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Electrochemical Detection in Microfluidic Devices

W. A. Collier and A. L. Hart
Dairy Science & Technology
AgResearch, Palmerston North, New Zealand
alan.hart@agresearch.co.nz

Abstract

Screen-printing was used to incorporate electrodes into a channel of a microfluidic device. These enabled, through the electrochemical oxidation of hydrogen peroxide, the determination of glucose which had been added to milk pre-treated by ultrafiltration. Screen-printing is a relatively simple, inexpensive technique and its use to add electrochemical detection to microfluidic devices is a step towards convenient chemical analysis at point of need.

Keywords: microfluidics, laminar flow, glucose, electrochemical detection, screen-printing, milk

1 Introduction

There is a demand for rapid chemical analyses in small or miniature formats at point-of-need. These formats offer speed of analysis, low usage of reagents and, where relevant, low levels of hazard compared to reactions on a larger scale. Chemical sensors in which analyte recognition and transduction are combined in a single device is one such format. Another format is microfluidics where homogeneous analytical reactions occur in small fluid-filled channels, often under conditions of laminar flow. ("Lab-on-a-chip" is a closely allied approach.)

Development of microfluidic devices has often used techniques based on silicon lithography from the semi-conductor industry but these are expensive and not always readily available. Various alternatives to fabricating structures from silicon have been suggested. One of these, "microfluidic tectonics" [1], entails making structures in plastic by placing monomers in a small mould on a microscope slide and polymerizing them by exposure to UV light. The devices are very easily and quickly built and the equipment and chemicals required are not expensive. The devices and the channels within them are not as small as can be made in silicon or polydimethylsiloxane, for example, but can be regarded as microfluidic devices as the Reynold's numbers for the flow in the channels are appropiately low and flow is laminar.

Analytical reactions carried out in microfluidic devices have often taken advantage of variation in diffusivity among chemical species to condition samples and bring reactants together [2, 3, 4, 5]. In an H-filter [2], for example, two laminar streams flow side by side. Particles which differ in their diffusivity will differ in the degree to which they move from one stream (donor) to the other (acceptor) thus allowing analyte molecules to be sorted from slower particles making up the sample matrix.

A further requirement for fully integrated devices is a means of detection of the endpoint of the analytical process. Optical detection, particularly detection of fluorescence, is one option, as is electrochemical detection [6]. Techniques of varying complexity have been used to incorporate electrochemical detection into microfluidic devices eg electroless deposition [7, 8], plasma etching [9], metal sputtering [10, 11], vapour deposition of carbon followed by dry etching [12] and micro-moulding with carbon ink and polydimethylsiloxane [13]. Screen-printing has been used to prepare electrodes for detection external to microfluidic devices [14]. Electrodes produced by screen-printing with inks cured at low temperature (the case here) are somewhat thicker (c. 20 - 40 μm) than dimensions found in many microfluidic devices but appeared to be compatible with the channel width and depth (e.g. 1 mm x 250 μm) of the devices used here. It is also a relatively simple, inexpensive technique [15]. The practicality of using screen-printing to place electrodes in the channels of tectonic devices was investigated. As a demonstration of electrochemical detection, the electrodes were used to monitor an enzymatic assay for glucose, in which glucose was extracted from a donor into an acceptor stream by diffusion.

2 Materials and Methods

2.1 Device construction

Microfluidic devices in the form of an H-filter were constructed after the methods described in [1]. The backing was removed from self-adhesive 22 x 44 mm HybriWell™ hybridisation chambers with a depth of 250μm (HBW2240, Grace Bio-Labs, USA) into which 1 mm holes had been punched for fluid

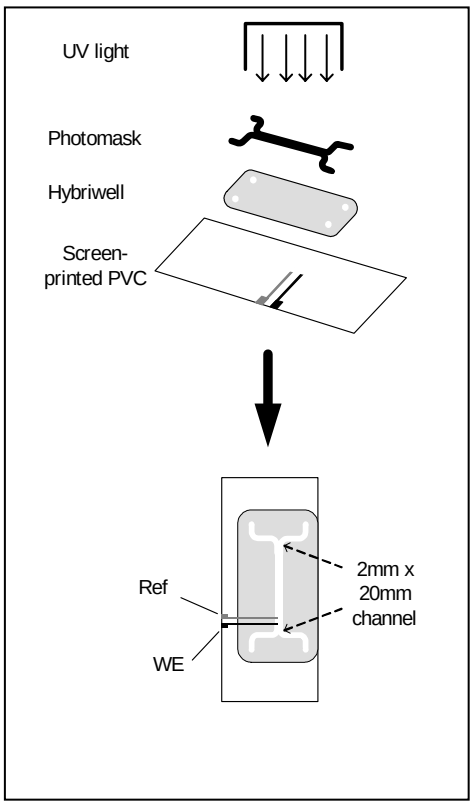


Figure 1: Schematic diagram illustrating the arrangement of parts during fabrication, and in the finished device. Ref: reference electrode; WE: working electrode.

connections (Figure 1). The chambers were attached to PVC bases onto which working and reference electrodes had been screen-printed so that the electrodes would end just before mid-channel in the completed device. The electrode tracks extended out beyond the chamber for connection to electrochemical equipment. The PVC bases were attached to glass microscope slides using double sided tape to stabilise the devices and to ensure uniform thickness among them. A pre-polymer matrix of 92% iso-bornyl acrylate (Aldrich), 5% tetraethyleneglycol dimethacrylate (Fluka) and 3% Irgacure 651 photo-initiator (Ciba Specialty Chemicals) was introduced into the HybriWell system through the connection holes using a pipette, a photomask was placed over the HybriWell, and the polymer was cured using a EFOS Acticure UV spot curing system (EXFO, Canada) set on relatively low intensity for 30s. Unpolymerised monomer was removed with air and the channels washed with methanol. Devices were made with main fluid channels 20, 50 or 100 mm in length.

Fluid connections were made by inserting a device into a holder made, in-house, from Perspex. Pressure seals were formed by screwing a barbed connector (Upchurch, USA) onto an O-ring at each entry / exit port. Fluid flow was controlled by a Baby-Bee syringe pump (Bioanalytical Systems, Inc, USA).

2.2 Screen-printing of electrodes

A DEK 245 (DEK Printing Machines Ltd, UK) screen-printer was used to print the electrodes onto the PVC bases which were cleaned with iso-propanol prior to printing. The screens were formed from polymer mesh blocked with light-sensitive emulsion. The working electrode was made from mediated carbon paste (C40511D8, Gwent Electronic Materials Ltd, UK) and the reference from silver / silver chloride (C50228D1, Gwent Electronic Materials Ltd, UK). The inks were cured at 60 C for two hours. The chloride ion concentration in solution was maintained at sufficient levels for reference electrode function. The electrodes were approximately 0.5 mm wide and 1.5 mm apart. They had a small pad at their distal end, and electrical connection was made to a MacLab and potentiostat (ADInstruments, New Zealand) via a PCB edge connector.

2.3 Electrochemical detection

The electrochemical cell was completed by inserting an auxiliary electrode, comprised of a stainless steel needle, into the outlet draining the working and reference electrodes. In experiments to establish that electrochemical detection was possible in these devices, materials were allowed to fill the entire central channel (2 mm wide). The electrodes were exposed to potassium ferrocyanide, catechol and hydrogen peroxide in 0.1 M KCl. Various potentials were applied between the working and reference electrodes; see Figure captions for details.

For the detection of glucose, as a standard solution in Sorensen's buffer [16], pH 7.4, 50 mM KCl, or in a more complex matrix (UF milk), it was introduced in a laminar donor stream (1 mm wide) which ran parallel to a laminar acceptor stream (1 mm wide) running over the electrodes. The acceptor stream contained glucose oxidase (Sigma, USA) dissolved in Sorensen's buffer [16], pH 7.4, 50 mM KCl. Three replicate determinations were carried out in the same device. Hydrogen peroxide arising from enzymatic action was detected at an applied potential of 600mV (vs. Ag/AgCl).

In order to provide a reference point for the enzymatic detection of glucose, the components of the enzyme assay dissolved in buffer were mixed together and then pumped into the entire channel and the current recorded.

2.4 Preparation of milk samples

Homogenised milk was passed through a Microza ACP-0053 laboratory module filter (Pall Ultrafine Group, UK) with a molecular weight cut off of 13,000 Dalton at an outlet pressure of approximately 12 psi. Eluate was used as sample material without further treatment except for being spiked with glucose. (Milk does not normally contain significant levels of glucose but it was used here as a convenient analyte.)

3 Results and Discussion

Currents which increased with substrate concentration were obtained when catechol and ferrocyanide solutions were introduced into the device indicating that a complete electrical circuit had been formed and electrochemical oxidations had occurred (Figure 2).

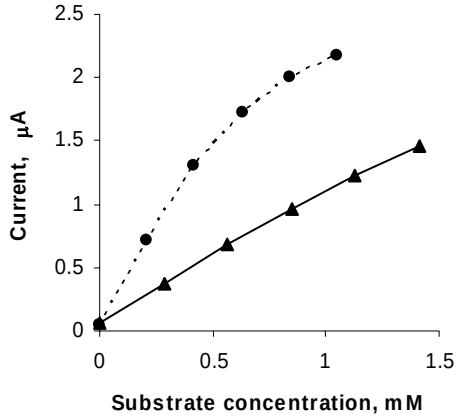


Figure 2: Currents generated at working electrodes by a flow (50µl. min^{-1}) of various concentrations of catechol (●) or ferricyanide (▲)in 0.1 M KCl. E_a = 600 mV vs. Ag/ AgCl. Channel length = 20 mm. Individual curves generated within the same device.

The devices were also found capable of responding to hydrogen peroxide, a product of glucose oxidase activity. (Figure 3a). A hydrodynamic voltammogram was generated, confirming that 600 mV was a reasonable applied potential to use for hydrogen peroxide detection (Figure 3b).

In devices of this kind there will be an effect of flow rate on current. Where the substrate was spread evenly throughout the channel, current increased with flow rate (Figure 4a) and the response time was inversely proportional to the flow rate (Figure 4b). Response time was defined as the time between the first response of the electrode

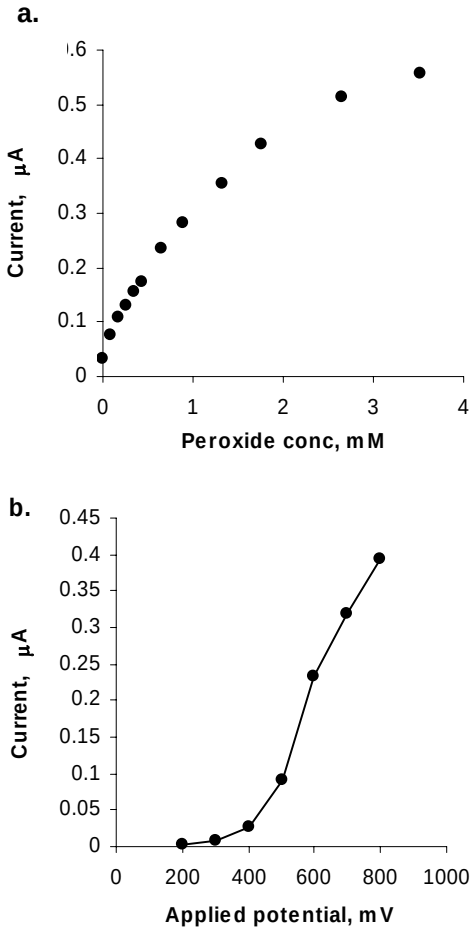


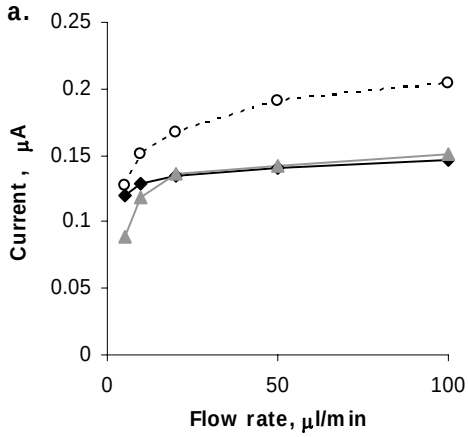
Figure 3: Currents generated **a.** by various concentrations of hydrogen peroxide in 0.1 M KCl; E_a = 600 mV vs. Ag/ AgCl, and **b.** by 1 mM hydrogen peroxide at various applied potentials. Channel length = 20 mm. Flow rate (a & b) = 50 µl.min^{-1}.

to the substrate and the subsequent steady-state current. When glucose was placed in the donor stream (the one not containing the electrodes) it was detected by glucose oxidase in the acceptor stream (Figure 5), having moved there by diffusion.

The characteristic diffusion distance, λ_b, for a particle moving from one laminar flow to the other in the devices is given by

$$\lambda_b = \sqrt{D_f H / v}$$

where D_f = diffusion coefficient, H = channel width and v = linear flow rate [2]
so, an interaction between diffusion, channel width and length and flow rate in determining current can be expected. Figure 5 illustrates the interaction between channel length, (volumetric) flow rate and diffusion. In this case (c.f. Figure 4) currents declined as flow rate increased, as the glucose arriving by diffusion, and the hydrogen peroxide subsequently generated,



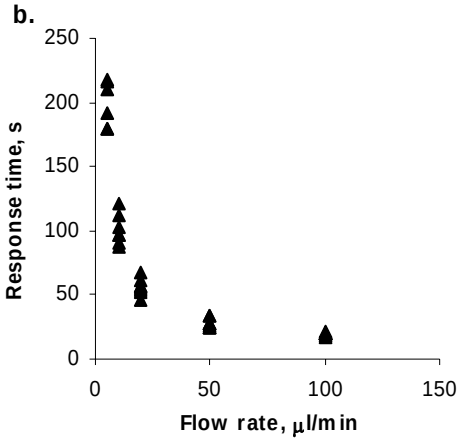


Figure 4 **a.** Currents generated by1 mM hydrogen peroxide in 0.1 M KCl at various flow rates. The curves are from replicates of devices, channel length = 20 mm.
b. Relationship between flow rate and response time for the devices in **a.**

were swept away from the working electrode. Currents increased with channel length as more time was available for glucose to diffuse to the acceptor channel, and the residence time of the peroxide would have been greater. Maximum currents were obtained at the lowest flow rate and greatest channel length.

Results for one replicate determination of glucose in UF milk are shown in Figure 6. The linear range for the analysis where the reagents were mixed before being pumped into the device was the same as that where the determinations were carried out entirely in the device. This is a reflection of the Michaelis-Menton constant for the enzyme. The lower slope of the relationship between current and concentration for determinations carried out

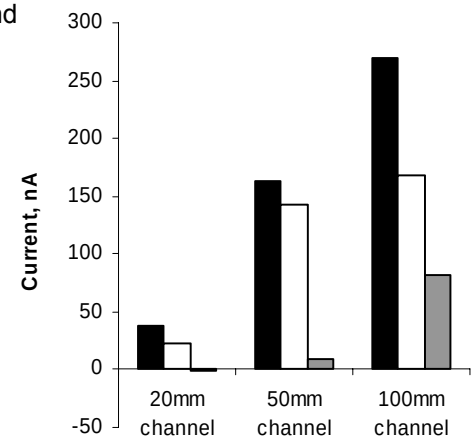


Figure 5: The detection of glucose (10 mM) in the acceptor stream of a microfluidic device. Currents generated in streams of various lengths, indicated on the X-axis, and at various flow rates: ■ 20 µl.min^{-1}; □ 40µl.min^{-1}; ■ 100 µl.min^{-1}. E_a = 600 mV vs. Ag/AgCl.

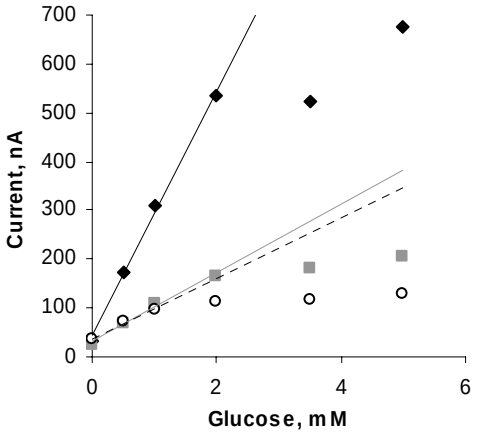


Figure 6: The relationship between current and glucose concentration in buffer and UF milk in an H-filter. Channel length = 100 mm.Flow rate = 20 µl.min^{-1}. ♦ detection reagents and glucose mixed before being placed in device; ■ glucose in buffer in donor stream; o glucose in spiked UF milk in donor stream (dashed line).

entirely in the channel can be attributed to the requirement for diffusion to move glucose from the donor to the acceptor stream. (Results for the second replicate were essentially identical; the third replicate showed a lower linear range than the other two for analyses carried out entirely in the device). Where more time is available for diffusion e.g. a longer channel length, currents arising from detection in the channel could be expected to approach those seen with pre-mixed material.

Detection by electrochemistry is always liable to suffer from interference by electroactive compounds that can be oxidized (or reduced) at the working electrode. The similarity between the currents generated by calibrant and by spiked UF milk suggests that very little if any electrochemical interference occurred. Analysis of more complex matrices may require further development of the electrodes such as the addition of size exclusion membranes.

A relatively inexpensive fabrication technique, screen-printing, was used to add electro-chemical detection to a device which was also inexpensive and easy to construct. Controlled laminar flow and diffusion were exploited to achieve a chemical analysis.

4 Acknowledgements

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5 References

[1] Beebe, D., Moore, J. S., Yu, Q., Liu, R. H., Kraft, M. L., Jo, B-H., Devadoss, C. "Microfluidic tectonics: a comprehensive construction platform for microfluidic systems", *Proc. Natl. Acad. Sci.* 97: 13488-13493 (2000).

[2] Brody, J. P., Yager, P. "Diffusion-based extraction in a microfabricated device", Sensors and Actuators A 58: 13-18 (1997).

[3] Kamholz, A. E., Weigl, B. H., Finlayson, B. A., Yager, P. "Quantitative analysis ofmolecular interaction in a microfluidic channel: the T-sensor", Analytical Chemistry 71: 5340-5347 (1999).

[4] Sato, K., Hibara, A., Tokeshi, M., Hisamoto, H., Kitamori, T. "Integration of chemical and biochemical analysis systems into a glass microchip", Analytical Sciences 19: 15-22 (2003).

[5] Weigl, B. H., Yager, P. "Microfluidic diffusion-based separation and detection", Science 283: 346-347 (1999).

[6] Wang, J. "On-chip enzymatic assays." Electrophoresis 23: 713-718 (2002).

[7] Kenis, P. J. A., Ismagilov, R. F. "Microfabrication inside capillaries using multiphase laminar flow patterning", Science 285: 83-85 (1999).

[8] Wang, J. Chen, G., Chatrathi, M. P. "Nickel amperometric detector prepared by electroless deposition for microchip electrophoretic measurement of alcohols and sugars", Electroanalysis 16: 1603-1608 (2004).

[9] Rossier, J., F. Reymond, Michel, P. E. "Polymer microfluidic chips for electrochemical and biochemical analysis", Electrophoresis 23: 858-867 (2004).

[10] Ko, J. S., Yoon, H. C., Yang, H., Pyo, H-B., Chung, K. H., Kim, S. J., Kim, Y. T. "A polymer-based microfluidic device for immunosensing biochips", Lab Chip 3: 106-113 (2003).

[11] Lacher, N. A., Lunte, S. M., Martin, R. S. "Development of a microfabricated palladium decoupler/ electrochemical detector for microchip capillary electrophoresis using a hybrid glass/ poly(dimethylsiloxane) device", Analytical Chemistry 76: 2482-2491 (2004).

[12] Hayashi, K., Iwasaki, Y., Kurita, R., Sunagawa, K., Niwa, O. "On-line microfluidic sensor integrated with a micro array electrode and enzyme-modified pre-reactor for the real-time monitoring of blood catecholamine", Electrochemistry Communications 5: 1037-1042 (2003).

[13] Kovarik, M. L., Torrence, N. J., Spence, D. M., Martin, R. S. "Fabrication of carbon microelectrodes with a micromolding technique and their use in microchip-based flow analyses", Analyst 129: 400-405 (2004).

[14] Wang, J., Tian, B. M., Sahlin, E. "Micromachined electrophoresis chips with thick-film electrochemical detectors", Analytical Chemistry 71: 5436-5440 (1999).

[15] Atkinson, J. "Screen printing: The art of thick-film sensors" In: Sensors: Technology, Systems and Applications, ed. Grattan, K. T. V., Adam Hilger, Bristol, pp. 541-546 (1991)

[16] Whittaker, M. "Cholinesterases" In: Methods of Enzymatic Analysis, ed. H. U. Bergmeyer, Verlag Chemie, Weinheim, Vol. IV, pp. 52-74 (1984).