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Development of a simple, low cost chronoamperometric assay for fructose based on a commercial graphite-nanoparticle modified screen-printed carbon electrode

Phil Nicholas[a], Robin Pittson[a], John P. Hart[b,*]

[a] The Gwent Group, Monmouth House, Mamhilad Park, Pontypool NP4 0HZ, United Kingdom
[b] Centre for Research in Biosciences, Faculty of Health and Applied Sciences, University of the West of England, Bristol, Coldharbour Lane, Bristol BS16 1QY, United Kingdom

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ABSTRACT

This paper describes the development of a simple, low cost chronoamperometric assay, for the measurement of fructose, using a graphite-nanoparticle modified screen-printed electrode (SPCE-G-COOH). Cyclic voltammetry showed that the response of the SPCE-G-COOH enhanced the sensitivity and precision, towards the enzymatically generated ferrocyanide species, over a plain SPCE; therefore the former was employed in subsequent studies.

Calibration studies were carried out using chronoamperometry with a 40 µl mixture containing fructose, mediator and FDH, deposited onto the SPCE-G-COOH. The response was linear from 0.1 mM to 1.0 mM. A commercial fruit juice sample was analysed using the developed assay and the fructose concentration was calculated to be 477 mM with a precision of 3.03% (n = 5). Following fortification (477 mM fructose) the mean recovery was found to be 97.12% with a coefficient of variation of 6.42% (n = 5); consequently, the method holds promise for the analysis of commercial fruit juices.

1. Introduction

The ability to precisely and accurately measure the sugar known as fructose has become of considerable interest, to many food companies. For example, the wine manufacturing industry use the concentration of fructose (along with glucose) to predict the alcohol content following fermentation (Bauer & Pretorious, 2000; Guillaume, Delobel, Sablayrolles, & Blondin, 2007). The fructose concentration in commercial fruit juices is also an important indicator of the freshness of the food product (Fadel, 2008).

Currently, few reports describe the development of amperometric assays for the measurement of fructose, compared with other sugars such as glucose and sucrose (Antiochia and Gorton, 2014; Biscay et al., 2012; Tsujimura, Nishina, Kamitaka, & Kano, 2009). One of the current methods, of determining fructose and other simple sugars, involves the °Brix test (Cejpek, 2012; Kawahigashi, Kasuga, Okuizumi, & Hiradate, 2013), which is based on refractometry; this provides the percentage of total dissolved solids present in the liquid sample. As this method involves refractive index measurements, alcohol can have a detrimental effect on the result, owing to the difference in refractive index between alcohol and water (Dongarea, Buchadeb, & Shaligramca, 2015). An alternative approach is based on Fourier transform infrared spectroscopy (Reru, Wibowo, & Rondonuwu, 2016; Wang, Kliks, Jun, Jackson, & Li, 2010), however this technique is not readily applicable to remote analysis and has a relatively high cost.

An attractive alternative approach, which we decided to explore, involves the development of a simple chronoamperometric assay, based on a screen-printed electrode. This is a low cost method, particularly when carbon materials are used in the fabrication of the electrodes. Screen-printed carbon based sensors have been previously developed by our group for the measurement of a wide variety of analytes, (Hughes, Pemberton, Fielden and Hart, 2016; Hughes et al., 2016). We recently demonstrated the possibility of measuring the sugar galactose, using the enzyme galactose oxidase in conjunction with a screen-printed carbon electrode, modified with the mediator cobalt phthalocyanine (Kanyong, Hughes, Pemberton, Jackson, & Hart, 2016; Kanyong, Pemberton, Jackson, & Hart, 2013). In another paper, we demonstrated the possibility of developing a biosensor for the measurement of glutamate, in a food sample using the enzyme glutamate dehydrogenase integrated with a screen printed carbon electrode. It was possible to carry out the analysis of commercial OXO cubes, after a very simple dissolution and dilution step (Hughes, Pemberton, Fielden, & Hart, 2015).

* Corresponding author.
E-mail addresses: philip@gwent.org (P. Nicholas), john.hart@uwe.ac.uk (J.P. Hart).

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Consequently, we decided to explore the possibility of developing a simple electrochemical sensor system, for the measurement of fructose in food samples, based on screen-printed carbon electrodes (SPCEs) in conjunction with fructose dehydrogenase. As the incorporation of nanoparticles in the chronoamperometric measurement of glutamate proved to be advantageous, we decided to investigate a novel nanomaterial in the present study.

This paper describes the optimization of the components and operating conditions, of a chronoamperometric assay for fructose; this incorporated fructose dehydrogenase with a nanoparticle modified screen-printed electrode. The possibility of measuring the sugar, in a commercial fruit juice, will be discussed.

2. Experimental

2.1. Chemical reagents

D-Fructose dehydrogenase was obtained from Toyobo Enzymes (Japan). (www.toyobo-global.com).

The graphite-nanoparticles (graphite modified with carboxylic acid) in solution (C2131210D1) were obtained from Gwent Electronic Materials. (www.gwent.org).

Apple juice was obtained from a local supermarket.

All other chemicals and reagents were obtained from Sigma-Aldrich (UK). (www.sigmaaldrich.com).

McIlvaine buffer was prepared by mixing 0.2 M citric acid (containing 0.2 M KCl) with 0.4 M disodium phosphate (containing 0.2 M KCl) to produce a final pH of 4.5.

2.2. Apparatus and instrumentation

All electrochemical measurements were conducted with a two-electrode system, consisting of a screen-printed working electrode (*GEM* code: C2030519P4), Ag/AgCl reference electrode (*GEM* Product Code: C61003P7) both screen-printed onto valox (a semi-crystalline material based on polybutylene terephthalate and polyethylene terephthalate polymers; Cadillac Plastics Swindon, UK). The diameter (6 mm) of the working electrode was defined using a dielectric ink (*GEM* Product Code: D2070423P5) a concentric silver/silver-chloride served as the counter/reference electrode, (*GEM* Electrode Design: BE2110916D1). For further studies, the surface of the working electrode was modified by addition of 10 µl of graphite-nanoparticles (1.787 mg ml^{-1}) (*GEM* code: C2131210D1).

The working and reference electrodes were connected to the potentiostat with *GEM* electrode connector (*GEM* Code: CON002). All electrochemical studies were performed using an AutoLab [µAutoLab Type II], with General-Purpose Electrochemical Software (The Netherlands). Data were further analyzed with Microsoft Excel.

Fig. 1 summarises the fabrication and operation of the fructose biosensor.

It should be noted that during the fabrication of the nano-particle modified electrodes, the deposited nano-particles were confined to the working area by the hydrophilicity of the carbon and the hydrophobicity of the underlying valox substrate. This ensured that the working area remained the same between the unmodified and modified working electrodes and was confirmed by visual inspections.

2.3. Procedures

Cyclic voltammetry was performed by depositing a 300 µl aliquot of 0.5 mM ferricyanide, in 0.1 M phosphate buffer pH 7.5 containing 0.1 M potassium chloride onto the surface of the screen-printed carbon electrodes. Cyclic voltammetry was performed using the following conditions: initial and final potential +0.8 V; switching potential −0.4 V; scan rate 10 mV s^{-1}. Potential held at +0.8 V for 20 s before initial cycle.

Calibration studies were performed using chronoamperometry with standard solutions of fructose, over the concentration range 0.20–32.00 mM, in water; FDH was dissolved in McIlvaine buffer to produce concentrations of either 50 U ml^{-1} or 200 U ml^{-1}. The measurement procedure involved the deposition of 20 µl of either enzyme solution, onto the screen printed transducer, followed by 10 µl of 12 mM ferricyanide and 10 µl fructose standard. Following an incubation time of 180 s (open circuit), with initial 20 s of agitation, the potential was stepped from open circuit to +0.3 V vs Ag/AgCl. Currents were measured 20 s after application of the voltage and these values were used to plot calibration graphs.

2.4. Analytical application

A preliminary study was performed with the commercial apple juice, to deduce an appropriate dilution procedure. A series of dilute apple juice solutions were prepared by mixing the neat sample with deionized water to produce final dilutions in the range of 1/2 and 1/512, of the original concentration. The analysis was carried out using chronoamperometry as described above, and from the results the optimum dilution that produced a signal within the linear range was deduced.

The method of standard addition was performed with the optimum dilution of the apple juice (with deionized water). This was achieved by mixing the diluted apple juice with different concentration fructose standards, so that the final concentration of the standard added was between 0.1 and 0.8 mM. This procedure was repeated 5 times, with each data point being replicated 5 times. The concentration of fructose in the original sample was obtained from this data, together with the precision of the measurements.

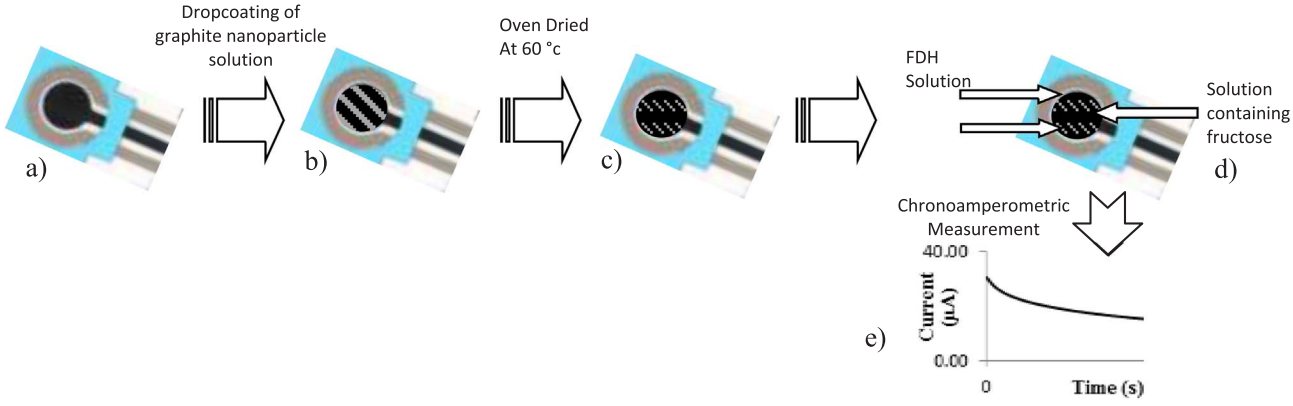

Fig. 1. Scheme showing the fabrication of the fructose biosensor and chronoamperometric measurement of fructose: a) Plain SPCE; b) SPCE with deposition of nanoparticles in solution; c) SPCE with dried nanoparticles; d) addition of 10 µl FDH, 10 µl ferricyanide; 20 µl of solution containing fructose; e) Chronoamperometric measurement.