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A Fill-and-Flow Biosensor

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An alternative design for an amperometric biosensor for lactate is described in which the analyte flows through a rectangular duct without the aid of external pumping or a flow system. With this deviation from the traditional "stacked layers" of the biosensor geometry, the biorecognition matrix is located upstream of the detector electrode rather than directly over the electrode; this general design is reminiscent of the "dipstick" technology of the home test kits, but in the latter case the "flow" is a function of the nature of the wicking material rather than the dimensions of the channel. A droplet of analyte, L-lactate, is placed in the inlet well of the channel which flows through the cell and reacts with an immobilized lactate oxidase surface matrix zone, and the coproduct, hydrogen peroxide, is swept downstream to the monitoring electrode, where it is detected. The flow through the channel biosensor is shown to be laminar, and the biosensor response varies with the rate of flow of the analyte, which could be controlled by the outlet porosity; the sensitivity was observed to increase, but the dynamic range decreased with an increase in flow rate until a threshold loading, where the biosensor response became independent of the enzyme loading.

The most popular description of a biosensor involves a planar geometry in which the biorecognition matrix is maintained in contact with and over the signal transducer.[1−4] For an enzyme electrode, such a geometry has been shown theoretically to be very sensitive to the thickness of the biorecognition layer.[5−8] This sensitivity of the immobilization matrix thickness is a crucial parameter in manufacturing reproducible biosensors and can become a major barrier for their transition from the research laboratory to the marketplace. The precision to which the thickness of enzyme layers can be cast onto electrodes is commonly insufficient to avoid significant variation in response between sensors.[4] It has been shown that a 10% error (±0.2 μm) in the thickness of a 2-μm biorecognition matrix resulted in a variable substrate concentration-dependent error of up to 10% in the detected current.[5] None of the current methods of applying polymer layers can manufacture biorecognition layers with greater precision than the 0.2 μm error of this example, and thus the attainable reproducibility of biosensor manufacture is severely limited. Many analytical applications do not require a result with greater precision than this, but, in cases where such errors in the analysis would result in a totally different interpretation or action, this is an obvious problem. However, the quest to improve the classical biosensor, to achieve greater precision, is likely to be fruitless without better manufacturing control.

One strategy to overcome this problem is to redesign the enzyme electrode geometry such that the response is independent of the thickness of the biorecognition matrix, but without the realization of the theoretical limitations of the popular designs; this approach has received scant attention. Gooding and Hall[9,10] have presented a biosensor geometry for oxidase enzymes where a permeable electrode was positioned on the solution side of the biorecognition polymer so that analyte diffused through the electrode and reacted with the enzyme, and the product of the enzyme reaction, reduced mediator, diffused back to the electrode to be detected. Such a geometry was shown, both theoretically and experimentally, to produce a response independent of the thickness of the enzyme layer once a threshold thickness was exceeded. However, this threshold thickness is greater than the traditional enzyme layers and results in response times which are prohibitively slow for many commercial applications, although it was ideal for the time-averaged workspace monitoring for which it was developed.[9,11]

In this paper, a different geometry for a "biosensor" is presented as shown in Figure 1. This departs from the classical biosensor geometry since it places the immobilized reagent(s) adjacent to rather than on top of the transducer; nevertheless, small standalone devices are equally viable. The operation of the system is reminiscent of the "dipstick" technology of the home test kits, but in the latter case the "flow" is a function of the nature of the wicking material rather than the dimensions of the channel. The basic channel geometry has been exploited in analytical flow channels and optimization achieved by adjusting the channel and electrode parameters. For example, an innovative signal enhancement was designed by adjusting the channel height and including

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(1) Hall, E. A. H. *Biosensors*; Open University Press: Buckingham, UK, 1990.
(2) *Biosensors: Fundamentals and Applications*; Oxford University Press: Oxford, UK, 1987.
(3) *Biosensors: A Practical Approach*; IRL Press: Oxford, UK, 1990.
(4) Hall, E. A. H.; Gooding, J. J.; Hall, C. E. *Mikrochim. Acta* **1995**, *121*, 119−145.
(5) Gooding, J. J.; Hall, E. A. H. *Electroanalysis* **1996**, *8*, 407−413.
(6) Parker, J. W.; Schwartz, C. S. *Biotechnol. Bioeng.* **1987**, *30*, 724−735.
(7) Leypoldt, J. K.; Gough, D. A. *Anal. Chem.* **1984**, *56*, 2896−2904.
(8) Mell, L. D.; Maloy, J. T. *Anal. Chem.* **1975**, *47*, 299−307.
(9) Gooding, J. J.; Hämmerle, M.; Hall, E. A. H. *Sens. Actuators* **1996**, *B34*, 516−523.
(10) Gooding, J. J.; Hall, E. A. H. *J. Electroanal. Chem.* **1996**, *417*, 25−33.
(11) Hämmerle, M.; Hall, E. A. H.; Cade, N.; Hodgins, D. *Biosens. Bioelectron.* **1996**, *11*, 239−246.

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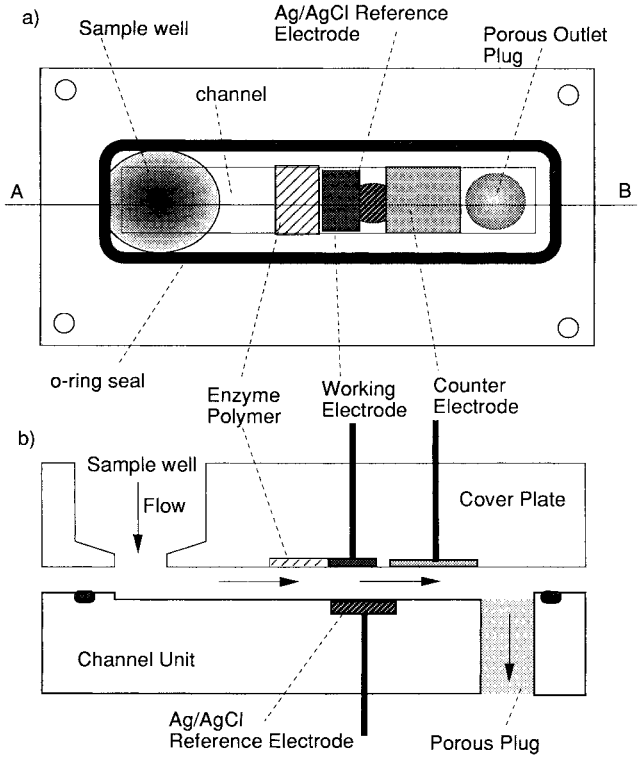

Figure 1. Schematic of the channel biosensor. (a) Top view; (b) section view through cut A−B.

parallel electrodes capable of electrochemical recycling.[12] Furthermore, the location of a reaction zone upstream of the detector electrode can also be compared with channel flow cells utilized by Compton and co-workers[13−17] to investigate heterogeneous reaction kinetics at the interface between a nonconducting solid and a reactant solution. The integrity of kinetic information from this channel cell was ensured by the well-defined laminar flow, with a Poiseuille velocity profile, enabling the calculation of the reaction kinetics from the variation in detector electrode response with flow rate for a given reaction mechanism. However, the assets of this geometry have not been considered previously for application in the analytical environment of biosensors. In fact, the modus operandi of these previous flow systems is different since the biosensor proposed here is not connected with a controlled flow sample reservoir but is developed for a "standalone" finite sample.

Flow through cells which are not standalone but are connected with a pumped flow system have been used for many analytical purposes, particularly in flow injection analysis (FIA)[18,19] and liquid chromatography (LC).[20−22] These flow lines have commonly required the injection of the sample into a flowing carrier stream, often containing reagents, and have incorporated the biorecognition component in an upstream reactor column with the detector in the downstream flow cell. Alternatively, the biorecognition component is immobilized over the transducer within the flow cell, as with the conventional geometry biosensor, and hence the sensitivity of the response to the membrane thickness is still as prevalent as in the classical biosensor. With FIA, the necessity for continuous flow lines, solution reservoirs, and a pumping system generally renders the technique too complex for small disposable devices which can operate at the site of analysis, although some microsystems are now beginning to be developed.

Thus, reports of portable channel cells for biosensor applications have all required pumping or do not include a continuous flow period in their operation.[23−25] The cell of Morris et al.[24] is a capillary fill cell without the continuous flow, whereas Moser et al.[23] have described an on-chip pumped flow cell which contained four enzyme-modified electrodes; with this latter device, the enzyme is immobilized directly over the electrode in the classical thickness susceptible geometry. The flow cell of Murakami et al.[25] is particularly significant, as the biorecognition matrix is immobilized into the entrance of the cell, and the hydrogen peroxide produced by the glucose oxidase reaction with glucose was detected in the flow cell at downstream electrodes. The cell was employed in two formats, either as a component of an FIA or as a disposable self-filling sensor. In the latter format, the cell filled by capillary action, but again the flow stopped once the cell was completely filled.

In fact, these systems do not specifically exploit the ability of the channel flow cell to precisely probe a reaction surface, due to well-defined laminar flow; it is this feature in particular which is attractive, since it is compatible with the requirement for a reproducible biosensor. To achieve this, a biorecognition matrix (e.g., an enzyme layer) would be immobilized upstream of the detector electrode. The flow system would be removed and replaced with an inlet well designed to take a sample of a volume such that it not only fills the channel but also flows through it in a prescribed manner, without pumping or other solution reservoirs. Thus, the detector electrode current will be a function of the analyte concentration, and the QA of the measurement should be ensured by the geometry of the channel. Since the enzyme reaction occurs predominantly at the surface of the biorecognition matrix, the influence of variations in the enzyme layer thickness on the biosensor response would be overcome.

The purpose of this preliminary communication is to demonstrate that such a fill-and-flow biosensor can be constructed with well-defined Poiseuille flow and to illustrate its viability as a biosensor geometry for an L-lactate biosensor employing lactate oxidase.

(12) Weber, S. G.; Purdy, W. C. *Anal. Chem.* **1982**, *54*, 1757−1764.
(13) Gooding, J. J.; Compton, R. G.; Brennan, C. M.; Atherton, J. H. *J. Colloid Interface Sci.* **1996**, *180*, 605−613.
(14) Gooding, J. J.; Coles, B. A.; Compton, R. G. *J. Phys. Chem. B* **1997**, *101*, 175−181.
(15) Gooding, J. J.; Brennan, C. M.; Atherton, J. H.; Coles, B. A.; Compton, R. G. *J. Phys. Chem. B* **1997**, *101*, 182−188.
(16) Unwin, P. R.; Compton, R. G. *Comp. Chem. Kinet.* **1989**, *29*, 173−296.
(17) Compton, R. G.; Harding, M. S.; Atherton, J. H.; Brennan, C. M. *J. Phys. Chem.* **1993**, *97*, 4677−4682.
(18) Hall, E. A. H. *Curr. Opin. Biotechnol.* **1991**, *2*, 9−16.
(19) Schügerl, K.; Hitzmann, B.; Jurgens, H.; Kullick, T.; Ulber, R.; Weigal, B. *Trends Biotechnol.* **1996**, *14*, 21−31.
(20) Wagner, P.; Hegner, M.; Kernen, P.; Zaugg, F.; Semenza, G. *Biophys. J.* **1996**, *70*, 2052−2066.
(21) Weber, S. G.; Purdy, W. C. *J. Electroanal. Chem.* **1980**, *115*, 175−187.
(22) Allison, L. A.; Shoup, R. E. *Anal. Chem.* **1983**, *55*, 8−12.
(23) Moser, I.; Jobst, G.; Aschauer, E.; Srasek, P.; Varaham, M.; Urban, G.; Zanira, V. A.; Tjoutrina, G. Y.; Zharikova, A. V.; Berezov, T. T. *Biosens. Bioelectron.* **1995**, *10*, 527−532.
(24) Morris, N. A.; Cardosi, M. F.; Birch, B. J.; Turner, A. P. F. *Electroanalysis* **1992**, *4*, 1−9.
(25) Murakami, Y.; Uchida, T.; Takeuchi, T.; Tamiya, E.; Karube, I.; Suda, M. *Electroanalysis* **1994**, *6*, 735−739.

EXPERIMENTAL SECTION

Materials. The enzyme lactate oxidase (LOD) from *Pediococcus* species was purchased from Sigma Chemicals (Poole, UK), as was the DL-lactic acid lithium salt (98% purity). The salts potassium chloride (AR grade), potassium dihydrogen orthophosphate (LR grade), dipotassium hydrogen orthophosphate (LR grade), potassium chloride (AR grade), sodium hydroxide (LR grade), and potassium ferricyanide (99% purity) were all from Sigma. The methyl methacrylate, butyl acrylate, and poly(vinyl acetate) (molecular weight 115 000 and 100% hydrolyzed) were from Aldrich (Dorset, UK), as was the initiator α,α′-azoisobutyronitrile (AIBN). The polymer preparation was conducted according to the procedure of Martens and Hall.[26]

All electrochemical experiments employing lactate oxidase were conducted in a 0.05 M phosphate and 0.05 M KCl buffer solution adjusted to pH 7.0 using hydrochloric acid or sodium hydroxide. Aqueous potassium ferricyanide solution were prepared with a background of 0.2 M KCl and 0.1 M NaOH. All solutions was prepared using distilled water.

All experiments were conducted with an EG&G 273A computer-controlled potentiostat with a JJ CR600 $Y-t$ recorder to record current/time traces. Computing was conducted on a 486-66DX personal computer.

Flow Cell. A schematic of the channel biosensor is shown in Figure 1. It was comprised of a channel unit and a coverplate, both of which were constructed from prospect. The channel unit had dimensions of 15 mm × 2 mm. The depth of the channel was ~0.3 mm. Embedded flush with the floor of the channel unit was a Ag/AgCl electrode (Clark Electromedical Instruments, Reading, UK) of diameter 1.5 mm. The channel unit also contained the flow outlet, which contained a porous plug supplied by Sintair Limited (Norfolk, UK). The hydrophilic porous plug was used in the outlet of the channel to draw solution through the channel and also control the flow rate through the channel.

The coverplate contained a well to hold the biorecognition matrix of dimensions 3 mm × 3 mm and depth of 0.1 mm. Downstream of, and directly adjacent to, the well was a flat platinum working electrode constructed from 1-mm-diameter platinum wire (Goodfellows, Cambridge, UK). The working electrode dimensions was 0.96 mm in length and 2 mm in width. The electrode and biorecognition matrix were masked using Teflon tape so as to confine the electrode to at least 0.2 mm from the channel walls. This was done in order to eliminate edge effects.[27] Downstream from the working electrode was a platinum counter electrode of dimensions 4 mm × 1.5 mm. The inlet of the sample reservoir, of volume 200 μL, was also housed in the coverplate. The cell was formed by mating the channel unit and coverplate with mechanical pressure. An O-ring around the channel formed a leak-proof seal.

With biosensing experiments, and some of the flow analysis experiments, the flow cell was used as a self-contained device. The small height difference between the cell inlet and outlet allowed the solution to flow through the cell under the influence of gravity. To determine the channel cell height and characterize the flow profile in the channel over a wide range of flow rates, the channel was connected to a solution reservoir via a flow line. The flow system was constructed of 1.5-mm-id silicone tubing, which connected the flow cell to a 250 cm^3 reservoir. Gravity was used to "pump" the solution through the flow cell, with the flow rate being controlled by the height difference between the reservoir and the cell outlet and additionally by the porosity of the outlet plug.

Biorecognition Matrix. The LOD was immobilized in the emulsion polymer Poly 14, as used by Gooding et al.[28] Aliquots of 5 μL of the mixture of LOD and Poly 14 were placed in the well in the channel biosensor coverplate and allowed to dry overnight. This quantity of enzyme polymer was found, after wetting, to sit flush with the surface of the coverplate. The enzyme loading was altered by varying the mass of LOD mixed with the Poly 14 emulsion.

RESULTS AND DISCUSSION

Channel Characterization. Prior to using the flow cell as a biosensor, the flow characteristics were investigated over a wide range of flow rates by connecting the flow cell to a flow line as described above. It was characterized using an electrochemical redox standard: a solution of 1.2 mM potassium ferricyanide was passed through the cell, and the diffusion-limiting reduction current was monitored as a function of flow rate. For a rectangular duct with laminar flow, a "plug" of solution entering the channel will develop a Poiseuille flow regime (parabolic velocity profile) downstream of the entry point due to the slowing of the solution at the channel walls. The entry length required for this to be established is given by

$$l_e = 0.1hRel \tag{1}$$

where h is the half-channel height and Re the Reynolds number, given by

$$Re = v_o h/v \tag{2}$$

where v is the kinematic viscosity and v_o is the solution viscosity at the center of the channel (related to the volume flow rate by $V_f = 4v_o hd/3$). For the flow rates between 0.001 and 0.02 $cm^3\ s^{-1}$, used for the evaluation of the flow cell, l_e is less than 1 mm. Therefore, the Poiseuille flow profile is established well before the sample solution reaches the electrode or any upstream reaction layer.

If the parabolic profile is approximated to a linear profile close to the electrode, the following relationship between current and flow rate is established from the Levich[29] equation for diffusional flow to the surface of a plate in a flowing fluid:

$$3I_{lim} = 0.925nFWc_\infty\left[\frac{V_f D^2 x_e^{\ 2}}{h^2 d}\right]^{1/3} \tag{3}$$

where I_{lim} is the diffusion-limiting current, n the number of electrons transferred, w the electrode width, c_∞ the bulk concen-

(26) Martens, N.; Hall, E. A. H. *Anal. Chim. Acta* **1994**, *292*, 49−63.
(27) Oldham, K. B. *J. Electroanal. Chem.* **1981**, *122*, 1−17.
(28) Gooding, J. J.; Hall, C. E.; Hall, E. A. H. *Anal. Chim. Acta* **1997**, *349*, 131−141.
(29) Levich, V. G. *Physicochemical Hydrodynamics*; Prentice-Hill: Englewood Cliffs, NJ, 1962.

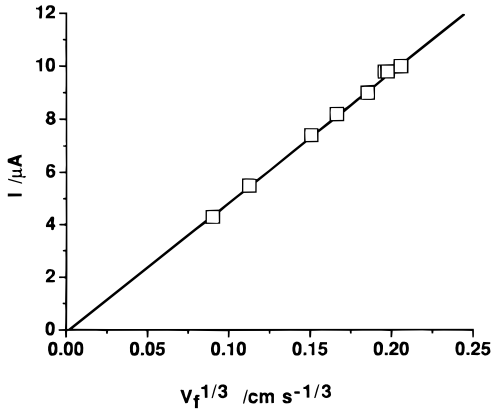

Figure 2. Plot of current versus (flow rate/cm^3 s^{-1})$^{1/3}$ for the channel biosensor, with best line of fit: $R^2 = 0.999$. The diffusion-limited current of a 1.2 mM potassium ferricyanide, 0.2 M KCl, and 0.1 M NaOH solution was measured. The electrode width was 0.2 cm.

tration of the electroactive species, V_f the volume flow rate, D the diffusion coefficient, x_e the electrode length, h the cell half-height, and d the channel width. Therefore, if the flow through the channel biosensor is laminar, then a plot of limiting current versus the cube root of flow rate will be linear, as shown in Figure 2. The gradient of this plot enables the exact calculation of the channel half-height, provided the diffusion coefficient of ferricyanide is known. As the current response of a channel flow cell is very sensitive to the cell height (and hence, so too will be the channel biosensor response), it is important that the flow cell could be assembled with a reproducible channel height. From the data sets shown in Figure 2, and using the reported[30] diffusion coefficient for potassium ferricyanide of 7.6×10^{-6} cm^2 s^{-1}, the channel half-height was calculated from eq 3 to be 0.145 ± 0.005 mm, which would translate into a variation in the current reading of $\pm 1.1\%$.

In the proposed "biosensor" application, this laminar flow channel would not be connected to a flow line, but the flow would be provided by "gravity feed" from the sample placed in the inlet cell. The inlet reservoir of the flow cell had a volume of only 200 μL; therefore, as solution passed through the channel, the volume in the inlet reservoir decreased. This creates a fundamental difference compared with the flow line experiment described above, since a decrease in volume results in a decrease in the channel head height and, therefore, the flow rate through the channel varies with time. The variation in flow rate with time can be characterized by monitoring the diffusion-limiting current of the reduction of ferricyanide with time (Figure 3a). This figure also shows the transition from the "fill" to the "flow", the current increasing during the initial filling stage as the sample passes the electrode and then decreasing as the sample reservoir begins to empty.

From this current, the flow rate can be calculated. At very slow flow rates, the diffusion layer extends to the center of the channel, and the flow cell enters the exhaustive electrolysis regime, where the variation in current becomes linearly proportional with flow rate,[16] with I_{lim}/V_f a constant. The 200-μL sample passes through the channel typically within 20−600 s, depending on the porosity of the outlet plug. From the diffusion-limited

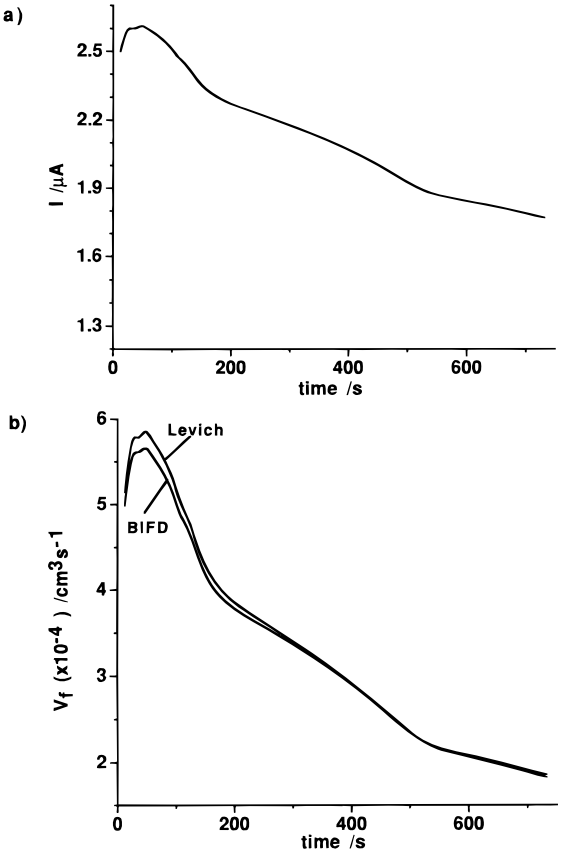

Figure 3. Variation in (a) diffusion-limited current of a 1 mM potassium ferricyanide, 0.2 M KCl, and 0.1 M NaOH solution with time and (b) flow rate with time calculated from (a) according to the Levich relationship and compared with the numerical solution of the convective−diffusion equation for laminar flow, using the backward implicit finite difference (BIFD) method. The outlet plug had a porosity of 10−20 μm, and the electrode width was 0.15 cm.

current flow dependence, as calculated by Anderson and Moldoveanu,[31] exhaustive electrolysis would be anticipated for flow rates below 10 pL s^{-1} in the channel employed here, whereas the flow rates, obtained are some orders of magnitude greater in the range of 1−20 μL s^{-1}. At these faster flow rates the relationship between the current and the cube root of the flow rate holds, and the convective−diffusion equation for laminar flow with the Poiseuille velocity profile can be applied.

The linear Lévêque approximation[32] of the Levich equation has normally been shown to be in good agreement with the more complete solution of the convective−diffusion equation for laminar flow, with the Poiseuille velocity profile for a simple heterogeneous electron-transfer process. However, it has not been tested for such a small-volume sample channel; to confirm whether the linear approximation is valid in this instance, the two approaches were compared. The backward implicit finite difference method employed by Anderson and Moldoveanu[31] and by Compton and co-workers[33] was utilized for this purpose, as this numerical solution can be used over the entire flow rate range. The variation in flow rate with time is shown in Figure 3b for the different

(30) Von Stackelberg, M. V.; Pilgram, M. *Z. Elektrochem.* **1953**, *57*, 342−0.

(31) Anderson, J. L.; Moldoveanu, S. *J. Electroanal. Chem.* **1984**, *179*, 107−117.

(32) Leveque, M. A. *Ann. Mines Mem. Ser.* **1928**, *12*, 201−0.

(33) Compton, R. G.; Pilkington, M. B. G.; Stearn, G. M. *J. Chem. Soc., Faraday Trans. 1* **1988**, *84*, 2155−2171.

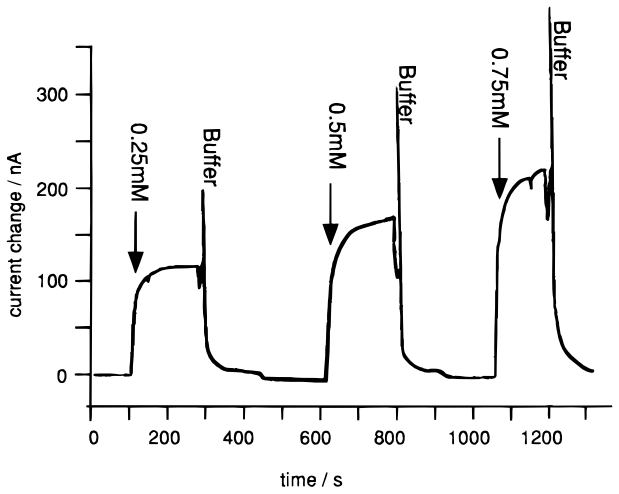

Figure 4. Current−time response curves for additions of lactate for the channel biosensor using lactate oxidase (LOD) immobilized upstream of the electrode. The porous plug was 20−35 μm, the enzyme loading was 25 wt % of LOD to Poly 14 emulsion, and the electrode width was 0.164 cm.

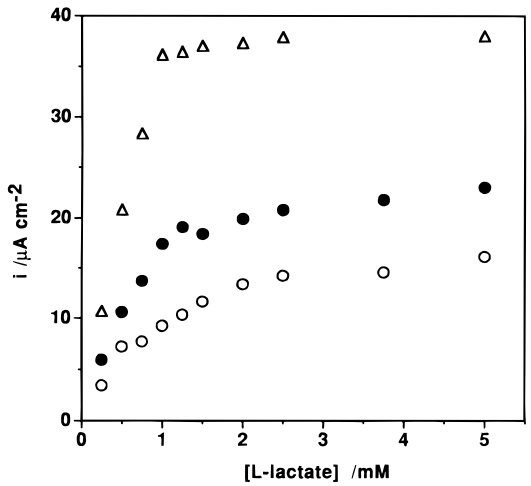

Figure 5. Influence of outlet plug porosity, and hence flow rate, on the lactate oxidase channel biosensor response, where the enzyme loading was 25 wt % of LOD to Poly 14 emulsion and where the plug porosity was (○) 10−20, (●) 20−35, and (△) 40−75 μm. The current densities were recorded 1 min after addition of the analyte.

approaches, and it can be seen that the curves converge completely at longer times and follow each other closely at short times during the "fill" phase. In this initial phase, the differences between the calculated flow rates cannot be directly interpreted in terms of linear versus parabolic profiles, but the good agreement during the "flow" phase confirms the application of the simple linear Lévêque approximation.

Biosensor Characterization. In the channel biosensor, the electroactive species is produced from reaction of the sample with the biorecognition layer upstream of the electrode. The flow dynamics will thus influence both this reaction and the electrode current. The response of the channel biosensor to L-lactate, with a LOD/Poly 14 biorecognition matrix, was determined by making 200-μL additions to the analyte reservoir. A series of example response curves are shown in Figure 4, and it can be seen that the current increases from a baseline and tends toward a maximum despite the decrease in flow rate with time. From Figure 3, this decreasing flow would be expected to be accompanied by a decease in current for a sample stream containing a constant concentration of electroactive species; however, in this instance the electroactive species (hydrogen peroxide here) is not in the sample but results from the reaction with the biorecognition layer (LOD) and at slower flow rates the reaction zone in the vicinity of this layer extends farther into the channel, whereas at faster flow rates there is a greater flux of analyte to the biorecognition matrix. The resulting downstream electrode current, therefore, becomes influenced by the thickness of the zone containing the electroactive product, and the current may become controlled by the enzyme kinetics.

The sensitivity and the dynamic range can thus be varied by changing the flow rate in the channel. With the channel biosensor, the flow rate is altered by changing the porosity of the outlet plug. As can be seen from Figure 5, for a given concentration of L-lactate, the faster the flow rate, the higher the current response. This increased sensitivity, however, is at the cost of a lower dynamic range. As analyte flows past the biorecognition matrix, it reacts with the enzyme, and thus there is a reduction in the concentration of the analyte near the reaction surface. With faster flow rates, further analyte is brought to the reaction surface to replace the consumed L-lactate. Therefore, for a given concentration, more analyte reacts with the enzyme when the flow rate is faster, although the depletion layer is thinner. As a result, the dynamic range is reduced, and the current response is greater. In effect, adjusting the flow rate in the channel biosensor is comparable to altering the membrane covering the classical geometry biosensor; the membrane acts as a partitioning barrier, modulating the concentration of analyte according to its partition coefficient between the sample and membrane. An increased flow rate, or partition coefficient, results in the enzyme being exposed to more analyte and, therefore, saturating at lower analyte concentration.

Under such conditions, the current response would also be expected to be modulated by the enzyme loading. The variation in current density with L-lactate concentration, 1 min after the addition of the analyte solution, is shown in Figure 6 for a variety of different enzyme loadings. These curves show several interesting features: an increase in the enzyme loading results in an increase in the sensitivity of the biosensor until a maximum is reached, and further increase in enzyme loading did not result in further increase in sensitivity or dynamic range. This indicates that the channel biosensor response is no longer limited by the amount of enzyme but by one of the substrates, i.e., the reaction zone has extended across the channel and exhaustive reaction is occurring. In the case of lactate oxidase, the limitation may be caused by either lactate or oxygen depletion. The concentration of dissolved oxygen in the buffer solution has been shown to be 0.2 mM,[34] whereas the lactate concentration is varied up to 8 mM, so that instinctively oxygen is the favored candidate here, but the order of magnitude difference in the diffusion coefficients of the two species means that the limiting substrate is less obvious than a simple comparison of concentrations. Nevertheless, the important result of this condition is that, beyond a threshold enzyme loading small changes in enzyme activity or loading, will not cause a change in the response. It has been shown theoretically [5] for

(34) Gooding, J. J.; Hall, E. A. H. *Biosens. Bioelectron.* **1996**, *11*, 1031−1040.

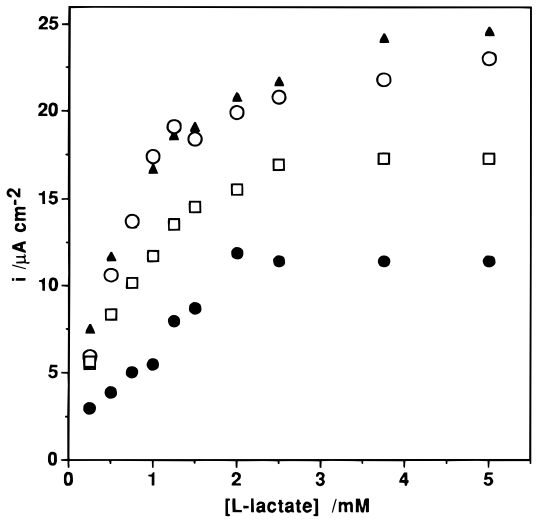

Figure 6. Influence of enzyme loading on the channel biosensor response with a 20−35 μm porosity outlet plug, where (●) is 12.5 wt % of LOD to Poly 14 emulsion, (□) is 19 wt %, (○) is 25 wt %, and (▲) is 50 wt %. The current densities were recorded 1 min after addition of the analyte.

an oxidase enzyme electrode of the conventional geometry, where the response is cosubstrate limited, that increasing the enzyme loading decreases the dynamic range but increases the sensitivity of the biosensor. The insensitivity of the biosensor response to the enzyme loading in this channel design is an advantageous feature for the long-term storage of the device, as loss of enzyme activity will not cause a serious deterioration in the operation of the biosensor.

CONCLUSIONS

The preliminary results from the standalone fill-and-flow channel biosensor indicate that this biosensor geometry has considerable promise. The channel design was shown to follow the simple linear Lévêque approximation for the relationship between current and flow rate, even when a sample of just 200 μL was employed. It was found that the biosensor response could be tuned to a given application by altering either the enzyme loading or the rate at which the analyte flows through the channel. The independence of the biosensor response to enzyme loading, once a threshold loading is exceeded, is a particularly advantageous feature, since loss of enzyme activity will not affect the biosensor response. The biosensor sensitivity can be increased by increasing the flow rate, but this will reduce the dynamic range. Alternatively, a slow flow region of exhaustive electrolysis can be selected, where, used together with the substrate limitation achievable at high enzyme loading, an integrated current response could accommodate sample-to-sample variations in flow character.

This is undoubtedly a difficult system to optimize fully using just experimental manipulations; theoretical development of the design parameters of the channel biosensor will thus be likely to be required in future work. The numerical simulation of the channel biosensor is simplified by the well-defined laminar flow of the channel biosensor and the ability to apply the simple linear approximation to the Poiseuille flow regime. Such modeling can be used to identify the flow rate, enzyme loading, and channel dimensions which will give the best response for a given application. This approach may offer the capability of using a "designer" analytical system which is inherently difficult in the traditional geometry.

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