

Microfluidics a Potent Route to Sample Delivery for Non-intrusive Sensors

George Kyriacou, Hong Chang, Joseph Gargiuli, Ajay Agarwal and Pankaj Vadgama

Abstract Biosensors offer wide opportunities for threat agent analysis, but practical analytical systems require these sensors to be integrated with pre- and post analytical steps to enable simplified, seamless operation. Perhaps the most important of these steps relates to sample handling and presentation. Advances in microfluidics now offer a realistic means for simplified, practical handling with the facility for compressing existing analytical platforms in biosensing. Small volume handling can not only allow for miniaturisation, but flow at this scale enables a different type of flow profile and the a facility for direct liquid-liquid exchange. Basic flow principles in microflow are presented followed by a description of aqueous/organic flows and how they cab be used both for solute partitioning and in situ membrane formation. The potential value of miniaturised separation membranes is described, including for sample cleanup, handling and biosensor protection. Finally, examples of sensor integration into microfluidic structures are given as pointer towards future developments. Overall, the chapter seeks to rebalance the traditional emphasis on biosensor design by highlighting the importance of controlled sample presentation as a potential route to low maintenance biosensors with improved response characteristics.

Keywords Microfluidics · Laminar flow · Liquid-liquid interface · Reynolds number · Electrochemical biosensor · In situ polymer membrane · Sample presentation · Miniaturisation

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1 Introduction

The miniaturisation of sensors has been driven by evermore sophisticated, high precision fabrication platforms available through centralised microelectromechanical foundries. Through this MEMs capability, an ever expanding repertoire of controlled geometry sensor surfaces, high density precision arrays and a kaleidoscope architectures have come about, never thought possible in the early, pioneering days of biosensor development [1, 2]. Much of this is centred on the machining of silicon, but with the advent of polymeric materials technologies, many using silicon as the forming template, it has also been possible to leverage, soft polymer structures in sensor design [3]. Perceived advantages have been the creation of devices that are small enough to be non-intrusive, requiring the smallest of samples and able to be intergrated into larger assay components through mechanical adaptability. Such considerations highlight the fact that a functional sensor is not itself the full answer to an analytical need, but must be a part of a cascade of functions from sampling, sample presentation/separation through to signal processing and presentation. Seamless sensor integration into a system is already well understood in the case of medical monitoring, particularly if measurement is to be undertaken in vivo [4]. However, systems having a small analytical ‘footprint’ for non-intrusive measurement are also needed in other sectors needing extra laboratory testing. This certainly applies to system deployment for bioterrorism/security where practical application demands a versatile capability as regards both location and stand alone operation.

Along with sensor miniaturisation, miniaturised sample presentation is also important; too little attention has been give to the fact that the way in which a sample is presented (dilution, flow, reagent content etc.) can make all the difference between a viable and non-viable system. Clearly, any fluid sample, but especially a liquid, can be controlled across a range of physicochemical variables; beyond sample diluent control, the volume requirement can be controlled, flow rate manipulated, calibrants interposed (one advantage of the early FIA approaches) and critically surface shear and turbulence at the sensing surface manipulated. This last aspect has been shown to be a critical influence not just on analyse flux to a sensor, but on the deposition of colloidal materials likely to foul a sensor surface. This chapter focuses on microfluidics developments as a research field in its own right and then on the way it serve as a sample presentation methodology for miniaturised sensors. As such only a part of the uTAS paradigm is covered; sample prove design and the use of miniaturised sample cleanup and chromatographic components are excluded. However, a sensor and microfluidic combination goes a long way towards underpinning the uTAS ideal.

2 Microfluidics: Basic Features

Analytical structures and devices operating at the micrometer scale are fabricated on either top down approach or bottom up principles, now well established [5]. For top down fabrication, a larger structure is machined without atomic or molecular control to the smaller dimension, while in the bottom up approach atomic scale manipulation is used to build up the larger structure. Whilst this especially applies to nano devices, the principles are the same for micron scale design. The inherent benefit of miniaturisation is the combination with other complex sub-components to create a monolithic structure that is simple in design, ergonomically efficient and appropriate for use by a non-skilled operator [6].

The governing principle around microfluidic sample management is embodied in the Reynolds number (Re). This defines the ratio of inertial versus viscous force during flow:

$$\text{Re} = \frac{DV\rho}{\mu} \quad (1)$$

Re is dimensionless and here, D is channel diameter (m), V is fluid velocity (m/s), ρ is fluid density (kg/m^3) and μ fluid viscosity ($\text{N} \cdot \text{s/m}^2$). In low dimension channels, the viscous force dominates and the tendency is then for turbulent flow to switch over to laminar flow. At approximately <3000 Re turbulent flow is avoided and the key analytical implication is that intermolecular exchange and solute transport are determined by Fick's Laws alone. Indeed, much of microfluidics research is aimed at overcoming the barrier to mixing by attempting to engineer turbulence in miniature flows, e.g. to effect more efficient sample and reagent mixing. However, in the present chapter, the special benefits of non-mixing will be highlighted. A particular flow model of value for sample delivery is that of dual, linear fluid flow. This is relatively easy to generate, though the situation becomes marginally more complicated at the inception of flow convergence, where turbulence and local recirculation, along with eddy currents can combine to create unwanted physical mixing [7].

3 Dual Flow for Mobile Liquid-Liquid Interfaces

The dual flow model has been reported by us, based on bifurcated flow channels with convergence of independent flow at the starting point of a simple linear flow system. (Fig. 1). Various designs have been tested empirically for both the angle of convergence and the method flow drainage [8].

Our flow channels avoided dependence on a MST foundry, and were simply formed by adhesive bonding of cut glass plates, which had the advantage of permitting optical microscopy of flow, not possible with Si and avoided the solvent

incompatibility of transparent polymers (*vide infra*). Ultimately, a single channel drainage design, rather than the dual drainage shown in Fig. 1 proved the most effective and convenient. Figure 2 shows a practical manifestation with a design for an integrated electrochemical wire sensor. What the design allows is for a mobile fluid layer to be sustained between sample and sensor. In this way, a colloid containing or contaminated sample can be separated from the sensor surface avoiding the dangers of surface biofouling and associated sensor drift, permitting calibrant/reagent to be introduced without interruption of the sample flow and permitting modulation of the concentration of solute achieved at the sensor surface [9].

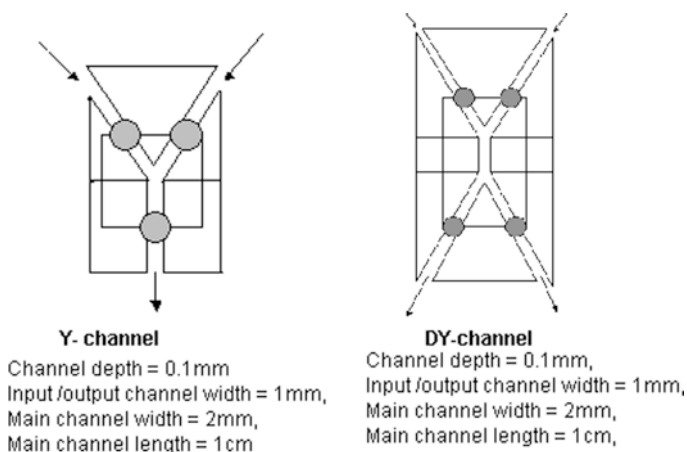


Fig. 1 Glass fabricated flow channels for laminar flow and incorporated distal fluid flow drainage design. The structures are configured to accept parallel fluid flow from micro flow pumps. The *shaded circles* indicate the location of pipette tips positioned vertically for fluid entry from external tubing. The glass construct allows for transillumination and for carriage of organic solvent. (From Ref. [8] with permission)

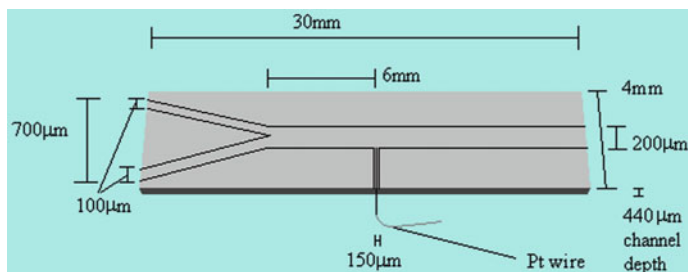


Fig. 2 A flow cell with integrated wire electrode for protecting the sensor tip using a contacting analyte, receiving, flow containing only buffer or reagent, while the non-contacting sample flow stream is kept physically away from the tip, enabling sensor protection from cellular and colloidal components liable to foul the electrode surface

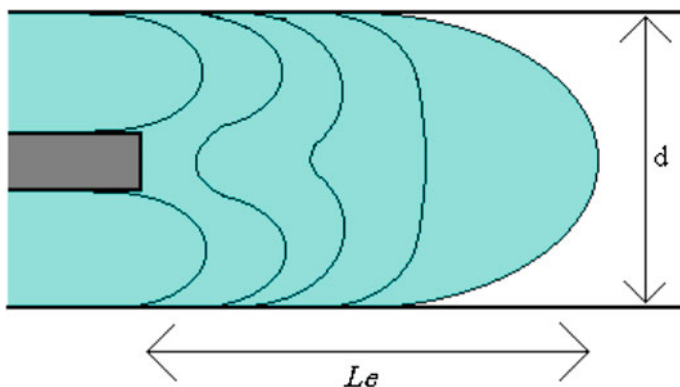


Fig. 3 Flow velocity profile as it develops from the point of dual flow contact on either side of a rectangular splitter palate. The initial curves represent dual parabolic flows, which over distance develop into merged and then a single parabolic flow with maximum velocity at the centre of the channel. The position of the single flow profile is defined as the Langhaar distance (Le)

Detailed consideration of flow and overall design are key to retention of this stable liquid-liquid interface and fluid ‘coating’ over the sensor. Importantly to adhere to laminar flow principles (Eq. 1) it is not necessary to have a micro dimension flow channel. Indeed, the latter has distinct disadvantages in that surface fouling of the channel wall is more likely to lead to channel blocking. At initial convergence of any such flows, there are inevitably two independent parabolic flow profiles, with their highest velocity, as in any pipe flow, at the centre and the lowest flow rate close to the channel wall converging to a no-slip boundary at the channel wall. Subsequently, a single, fully developed flow profile results attained at a channel location defined as the Langhaar distance (Le):

$$Le = 0.058. Re. d \quad (2)$$

where d is the characteristic length of the channel, which for a particular channel flow geometry can be taken as the hydraulic diameter—non-tubular flow. The flow profile convergence is illustrated in Fig. 3. The dimensions of this convergence is important because it determines the type of transport to the sensor surface and the expected response magnitude.

The velocity of flow at the liquid-liquid interface in the middle of the channel is inevitably the same for both liquids and depending upon the time available for their interaction along the channel, Fickian, concentration gradient driven diffusion directs solute transport orthogonally to the flow direction. This can be visualised in Fig. 4, where amylose in one flow solution meets an iodine indicator in the second flow stream. An iodide—amylose complex forms, is readily seen as a line along the length of the fluid interface, but importantly, its formation is determined by the

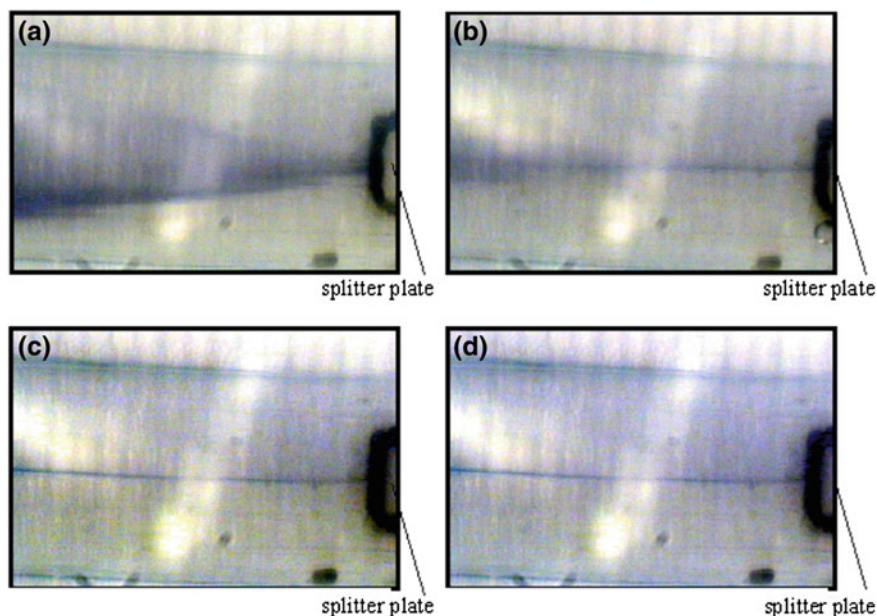


Fig. 4 Digital images of left to right flow near a splitter plate and the creation of flow dependent interaction zones, respectively between amylose loaded and iodide loaded flow streams. Flow is from *right to left*, and shows the interfacially generated amylose-iodide complex, known to be visible at sub-micomolar levels. **a** 10 $\mu\text{l}/\text{min}$ per inlet, **b** 50 $\mu\text{l}/\text{min}$ per inlet, **c** 100 $\mu\text{l}/\text{min}$ per inlet, **d** 999 $\mu\text{l}/\text{min}$ per inlet

degree of contact at the interface, dictated by the flow rate; higher flow permits only a reaction at the interface and slow flow permits a widening interaction to give a pyramidal zone of extended diffusive cross channel interactions.

4 Transport Phenomena Under Flow Conditions

The protective principle of dual laminar protective flow, described above, rests on the fact that surface active fouling agents are largely colloidal and cellular, whilst molecules of interest are typically micro solutes. The diffusion coefficients of a micro solute, being at least an order of magnitude greater than the effective diffusion value for a colloid or cell ensures that just a finite liquid layer forms a screen for protecting the sensor surface. Of course, part of the protective feature relates to the fact that the liquid layer is mobile and so a laterally diffusion colloid/cell is swept along the channel before reaching the sensor, so access to the sensing surface (Fig. 2) is much greater than that a fouling agent. The location of an intramural sensor along the flow channel is thus of importance because there is the balance of the increased time available for lateral transport of target molecule versus that of a

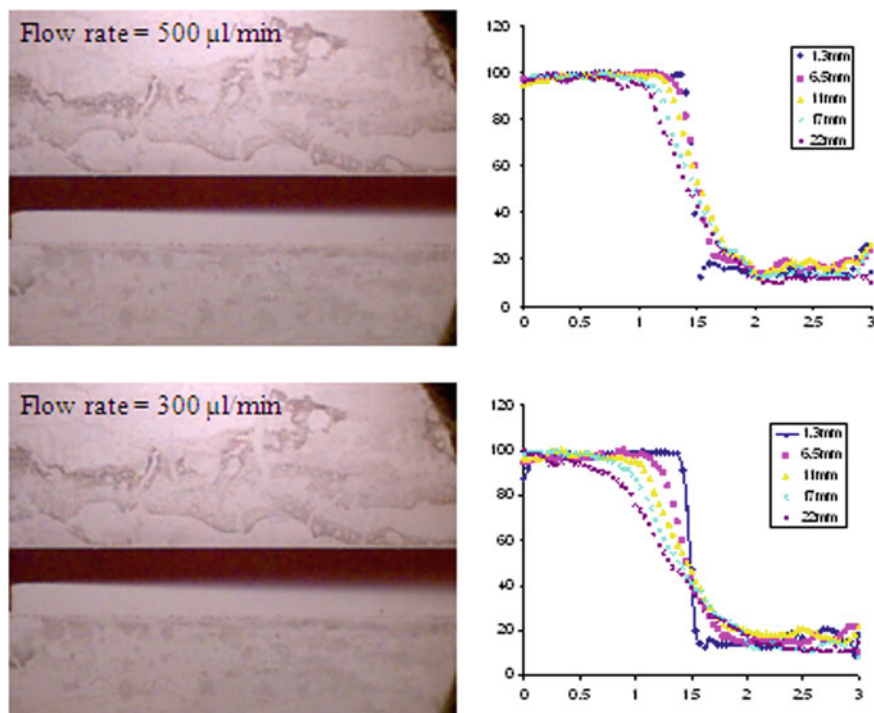


Fig. 5 A cross-channel densitometry analysis showing the location of the intense colour of permanganate solution as permanganate from such a solution diffuses laterally across the direction of flow into an aqueous receiving stream. The image represents a stop-flow experiment with loss of the sharp liquid-liquid interface as the permanganate diffuses across. Quantitative profiling was achieved by *grey* scale imaging. The horizontal axes is in mm and the key refers to distance along the flow channel

colloid, and so the sensor should not be too far down the channel, whilst positioning too near the flow entry means it is within the Languor distance. A quantitative evaluation of the diffusion process for a micro solute was undertaken using permanganate solution with stopped flow (Fig. 5); the concentration drop to the non-permanganate side becomes less distinct over time as lateral diffusion progresses; this applies to both micro- and macro-solutes, and of course the concentration profile itself offers a route to determining the diffusion coefficient value of a solute within a given sample matrix.

Laminar flow has been exploited in other sectors. Thus, in the case of electrochemical fuel cell design [10], non-mixing fuel and oxidant streams have been used in order to avoid the need for a membrane separator. Proposed advantages parallel those for sensing; a single channel simplifies packaging and sealing requirement, problems of membrane damage are avoided, fouling and permeability issues cease to be a worry and there is greater compatibility with Lab on a Chip design with elimination of one structural component. Arguably, whatever the advantages

regarding the non-fouling liquid-liquid interface, it may still be necessary to enhance solute transport to an intramural sensor. This has been proposed, variously, through the use of additional, multiple inlets to provide additional solute sources, to re-supply zones of depletion, drainage of depleted solution via multiple outlets and a creation of angular ridges along the flow channel to enhance solute supply from central flow via advection [11]. Also, solute residence time at any particular location in the cross-section of a channel is not just a function of flow velocity, but affected by channel geometry. If the inter-diffusive distance is small compared with the channel height, molecules at the top and bottom of the channel have longer for inter diffusion because as with any wall proximity, there is a much slower flow velocity at the top and bottom wall margins. This gives the typical ‘butterfly’ distribution of concentrations in the cross-section of a channel [12].

5 Electrophoretic Field Effects

For enhancing an electrochemical reaction itself, it has been shown in the case of the glucose biofuel cell that the reaction depletion layer at the electrode surface can be reduced by reducing the electrode surface length along the length of a micro flow channel [13]. The depth of the depletion zone over an electrode can also be reduced by dividing up an electrode surface to create spaced a array of smaller electrodes [14], in one instance with a separation that was $\times 3$ the length of electrode length a distinct enhanced power density was achieved at a microbiofuel cell [15]. This type of realignment will have exactly the same advantage for sensing, and may contribute to accessing low target molecule levels.

The differential diffusion of proteins and other solutes has been used as a means for their purification [16]. If the receiving stream in a dual flow channel has an affinity for these proteins, as has been observed in the case of a system for cell membrane proteins, then both an enhancement of concentration and purification by the receiving stream becomes possible [17]. This principle could be adapted to low level micro solute monitoring if the appropriate partitioning interface can be provided for the receiving stream.

Lateral application of an electric field induces electrophoretic transport of large charged molecules [18, 19], either as a basis for removing them from the assay sample or for increasing their concentration; this has analogy with field flow fractionation (FFF). A typical technical arrangement involves electrodes recessed behind a gel phase facing the channel, thereby avoiding flow disturbance at the electrode whilst achieving electrical connectivity to the sample. Quite a dense, narrowed, highly localised distribution of a mobile protein phase can thereby be achieved. This topic has been considered recently [20] and may have value for sample manipulation during sensor based monitoring. Such driven solute transfer between two distinct solvent phases may be asymmetric, thus transport from a PEG solution to a dextran receiving stream occurs readily, but that in the reverse direction is constrained and non-linear,

showing threshold effects [19]. In this context, the lateral transfer of protein was not seen to be pH, and therefore charge, dependent, but partition coefficients seemed to be important and a developed interfacial double layer at the liquid-liquid boundary will affect cross-phase mobility. A phosphate buffer was described as generating a high double layer potential leading to accumulation of protein at the interface whilst a Tris combination with BES buffer lowered the interfacial potential and thereby protein distribution [21, 22]. A rather different situation occurs with DNA fragments. Here, an apparent energy minimum is attained at the liquid interface, promoting electrostatically driven adsorption via an electrophoretic field. The complexity of the phenomenon is indicated by the promoted detachment of DNA fragments with increase of the electrical field gradient; this appeared to be thermally promoted, with detachment of fragments occurring in a size dependent manner. It appears that the local electrostatic field strength is substantially greater than any applied field, but the latter affects the local field by both a narrowing the thermodynamic potential energy well and a reduction of the height of the barrier to thermal displacement [23]. The much higher diffusivity of a protein versus DNA also, potentially, will help to separate these components for measurement and such dual liquid components could additionally allowing some size based discrimination of DNA prior to sensing.

6 Cell Resolution

When red and white cells from blood are brought into contact with a flowing liquid-liquid interface, the red cells tend to diffuse out into the bulk phase whereas the white cells are retained because of their larger size and lower mobility; the energy minimum experienced by the cells is also a factor and is determined by cell surface area, greater for white cells [24, 25]. Discrimination is also described in general between different nucleated cell types and between live/dead cells [26, 27]. So cell loaded samples can be manipulated in microfluidics for say accessing cell sub-types for toxin measurement or for avoiding cell and sensor contact.

7 Dual Flow Heterogeneity

A dual flow that involves high and low viscosity liquids shows significant flow asymmetry. At low Re in particular, the more viscous fluid occupies the larger cross-section of the flow channel, compressing the less viscous fluid (Fig. 6), with net flow rate in each fluid being the same; in this way, also, the balance of pressures across the flow interface is maintained [28, 29]. A solvent juxtaposition with inevitably different viscosities has been studied using a combination of phenol/chloroform/isoamyl alcohol and water [30]. The liquids are immiscible, but yet flow stability, and avoiding flow breakup, was a challenge, with surfactant

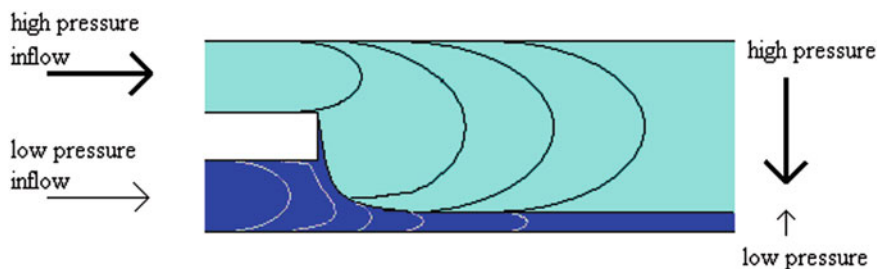


Fig. 6 A typical asymmetric width distribution for dual liquid-liquid flows unseparated by a physical barrier where a high and a low viscosity flow is juxtaposed. The asymmetry can be resolved by controlled, asymmetric pumping of flow

required to avoid initial interfacial curvature and later droplet formation. In this system, protein could be precipitated at the interface, but here it was not sufficiently to be able to completely remove protein that had been on the aqueous side. Where, however a more diffuse, miscible interphase is developed, solvent is present on both sides, determined by mutual solvent molecule diffusion; the result is a wider zone for protein accumulation and a more pure aqueous phase can be generated; the same effect is seen with DNA. Thus a DNA product of cell lysis could be extracted on a continuous basis.

A range of dual organic—aqueous flows have been investigated [31, 32], e.g. for sample extraction and increased solute concentration [33, 34]. Here, however, special flow units are usually required with etched glass typically used to give solvent resistance and allow optical monitoring. Examples of extraction include that of cobalt into a toluene phase [35], amphetamine like agents from urine into a chlorobutane phase [36] and ion pair extraction into chloroform [37]. One reported alternative to glass that simplified fabrication has been UV curable thiolene and a perfluoropolyether [34]; this was respectively used to establish the lipophilicity of caffeine through extraction and as a means of removing radioactive copper from aqueous solution as a possible system for radionuclide separation. In a further example, strychnine was extracted and then back extracted into a chloroform phase [38], and for a carbamate pesticide assay a sequential micro flow system achieved initial generation of a naphthol hydrolysis product, then a coloured diazonium adduct which was continuously extracted into a butanol phase for assay [39]. A further advancement of dual phases into triple parallel phases has been reported by Tokeshi et al. [35]. Here, sample mixing, reaction and extraction took place in a single chip and was defined as continuous flow processing (CFCP), illustrated, for example, for Co^{2+} with extraction of a 2-nitroso-1-naphthol complex from an aqueous phase into a non-polar xylene phase where the complex dissociated, for release of the free Co^{2+} into an acidic trapping phase.

8 In Situ Polymer Membrane Formation

Microfluidics has provided a versatile platform for sample extraction and assay as described above, recently reviewed by Atencia and Beebe [40]. Provided the balance of flows is established, stable, centralised flow patterns can be achieved. However, most descriptions above utilise the above type of direct liquid-liquid interfaces. There is a concern, however if flow disruption occurs, arguably during long term use a disruption to the flow dynamics could destabilise the co-linear flow and lead to measurement instability. Rather less work has been done to incorporate formal barrier structures that could both control solute transport and also stabilise flow. We have specifically addressed this challenge, along with other Groups, using flow balanced organic-aqueous flows to create reactive interfaces for generating polymeric barriers directly in situ. Rapid flow proves an advantage here as it promotes flow stabilisation, whereas slow flow is known to lead to segmentation [41]. Creation of distinct contralateral hydrophobic and hydrophilic flow surfaces also can provide stabilising pinning contacts for an organic and aqueous liquid, respectively. One hydrophobic surface modifier is organosilane on glass [42]; this has proved useful in protecting flow stratified, non-segmented flow. However, the approach does require a selective pre-deposition of the silane surface modifier, and a rather complex, ridged, channel design was necessitated to avoid channel crossover during surface treatment. Different fabrication materials for either side avoid this, e.g. PDMS and glass [43], but here the initial fabrication becomes more demanding. However, such balanced flows have not been used for interfacial membrane formation or synthesis, and focus essentially on extraction [44].

In our studies we used glass only fabricated Y-channels for hosting both solvent and aqueous flows, which considerably simplified construction. Stabilisation of flow and centralisation of the flow interface appeared to be achievable just through differential control flow control, avoiding viscosity induced flow asymmetry flow (Fig. 6). Initially, solvent flow was studied in an essential impermeable flow model system where the polymer generating reaction was guaranteed to be ultra-fast; the flows here delivered reactants for the formation of nylon 6,6 [8] based on a duality of water/xylene. The interface between the solvents moves naturally towards the xylene phase because of the lower solvent viscosity, but was countered by differential flow. The nylon system does not really serve as a convenient solute permeable membrane, except for gas molecules, and so an alternative fast reaction needed to be developed to create a membrane that had permeability and whose permeability and structure could be modified as a continuum, with the potential to host immobilised bioreagents, e.g. as a component in a bioreactor or a sensing system.

Accordingly, protein chemical cross linking was used to create the new class of self-standing membranes. This, of course, is the classic mode of protein immobilisation for supported protein layers for biosensors,. However, the standard cross linker reaction rates are too slow and in micro channels lead to channel blockage. By instead using a diacylchloride crosslinker (Fig. 7) an ultrafast interfacial

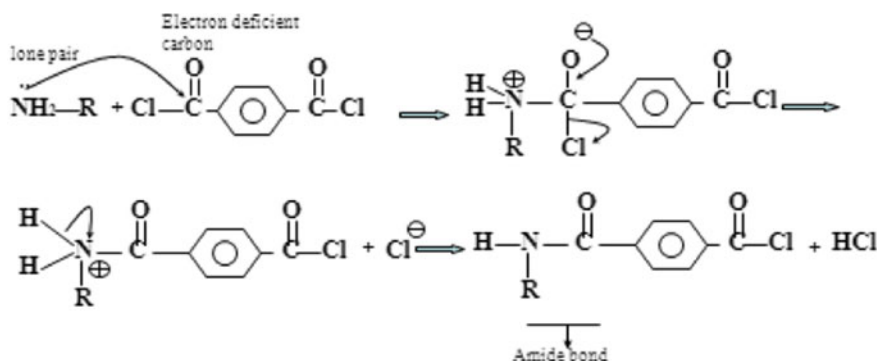


Fig. 7 Amide bond formation at a potential basic amino acid side chain. The rapid tetrachloro-4-phenylbenzoyl chloride crosslinking reaction used for protein cross linking in micro channel. The reaction mixture comprises flow balanced organic and aqueous flow to avoid flow asymmetry.; the strength of this ensures that it is self standing and adherent to the top and base of the channel

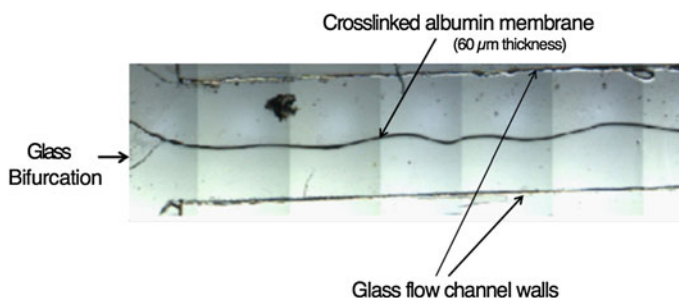


Fig. 8 The reaction interphase generated by the cross linking reaction in Fig. 7 leads to a self-limiting phase whereby a protein mesh limits further diffusive mixing. The edge of the self-limiting membrane is shown, and has the strength to be self supporting while also strongly adherent to the top and base of the glass flow channel

crosslinking occurs and a central, self standing, self limiting membrane results that has a cross linked mesh, overall thickness and permeability that can be tuned by controlling reactant concentrations, flow rates and choice of starting protein [45].

Additionally, a charge based separation is feasible, based on choice of protein isoelectric point and the operating pH for the solute transfer. A typical asymmetric flow was 400 uL/min water for the protein and 700 uL/min xylene for the cross linker in a 2×0.1 mm flow channel. Figure 8 shows a centralised, suspended membrane from cross linked albumin. A further benefit of this is the opportunity for using counterflow to accelerate solute transfer, and the operation of organic and other high partitioning solutes for analyte pre-concentration.

With protein based membranes, there are, of course, the readily available amino acid side groups for further facile immobilisation of bioreagents, including enzymes and antibodies. In the case of the former, not only can the enzyme be part of the assay chain, but it could be used to break down interferent species. Antibodies, alternatively, should permit immunosensing formats.

Nominally, a protein phase is biocompatible, though this would need to be determined on a case by case basis. By contrast, with the protein membrane hosting an RGD motif through co-retained fibronectin, laminin, vitronectin and other tissue matrix proteins, cell adhesion can be deliberately facilitated. The potential is there in that case for immobilised cells to serve as a toxin reporter, and a new embodiment of cell based biosensors could be achieved. Monitoring of such cells could be through localised sensor arrays or through optical tracking of the morphology of cell monolayers. A surrogate means of doing this is with electrode arrays in each channel for AC impedance spectroscopy. Cell layer retention on the side of the membrane opposite to the sample would allow delivery of effective growth medium and enable cell survival in a sample stream that no longer needed to be itself mixed with nutrient.

Considerable innovation is represented by work on interposed barrier structures, though the literature remains limited. Hydrogel barriers using, for example, polyacrylamide [46] and alginate [47] have been reported, though such gel phases with their high water content would have limited mechanical integrity, so would be used as a broad interphase and may lack the molecular discrimination of dense membranes. The environmental responsiveness and swelling of such gels could, however, have reactive cell and reagent reservoir release, and perhaps even serve as addressable valves. A rather more membrane like structure has been reported, made using alkali-acid gradients across a flow for the generation of chitosan membranes; these were shown to be able to resolve particulates and antibodies from micro solutes [48]. Nylon membranes of only ~ 10 μm thickness were formed in situ in an earlier study using an X-shaped micro channel, alternative to our Y-shaped flow [8]; interestingly this ultra thin barrier had permeability to H_2O_2 [32]. A further polyamide membrane has been reported, formed using a xylene carrier at a water interface [49] and a palladium complex membrane formed from a reaction between a phosphine polymeric ligand and a palladium compound [50]. Overall, the interest in in situ membrane formation, including our own, was stimulated by the work on microfluidics by Whiteside's Group [51, 52].

Use of preformed membranes is certainly an alternative, perhaps easier, approach, though a less elegant solution to sample separation for microfluidics, and the smaller the channel diameter, the more difficult to achieve. However, in one report a simple commercial microporous membrane was sandwiched between two micro flow chambers and used to discriminate low molecular weight peptides from a protein containing biological mixture for later SERS analysis [53]. For blood plasma separation, a microporous polysulphone membrane was used where vertical deployment of the membrane avoided cell sedimentation onto the membrane so avoiding membrane blockage [54].

9 Sensor Incorporation in Microfluidics

Dual flow microfluidics offers the opportunity not only for sensor protection but for reagent loading into the receiving stream; this could include bio- as well as non-biobioreagents. In a simple model with amperometric detection of glucose using glucose oxidase we generated stable sensor responses (Fig. 9). So despite a continuous flow of solution enzyme orthogonal to the diffusion of glucose, a ~ 130 μm Pt working electrode retained in the channel wall with no barrier membranes operated reliably; there would have been little concentration variation across the low diameter measurement surface. Importantly, the response is extended to well above the typical functional glucose k_m of an immobilised enzyme (~ 6 mM), and reflected solution enzyme k_m (>30 mM). This is likely to be due to two factors, the diffusion limitation to glucose transfer across the liquid stream and the continuous re-supply of oxygenated solution through ongoing flow. We have also found linear responses with combined enzyme and substrate in the supply stream. In principle it would be possible to operate with reagent requiring enzymes such as the dehydrogenases to broaden the analytical repertoire. Also, if an immunoreaction takes place rapidly as is the case for some homogeneous competitive immunoassays, then

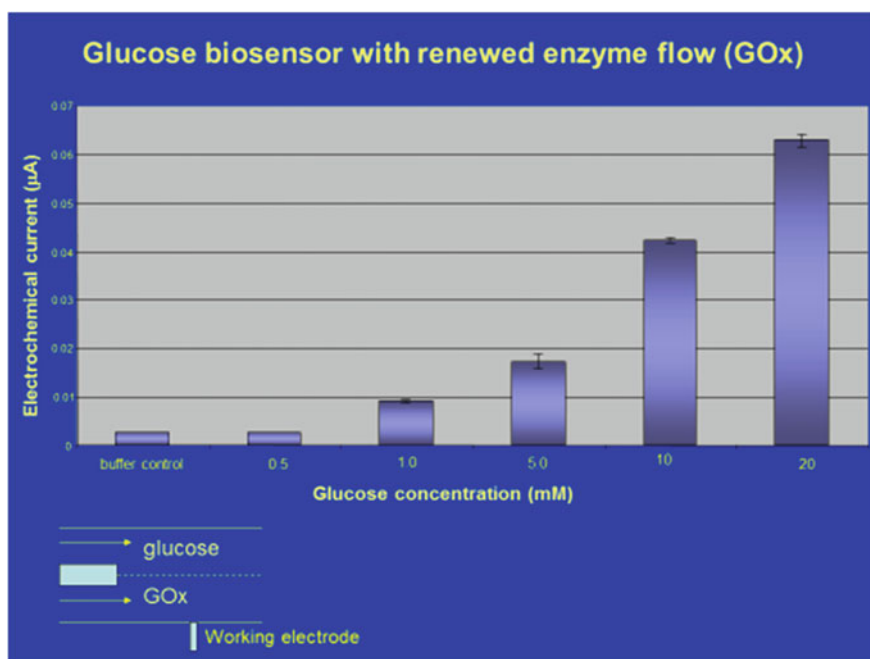


Fig. 9 Extended linearity state responses of a Pt wire working electrode to the H_2O_2 product of a glucose oxidase catalysed reaction using glucose oxidase dissolved in the flowing stream in the flow juxtaposed to the electrode surface, while glucose is present in the non-contacting sample stream

with the continuous re-supply of immunoreagent in the receiving stream, a quasi-continuous readout for immunosensing might be achieved for continuous tracking with immunosensors.

Microfluidics integrated biosensors based on enzymes have been reviewed recently [55], where the various alternatives of particle loaded, wall- and sensor immobilised configurations were highlighted. At the opposite end of the measurement spectrum, addressing multistep sample preparation procedures, a labelled DNA modified electrochemical sensor was integrated into a microfluidic device to registering hybridisation initiated change in the labelled DNA; however, this reagents configuration also included an on chip PCR amplification [56]. In a similar strategy, arrays of electrochemical sensors and heaters have produced for quantitative PCR utilising metal printed circuit board engineering [57].

For toxin detection other than metabolic disruption to cells, inhibition of enzymic reactions has provided a versatile routed for measurement. Thus, a microfluidic chamber incorporating an SPE and AChE [58] has been used for sarin detection. Amperometric detection of a thiocholine product; served as the reporter end point here. By an optical approach an aptamer was used to bind to polychlorinated biphenyls [59]. Here, an Ag based SERS detector surface functionalised with aptamer was integrated into a PDMS microfluidic channel, enabling assay of small sample volumes, whilst also providing for an aqueous interface that was more representative of a biological sample. SPR provides the ultimate surface biosensing technique, and localisation of such detection surfaces (LSPR) [60] has allowed multiple, simultaneous measurements on small sample volumes enabled through parallel microfluidics delivery. The general area of optical biosensors combined with microfluidics is thus receiving increased attention, and that in the near future advances in micro lens technology, organic LEDs and new materials for thin film photodiodes and high density optical pathway devices will lead to miniaturisation of optics with fluidics [61].

More adventurous bio-equivalent electrochemical configurations in microfluidic systems are being reported. Drug and chemical toxicity has been determined, for example, by using a polarised electrode to substitute for natural electron donation to immobilised Cyt P450; this powered oxidative catalysis by this liver enzyme system and enabled evaluation of resulting genotoxic metabolites from chemical agents. DNA damage here was registered by square wave voltammetry at a RuPVP coated electrode [62]. Nanowires offer a further route to low intrusion sensing, complementing microfluidics. In one study, the micro channel itself was use to direct flow for the formation of nanowire patterns [63]. As a departure from solid state flow channels, a soft gel micro channel has been reported with incorporated carbon nanowire arrays of 20 μm diameter comprising core-shell titanium carbide with carbon; this was used for monitoring NO release from cultured endothelial cells [64]. Whilst not part of a microfluidic structure, an individually addressable, compressed array of 110 gold micro electrodes for NO/peroxynitrite detection shows the likely detection scale up that could be achieved for future microfluidics [65]. Miniaturisation and array compression with nano sensors has been reviewed [66], indicating the diversity of platforms possible including impedance

measurement, potentiometric ion monitoring and nano-scale surface modification for improved bioreagent loading [66]. Extreme miniaturisation of ion selective electrodes, whilst retaining robustness, remains a challenge, and whilst the ISFET model is usable with appropriate MEMs engineering, there is now interest in wire type structures based on metal such as a Pt device where an ionophore coating was used for K^+ and Ca^{2+} , and iridium oxide for pH. Thin film sensors as have been designed for PDMS micro channels [67]. So diverse range of integrated structures is emerging, and is likely to enable multi parameter ion monitoring perhaps as future hyphenated systems with voltammetric and optical detection [68].

Immunosensors have also been under investigation at minaturised levels, and a substantial range of surface immobilisation chemistries and flow designs are reported [69]. As with other measurement modalities the drive for such research is miniaturisation, multiple analysis and improved response to background ratios. What is really needed is a re-engineering of the affinity binding chemistry itself so either the limit of sensitivity can be enhanced, or the dynamics of binding reversal can be accelerated to enable continuous immunosensor based tracking in microfluidics. However, the scene is set for microfluidics to at least simplify the multiple reagent flows, binding and separation steps in immunoassay to allow remote use by non-technical operators.

10 Conclusions

A broad range of technical achievements with microfluidics and sensors has been covered. Whilst these descriptions do not themselves amount to a comprehensive overview of uTAS, they do provide information about the key elements of detection and sample/reagent transfer at smaller scales. This is qualitatively distinct from macroscale fluid movement, highly exploitable, and the likely focus of future investigations. This is likely to move on to nanoscale fluid handling where wall effects dominate even more, so allowing greater materials based solute and fluid manipulation. The flow emphasis in this chapter attempts to presage an era when continuous sampling and operation will be the norm for decentralised measurement. The eventual goal is continuous monitoring, at least as a way of setting up alerts for threat agents and for identifying trends. We have seen considerable advances here with respect to small molecules, but there remains a need to move on to (bio-)macromolecular measurement with, if not continuous, then quasi-continuous measurement with rapid, repeat assays enabled through rapid microfluidic handling. Here also it can be envisaged that if arrays can be compressed into such miniature systems, then microfluidic delivery can become a part of high throughput arrays. This will become easier for a future when miniaturisation technologies shift from the micro to the nano scale as a norm through advances materials and engineering science. It is possible that health care monitoring will be the initial focus of attention, but the follow on to threat agent monitoring can only thereby be accelerated.

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