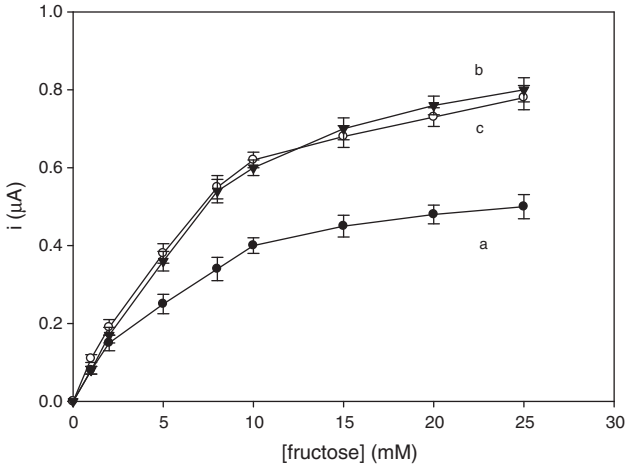

Fig. 4. Calibration graphs for the fructose biosensor with the following FDH amounts immobilised into an albumin hydrogel matrix: 2 U (a); 5 U (b); 10 U (c). R.S.D. values calculated for each point of the calibration curves are between 1% and 3.1% ($n = 6$). Applied potential: 0.2 V (vs. Ag/AgCl). Experimental conditions: as in Fig. 3.

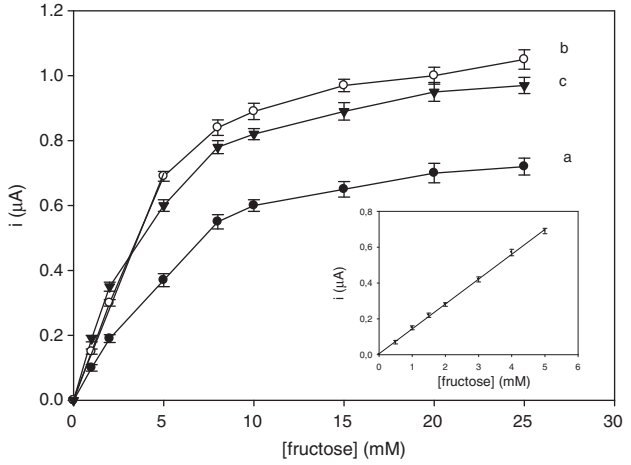

Fig. 5. Calibration graphs for the fructose biosensor with the following FDH amounts directly immobilised into the Os-polymer hydrogel: 2 U (a); 5 U (b); 10 U (c). The inset shows the linear part of curve b. RSD% values calculated for each point of the calibration curves are between 0.8% and 3% ($n = 6$). Applied potential: 0.2 V (vs. Ag/AgCl). Experimental conditions: as in Fig. 3.

again, an amount of 5 U of FDH was found to give the maximum current (curve b) and therefore 5 U of FDH was used to prepare the biosensor in further experiments. The results obtained can be explained by the fact that the enzyme is now wired through the redox hydrogel of the Os-polymer onto the CNTP electrode surface. The hydrogel allows the immobilisation of the enzyme without suffering major structural changes or loss of activity but at the same time the high surface area of the SWCNTs allows a bigger exposure of the enzyme's catalytic sites as well as a high loading of FDH with a possible enhancement of the resulting signal.

Fig. 5 shows the calibration curve for fructose registered by the optimised biosensor and in the inset it is possible to see the linear part of the calibration curve. It shows a good linearity over the range of 0.1–5 mM. At higher fructose concentrations the curve becomes nonlinear approaching a saturation value ($K_M = 3.66$ mM). Statistical analysis gave the following equation $y = 0.14x + 5.24 \times 10^{-3}$ ($r = 0.9992$, $n = 6$), where y indicates the current (μA) and x the fructose concentration (mM). The sensitivity was found to be 1.95 μA cm^{-2} mM and the detection limit was found to be about 1 μM, calculated using the relation 3S.D.a/b, where S.D.a is the absolute standard deviation of the intercept and b the slope of the calibration curve.

The biosensor showed also a fast response time of about 4 s.

In the literature several FDH biosensors based on platinum, glassy carbon and carbon paste electrodes have been reported. The comparison of some analytical characteristics of the previously reported FDH biosensors and the proposed biosensor was given in Table 1.

3.3. Effect of pH and temperature

The influence of the pH solution on the biosensor response for fructose was investigated in the pH range between 4 and 5.5 in 0.1 M acetate buffer and in the range between 6 and 7.5 in 0.1 M phosphate buffer. As shown in Fig. 6, the relative response is enhanced by increasing the solution pH from 4 to 5, reaching the maximum value in acetate buffer at pH 5.0, which was subsequently utilised for further experiments. At pH values higher than 6 a progressive marked decrease of the relative response is registered and at pH values higher than 7.0 a loss of response of about 80% is observed. This result is in good agreement with those obtained in literature for FDH modified electrodes (Antiochia et al., 2004b; Trivedi et al., 2009) and with the optimum pH of the free enzyme, which is 4.5 (Ameyama, Shinagawa, Matsushita, and Adachi (1981)).

The effect of the temperature of the buffer solution on the response of the sensor was studied in the range 10–40 °C and is reported in Fig. 7. As can be observed, the relative response initially increases with the temperature up to a maximum value at about 30 °C and decreases after a further increase. This behaviour can be attributed to thermal inactivation of FDH at higher temperatures. However, a temperature of 25 °C, at which only a

Table 1
Comparison of analytical characteristics of various FDH-modified electrodes reported in literature.

Electrode configuration	Mediator	Linear range	LOD	Operational stability	Refs.
Au	Hexacyanoferrate	0.25–5.0 mM	–	30% decrease after 5 h	Damar and Demirkol (2011)
CPE[a]	$Os(bpy)_2Cl_2^{2+/+}$	0.22 mM (batch)	–	No decrease after 4 h	Yamada et al. (1966)
Pt	Hexacyanoferrate	1.0 μM–1.0 mM		50% decrease after 3 months	Moscone et al. (1999)
SPGE[b]	Phenazine methansulfate	0.05–0.5 mM	–	10% decrease after 15 days	Tominaga et al. (2007)
CPE	Tetracyanoquino-dimethane	10 μM–0.8 mM	10 μM	60% decrease after 6 day	Bassi et al. (1998)
CPE	Meldola Blue	0.1–0.8 mM	–	No decrease after 2 months	Garcia et al. (1996)
CNTP[c]	3,4-dihydroxy benzaldehyde	5 μM–2 mM	1 μM	10% decrease after 1 week	Antiochia et al. (2004a, 2004b)
SWCNTP[d]	$Os(4,4'\text{-dimethyl-}2,2'\text{-dpy})_2Cl_2)^{2+/+}$	0.1–5.0 mM	1 μM	No decrease for 4 months	This work

[a] Carbon paste electrode.
[b] Screen printed graphite electrode.
[c] Carbon nanotube paste electrode.
[d] Single-wall carbon nanotube paste electrode.

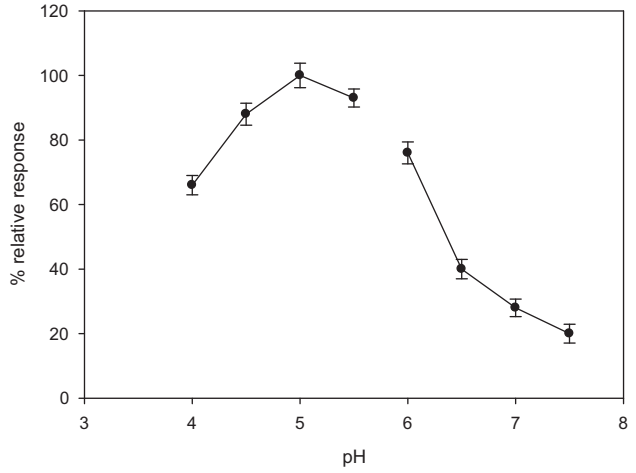

Fig. 6. Effect of pH on the maximum response of the fructose biosensor. [fructose] = 5×10^{-4} M; buffers employed: 0.1 M acetate buffer, 4 < pH < 5.5; 0.1 M phosphate buffer, 6 < pH < 7.5. RSD % values calculated for each point of the curves are between 2.7% and 3.8% ($n = 6$). Other conditions as in Fig. 5.

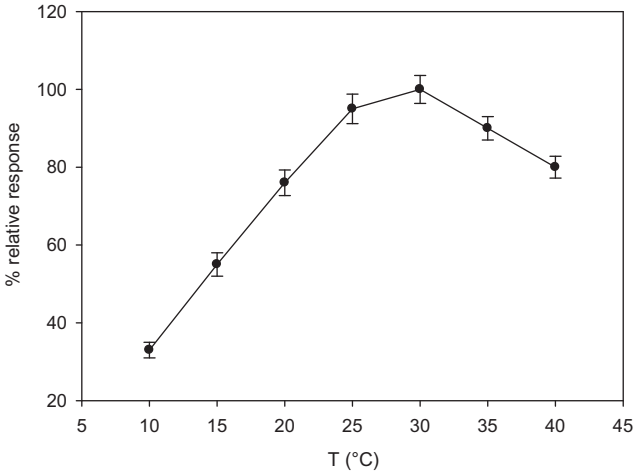

Fig. 7. Effect of temperature on the maximum response of the fructose biosensor. [fructose] = 5×10^{-4} M. RSD values calculated for each point of the curve are between 2% and 3.8% ($n = 6$). Other conditions as in Fig. 5.

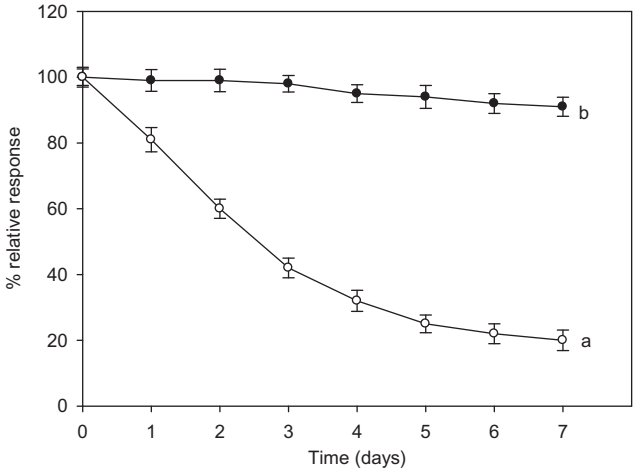

Fig. 8. Lifetime of the fructose biosensor stored at 4 °C in dry conditions (a) and wet conditions (b). RSD% values calculated for each point of the curves are between 2.5% and 3.7% ($n = 6$). Other conditions as in Fig. 5.

Table 2
Influence of interfering compounds on fructose response of the FDH-Os-polymer-CNTP fructose biosensor. The amounts of interferents added were 500 μM.

Interfering compounds	[fructose] (μM)	Recovery (%)
Ascorbic acid	460	92
Ethanol	490	98
Glucose	480	96
Sucrose	470	94
Uric acid	495	99

Table 3
Results for fructose determination in real samples using the FDH biosensor and the reference enzymatic kit.

Sample	[fructose] (%)[a] fructose biosensor	[fructose] (%)[a] enzymatic kit
Orange juice	20.82 ± 0.15	23.15 ± 0.22
Apple juice	27.34 ± 0.20	29.12 ± 0.30
Pineapple juice	18.30 ± 0.34	20.14 ± 0.25
Peach juice	29.38 ± 0.52	31.68 ± 0.35
Cherry juice	27.68 ± 0.28	28.94 ± 0.34
Honey (thousand flowers)	36.22 ± 0.44	37.11 ± 0.24
Honey (rosemary)	39.05 ± 0.37	39.98 ± 0.22
Coco-cola	13.95 ± 0.18	15.14 ± 0.30
Energy drinks	37.55 ± 0.48	39.58 ± 0.31

[a] All measurements were repeated six times and reported as average ± RSD ($n = 6$).

5% decrease in the maximum response was registered, was chosen for further experiments, because it is closer to room temperature.

3.4. Reproducibility

The reproducibility of the biosensor was found to be very good, obtaining an RSD of 2.1% for a 0.5 mM fructose solution for $n = 6$, where n represents the number of sensors used for the test.

3.5. Storage stability and lifetime

The stability and lifetime of the biosensor were also tested by consecutive measurements of the current response to a 1 mM fructose solution. As most enzymes lose their activity if not stored in the refrigerator (4 °C), the fructose biosensor was carefully stored at 4 °C when not in use. Fig. 8 shows the biosensor lifetime under dry and wet storage conditions at 4 °C. Under dry storage conditions, the biosensor lost about 20% of its initial activity after 1 day of 8 h/day operation and more than 80% after 1 week. Under wet conditions, the biosensor retains almost 100% of its original response after 3 days of 8 h/day operation and about 90% after 10 days. Under wet conditions with a use of a maximum 8 h/day operation and storage at 4 °C, the biosensor is able to retain 80% of original response even up to about 4 months. The stability presented for the proposed biosensor is quite superior to most of the amperometric biosensors for fructose described in the literature (Damar & Demirkol, 2011). This result is consistent with the fact that under wet conditions the biosensor is kept in a closed bottle with cotton wet with buffer solution and this allows the maintenance of a higher water content in the immobilised hydrogel layer.

3.6. Selectivity of the biosensor

In order to confirm the selectivity of the biosensor, possible interfering species such as ascorbic acid, ethanol, glucose, galactose and sucrose were checked by adding equal quantities of the

interferent and fructose. The results are shown in Table 2. No significant influence was observed for all interferences with the only exception of ascorbic acid, which showed an interference of about 8%. However, despite the slight interference of ascorbic acid, these results indicate that the biosensor can be used in fructose determination in real samples as these species do not interfere during detection of fructose.

3.7. Analysis of food samples

The proposed FDH fructose biosensor was applied to the analysis of foodstuffs like commercial fruit juice samples, honey, soft drinks and energy drinks. The fructose content was determined applying the calibration graph method and the samples were analysed in five replicates. The results were compared to those obtained with the enzymatic commercial spectrophotometric assay kit. As can be seen from Table 3, there is a good agreement between the results obtained with the biosensor and the reference spectrophotometric kit. It is interesting to observe that the results obtained with the reference spectrophotometric kit all show slightly higher values, probably because of the multienzyme cascade reactions, which take place in the kit and that can cause interferences from other compounds, especially other sugars, whereas the FDH biosensor is strictly specific for fructose, as demonstrated above.

4. Conclusion

The presented fructose biosensor, based on the combination of fructose dehydrogenase, carbon nanotube based electrode and a highly original osmium redox polymer, will form the basis of a new, rapid and portable method for determination of fructose in food analysis. The preparation of the biosensor is very simple, cheap and not time-consuming. The biosensor showed a good linear range, low detection limit (1 μM), good reproducibility, good stability and a fast response time. It was also not influenced by possible interferents which can be present in food matrices.

For these reasons, this new, low cost and disposable fructose biosensor prototype would be suitable for possible use in food industries.

Acknowledgements

The authors thank the following agencies for financial support. (LG) The Swedish Research Council and the Nanometer Consortium at Lund University.

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