Research Highlights

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Nanolitre viscometer

The ability to accurately measure changes in fluid viscosity is of great importance in a number of disciplines in the chemical and biological sciences. At a fundamental level viscosity is a measure of the resistance of a substance to a change of shape. More often it is used to describe the resistance to flow of a fluid. This resistance acts against the motion of a solid object through the fluid and also against motion of the fluid itself past stationary obstacles. Although viscosity measurements provide little information relating to chemical identity, they can prove invaluable in characterising the overall chemical composition of many fluids. For example, viscosity measurements have been widely used in the polymer industry for many years to assess polymer molecular weights, and as a powerful characterisation tool when applied to the study of paints, ink and varnishes. Furthermore, important information relating to the mechanism and dynamics of molecular and biological processes can be obtained by studying the dependence of reaction kinetics on solvent viscosity.

Cone and capillary instruments are the most widely used tools in viscometry. Cone (or plate) viscometers provide for high information content viscosity analysis but are relatively complex and expensive to implement. Conversely, capillary systems are inexpensive and simple to operate but provide more limited information. Moreover, both approaches are difficult to miniaturise and thus are limited to laboratory environments. To address this limitation, Mark Burns and colleagues at the University of Michigan, Ann Arbor have recently reported the design, fabrication and testing of a microfabricated viscometer that can be applied to the rapid and accurate measurement of viscosity in a variety of fluid media.

The silicon–glass hybrid microdevice (Fig. 1) is manufactured using standard photolithographic methods, incorporates no moving parts, requires minimal amounts of fluid and is self-calibrating.

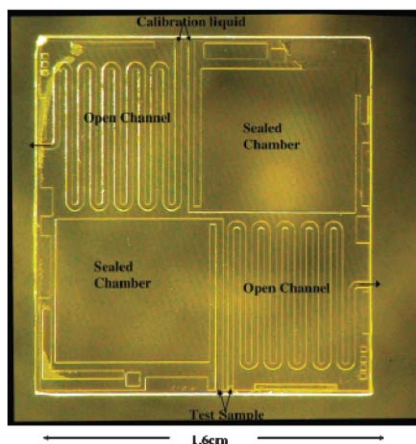

Fig. 1 Self-calibrating nanoliter viscometer. (Adapted with permission. Copyright 2005, The American Chemical Society.)

Furthermore, since fluid introduction and motivation is controlled through capillary forces, external actuators are not required. Viscosity measurements are based on pressure-driven laminar flow within microchannels that have a width of approximately 300 microns and a depth of 30 microns. Fluid viscosity can be calculated through knowledge of the channel depth, the length of the liquid column, the pressure drop across the liquid, a geometric factor, and the fluid velocity. Consequently, measurement of the pressure drop and the mean velocity allows direct calculation of the liquid viscosity. In a simple configuration a fluid drop (of known volume) is placed at the sample inlet. Capillary forces drive fluid into the microchannel and the liquid velocity is measured. Capillary pressure is then calculated through use of the Young–Laplace equation. Using this approach the authors were able to estimate the viscosity of water at 22 °C to within 1% of the literature value within one minute. Although successful, the approach is limited since analysis requires *a priori* information and does not account for variations in channel geometry. Consequently, a self-calibrating viscometer was developed to allow for improved precision. The elegant design (shown in Fig. 1) incorporates two sets of open and sealed channels (one set for the sample under investigation and one set for a calibration liquid). Since all channels are made using the same fabrication method the channel geometries are identical for each set, thus allowing high precision viscosity measurements to be performed. Using this self-calibrating device the authors were able to calculate the viscosity of blood plasma samples (collected from patients with symptoms of hyperviscosity) to an accuracy of 3%.

These proof-of-principle studies demonstrate that viscosity measurements can be easily performed on nanolitre sample volumes in less than 100 s. Importantly, the reported microdevices can be used to interrogate solutions with viscosities ranging from 1 to 5 cP, and can deal with relevant biological fluids such as blood plasma. It is expected that such self-calibrating nanoliter viscometers may find widespread use in diagnostic and personal health care applications.

Anal. Chem., 2005, **77**, 383.

Electrokinetic micromixing

The reduced feature dimensions associated with most microfluidic systems typically dictate that flow within such devices is typified by reduced Reynold's numbers. Consequently, flow is laminar rather than turbulent. This means that turbulence is rarely achievable and diffusion is the predominant mechanism by which fluid streams unite and mix. Although diffusive mixing can be slow, especially for analytes with large diffusion coefficients, a large number of approaches to efficient mixing within microchannel environments have been reported. Many of these structures have incorporated external actuators (based on ultrasonics, piezoelectric actuation and magnetic actuation) to enhance mixing speeds. Using similar ideas Hsin-Yu Wu and Cheng-Hsien Liu at the National Tsing University in Taiwan have recently demonstrated a microfluidic mixer that uses dynamic manipulation of local flow fields to enhance mixing phenomena in microchannels.

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When a silicon dioxide microchannel surface is contacted with an electrolyte an electrical double layer (consisting of tightly bound and diffuse cationic layer) forms. When an external electric field is applied along the channel cations in the diffuse layer are attracted towards the cathode and drag the surrounding bulk liquid in the same direction. This fluidic motion is commonly termed electro-osmosis. Importantly, the magnitude of electroosmotic flow can be controlled by variation of the zeta-potential (the potential difference between the tightly bound and diffuse layers). By embedding electrodes below the surface of the micro-channel bed the surface charge in the electric double layer can be modulated. Moreover, by varying this charge (and thus the zeta potential) in a periodic manner a local circulation zone can be generated which can force a portion of the mixed 'downstream' fluid back into the unmixed 'upstream' region and thereby reduce mixing lengths and timescales.

To test this concept the authors fabricated a T-shaped microfluidic mixer consisting of a structured PDMS layer to define fluidic channels and a silicon substrate containing an electrode array. Electrodes were fabricated on a silicon substrate by first growing a 1 μm silicon dioxide insulation layer on the surface. A series of asymmetric-herringbone electrodes (made of aluminium) were then deposited and patterned *via* a lift-off process to a thickness of 200 nm. This layer is then isolated by deposition of a 500 nm PECVD layer of silicon dioxide. Finally, a structured PDMS layer (fabricated using standard moulding techniques) is bonded to the silicon substrate following exposure to an O_2 plasma to form the complete fluidic device (Fig. 2).

The mixing process is enhanced by a combination of temporal and spatial modulation of the zeta potential at the microchannel walls to generate complex flow field for mixing enhancement. The temporal modulation is generated by periodic out-of phase AC radial voltage control whilst the spatial modulation is facilitated by the asymmetric-herringbone-electrode structure. Through experimental and theoretical studies, the authors demonstrate that the electrokinetic micromixer shows excellent mixing efficiencies (over 90%) after the solutions passed along a 5 mm long microchannel. An obvious change in velocity profile resulting from non-uniform zeta potential was also observed. Such a design provides real mixing enhancements for microfluidic systems operating within low Reynolds number regimes, and will be useful in creating complex flow fields within straight microchannels.

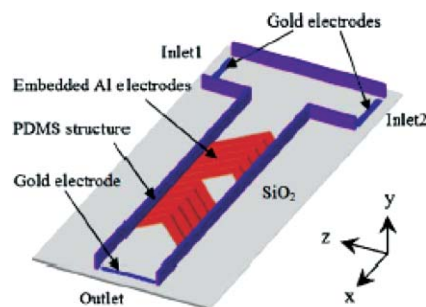

Fig. 2 T-shape micromixer with a 200 μm by 60 μm cross-section. Embedded electrodes are 500 μm long with a 5 μm gap between adjacent electrodes. (Adapted with permission, Copyright 2005 Elsevier B.V.)

Sens. Actuators, A, 2005, **118**, 107.

Glycosylation optimization

The optimisation of reactions in fine or bulk chemistry is vital to the economic success of any synthetic route. Every stage of such a process requires investigation to ensure that the ideal balance between cost and product yield is met. Reaction optimisation is normally a time consuming process, where data for each system can only be gathered by the repeated performance of the reaction under varied conditions. Although robotic systems have been employed to provide a degree of automation when carrying out an optimisation studies batch, macroscale reaction systems are unsuited to rapid reaction optimisation under most circumstances. In recent times, microfluidic systems operating within continuous flow have been shown to provide for a more efficient route toward reaction optimisation due to the precise control of reaction variables and the ability to efficiently process small volumes of reagent. To this end, Peter Seeberger and co-workers at the Swiss Federal Institute of Technology Zurich (ETH) and the Massachusetts Institute of Technology have demonstrated a microfluidic reaction system for the optimisation of organic reactions in continuous flow. Specifically, continuous flow microreactors were used to systematically study glycosylation reactions. Glycosylation reactions involve the addition of glycosyl groups to a protein to form a glycoprotein and are the first of the principal modification steps in the synthesis of membrane proteins and secretory proteins. Studies focussed on yield optimization (by control of reaction times and temperature) of glycosylations of trichloroacetimidate activated sugars. Significantly, these reactions are heavily dependent upon temperature and reagent concentration and can produce undesirable orthoester by-products.

Reagents were fed through a glass/silicon microreactor (Fig. 3) containing a simple structure to allow for the mixing of three components (donor, acceptor and activator) in a specified ratio. The same device then allows the reaction to proceed for a given period of time before it is quenched by the addition of a base (triethylamine). At this point, the reaction mixture can be analysed by in-line HPLC using a UV detector.

Using such a microdevice the authors were able to demonstrate optimisation of two model glycosylations, with optimal temperature/concentration/flow rate protocols being established by HPLC analysis of the chip effluent. For example, in the mannosylation of 2,3,4-tri-*O*-benzyl-methyl mannoside, optimal yields are achieved at −60 °C and a reaction time of 213 s. However, it is also shown that almost comparable performance can be achieved at a temperature of −35 °C and a reaction time of 26 s. This valuable information shows that much improved product synthesis in a production environment could be achieved by operation of the system under 'non-optimal' conditions (through an improved space–time yield and a less extreme reaction temperature). Such an approach to reaction optimisation is clearly highly advantageous due to the minimal reagent volumes and large number of analyses per unit time.

Chem. Commun., 2005, 578.

Large scale generation of fluorine using microreactors

Over recent years, Richard Chambers and colleagues at Durham University

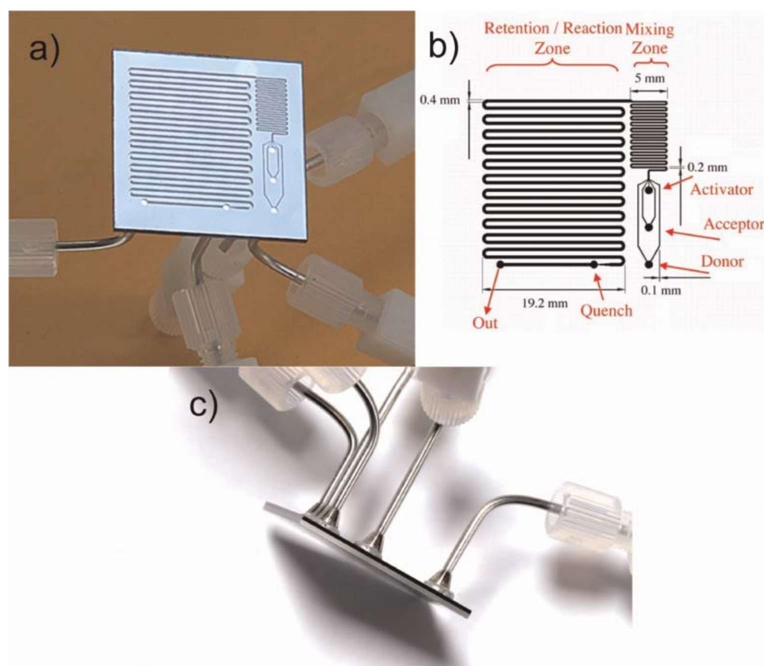

Fig. 3 (a) Silicon microfluidic microreactor. (b) Schematic of microreactor system, comprised of three primary inlets, a mixing and reaction zone, a secondary inlet for quench, and an outlet for analysis/collection. (c) Soldered joints of microreactor (perspective of device from side).

have described a number of microfluidic reaction systems for direct fluorination and perfluorination reactions. The primary concern when using elemental fluorine as a reagent in synthetic chemistry is the safe handling and control of thermodynamically exothermic processes. The application of microreactors to direct fluorination reactions has been shown to dramatically reduce safety considerations and constraints (due to the efficient heat transfer and low fluorine inventories) and moreover allow for selective and high-efficiency fluorination of a variety of organic substrates.

Subsequent to laboratory-scale optimisation of a reaction, scale-up of the reaction scheme is required to afford production-scale quantities of material and industrial viability. Under normal circumstances, the reaction system has to be radically adapted to accomplish this process, since reaction behaviour on the laboratory-scale is rarely mirrored in the system's performance on a fine to bulk scale. Safety procedures often add to large scale-up costs, and can lead to the abandonment of a synthesis if a particular hazard becomes apparent. When using microfluidic systems in continuous flow an alternate approach may be taken using the concept of 'scale-out'. Here, the manufacturing process is constructed of multiple reactors used in parallel. To investigate the 'scale out' of fluorination chemistries Chambers has extended his seminal work on fluorine gas/liquid reactors to produce an effective scale-out device.

In these studies, micromachined stainless steel reactors replace nickel substrates used in earlier work, with transparent top plates (made from PCTFE) allowing visualisation of the flow mode. In an initial scheme, nine channels, each 500 μm deep and 500 μm wide were coupled *via* a simple manifold interface to flows of fluorine and reagent (Fig. 4). The reactor was then used in the fluorination of a model compound, ethyl 3-oxobutanoate.

Near quantitative conversions could be observed under many conditions. Moreover, the reaction proved simple to conduct and easy to optimise, and the reactor itself easy to scale out. Indeed it was found that scaling from 9 to 30 channels gave no alteration in reaction performance. The test reactor showed that a back-to-back 60 channel reactor would produce 30 g day^{-1} of fluorinated product, meaning that 10 such reactors would produce 300 g day^{-1}. This represents a small pilot plant operation, running under the same conditions as the laboratory synthesis. Importantly, the reactors are easy to maintain, operate continuously, require single source fluid delivery, operate under safe conditions and provide for high efficiency synthesis of fluorinated products.

Lab Chip, 2005, **5**, 191.

Single quantum dot detection in microchannels

A significant challenge arising directly from the adoption of small volume analytical systems is the ability to efficiently detect analyte molecules. For example, when performing capillary electrophoresis within chip-based microfluidic systems, injection volumes are commonly no larger than 50 pL. This

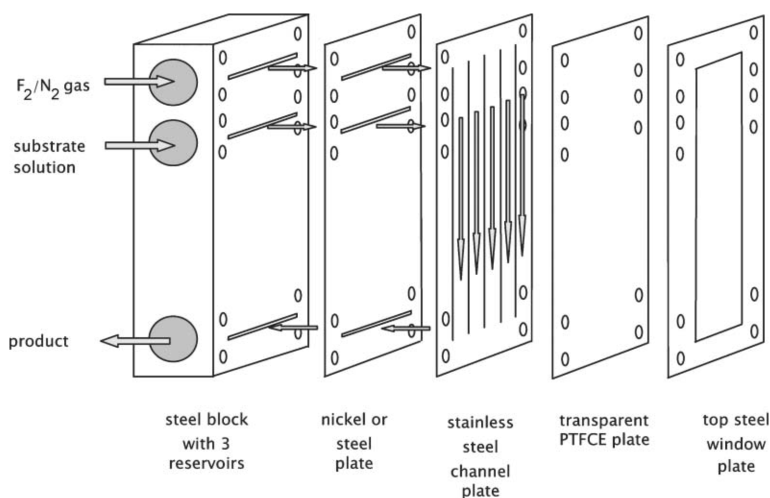

Fig. 4 Schematic representation of modular microreactor device for direct fluorinations.

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means that for an analyte concentration of 10 nM, only 3 $\times$ 10^5 molecules are available for separation and detection. This simple calculation demonstrates that detection is undoubtedly one of the primary issues determining the practicality and application of microfluidic systems. Taken to the extreme, detection of single molecules or particles within fluid media represents the ultimate limit of detection for analytical scientists opening up the possibility of performing single molecule immunoassays and single-molecule DNA sequencing.

Recently, further progress in the area of single molecule detection (SMD) within confined environments has been reported by Harold Craighead and associates at Cornell University. Specifically, fluidic channels with submicron dimensions were used to isolate and detect the binding of single molecules with functionalised quantum dots. Compound semiconductor quantum dots have attracted much attention as potential optical labels in biosensing applications due to their high fluorescence quantum efficiencies, high extinction coefficients and low photodegradation rate coefficients. As such they represent ideal candidates for SMD applications. To effect efficient SMD, the authors use both confocal optics and channels with sub-micron dimensions to reduce detection volumes to a few hundred attolitres and in turn significantly reduce background noise (Fig. 5). To demonstrate multicolour detection of single molecule binding events, the authors bind a commercially available functionalised quantum dot (Qdot 655 Streptavidin conjugate) to a short DNA oligomer labelled with an organic dye molecule (Alexa Fluor 488). When bound, both species can be detected coincidently as they are driven electrokinetically through the detection volume, whilst unbound DNA probes and quantum dots are detected as temporally separated photon bursts. The authors use photon counting histogram analysis to confirm coincident detection and also show that the relative dimensions of the fluidic channel and probe volume allow rapid detection and identification of nearly all fluorescent species flowing through the system. Such multiplexed single molecule studies will undoubtedly prove highly useful in many applications

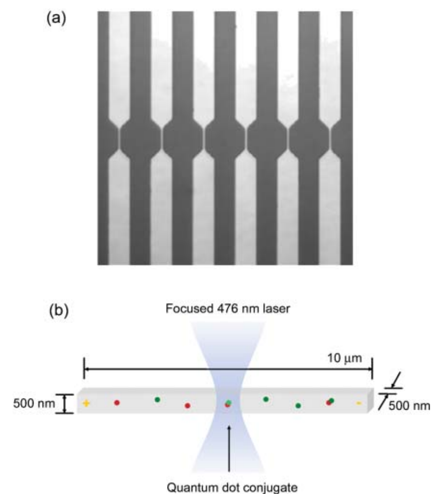

Fig. 5 (a) Optical micrograph of an array of nanofluidic channels. Each fluidic channel narrows from a width of 10 µm to 500 nm for a length of 10 µm. Detection of single quantum dot conjugates occurs in this narrow region. The channels were etched to a depth of 500 nm. (b) Schematic of a fluidic channel and the associated detection volume defined by a focused laser beam.

including ultra-high sensitivity biological assays.

Lab Chip, 2005, **5**, 337.

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