

# Thermal optimisation of the Reimer–Tiemann reaction using thermochromic liquid crystals on a microfluidic reactor

Alexander Iles,<sup>\*a</sup> Robin Fortt<sup>b</sup> and Andrew J. de Mello<sup>c</sup>

Received 20th December 2004, Accepted 9th March 2005

First published as an Advance Article on the web 22nd March 2005

DOI: 10.1039/b419081e

Microreactors incorporating thin film resistive heating elements for continuous flow organic synthesis are presented. Internal thermal conditions were monitored in real time using reflectance spectra of temperature sensitive thermochromic liquid crystals (TLC) in a collateral microfluidic network. To demonstrate the precise temperature control provided by this method, the thermal optimisation of the Reimer–Tiemann formylation of  $\beta$ -naphthol was performed under hydrodynamic pumping regimes.

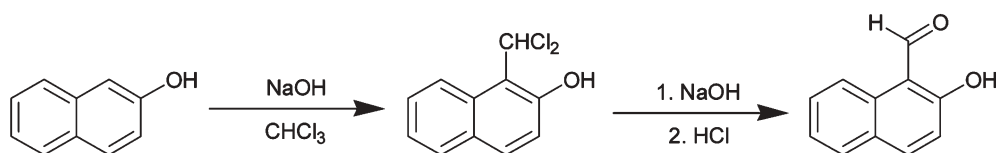
## Introduction

The use of microfluidic devices for chemical synthesis has generated considerable interest.<sup>1–3</sup> However, it is insufficient to demonstrate idealised, model reactions using these devices. A miniaturised system for small molecule synthesis must also be capable of performing reactions that are difficult or problematic. The Reimer–Tiemann reaction is a typical example of a problematic reaction that is nonetheless, extremely important in chemical synthesis. This reaction is utilised in the formylation of activated aromatic rings, namely phenols and pyrroles.<sup>4</sup> Direct introduction of an aldehyde group into an aromatic ring is possible using a variety of other methods, for example the Gattermann, Gattermann–Koch, Vielsmeier and Duff reactions. However, each method has its limitations and in certain circumstances the Reimer–Tiemann reaction is the only appropriate method for the direct formylation of phenols (Scheme 1).

There are two main factors that make the Reimer–Tiemann reaction problematic. Firstly, the biphasic nature of the system acts to limit the reaction interface. Since the phenolic substrate remains in the aqueous alkaline layer and the dichlorocarbene is largely present in the chloroform layer, inefficient transfer of reagents between these layers can stifle the rate of reaction and decrease yields. Secondly, to obtain a satisfactory reaction rate, the process should be performed at reflux. However, if reflux occurs within an enclosed microfluidic structure, the contact time in the mixing device becomes drastically reduced. This is due to the rapid expansion of the boiling solvents, which leads to the premature expulsion of the reaction mixture from the chip. The generation of gases also prevents down stream on-line analysis. To maximise the reaction efficiency without boiling, the temperature within the microreactor

channels must be carefully maintained and monitored. In addition, the viscosity of the reactants poses a significant problem for microreactors. Traditionally, a concentrated solution of alkali hydroxide (50% aqueous sodium hydroxide = 78 mPa s) is utilised for the hydrolysis of chloroform to generate the dichlorocarbene reactive intermediate and for the hydrolysis of the neutral intermediate (Scheme 1). Viscous reagents produce significant back pressures when operating under hydrodynamic pumping regimes, thus limiting flow rates. A further source of experimental complexity arises from the protonation of the phenoxide ion by hydrochloric acid. Following the acidic quench of the reaction mixture, substantial quantities of the alkali salt are produced (Scheme 1). This leads to precipitation and consequently, frequent blockage of the microreactor device.

The Reimer–Tiemann reaction is highly exothermic and, on the bulk scale, the heat source must be removed or controlled after initiation of the reaction. This is done to prevent overheating and thermal runaway. When using microreactors, the active removal of energy from reactions is rarely required. These reactors have excellent inherent heat transfer<sup>5</sup> and radical termination properties<sup>6</sup> and so the heat generated by a reaction can be efficiently dissipated. However, the Reimer–Tiemann reaction is not a straightforward case; careful balancing of heat input and heat generation is required to prevent thermal runaway. Several heated microreaction systems have been reported in which Peltier elements<sup>7</sup> or cartridge heaters<sup>8</sup> were used. Unfortunately, these systems have large thermal masses and hence slow heating/cooling rates. This makes it extremely difficult to maintain a satisfactory balance between the energy supplied to the reactor and heat generated by the reaction. It is possible to achieve rapid heating and cooling cycle times by incorporating micro or thin



Scheme 1 The Reimer–Tiemann formylation of  $\beta$ -naphthol.

film heaters into the microreactor itself. Thin film heating elements have a significantly smaller thermal mass and hence consume less power and provide much greater thermal control.<sup>9,10</sup> However, due to the added complexity of heating element incorporation, integrated thermal heaters remain underutilised in microfluidic reactors.

In heated organic syntheses it is important to determine accurately and maintain the reaction temperature due to the exponential relationship between reaction rate and temperature. This is achieved in large scale syntheses by simply inserting a thermometer or thermocouple into the reactant mixture and the reaction temperature at the centre of the mixture is taken as the bulk average. The channels inside a microreactor are much less accessible. It is possible to measure the surface temperature of a microdevice, however, for exothermic reactions, if the measured temperature does not correspond to the in-channel temperature, then the reaction may progress more aggressively than predicted. In addition, inaccurate temperature determination may lead to the increased formation of unwanted by-products. Measurement of temperature on microfluidic devices has been performed previously by the integration of thin film thermocouples or temperature sensitive resistors.<sup>10–12</sup> However, in microfluidic devices, where heat transfer coefficients are high, the temperature of the bulk material may differ considerably from the in-channel temperature. Additionally, this may be accentuated by the difference in the thermal conductivities of the chip material and the fluid filled portion of the device. Several optical methods for temperature measurement have been investigated, including the use of fluorescent dyes, molecular beacons and refractive index change.<sup>13–15</sup> Fluorescence based methods are prone to a number of problems, including photobleaching and the effects of the local environment surrounding the fluorophore (for example, pH and/or the presence of quenching species). For both fluorescence and refractive index measurement, it is essential to maintain precise optical alignment during the entire experiment, or calibration will be lost. It may also be difficult to apply refractive index measurement to an on-chip, two phase reaction where the refractive index would be continually changing, due to varying quantities of different phases passing along the channel and the distribution of the reactants/products between those phases.

Ideally, a method for in-channel temperature determination should reflect the temperature condition within a microfluidic channel directly. Furthermore, temperature measurement should not be performed at just one location; distributed readings should be obtained. This should be performed to identify any hot spots in the device. Any such method should be rapid to implement and flexible, requiring minimal additional support equipment. If possible, it should be based on the measurement of a parameter that is not related to an absolute intensity level, so that calibration is straightforward and does not have to be repeated every time a different microreactor is used or a different reaction is performed.

Thermochromic liquid crystals (TLCs) are organic molecules that demonstrate properties of both liquids and solids and exhibit a number of phases between these two extremes. The transition between phases is triggered by heat and results in a colour change due to the chiral nature of the molecule.

Temperature-sensitive liquid crystals exhibit a range of colours reversibly from colourless (black against a black background) through the colours of the visible spectrum in sequence from red to violet before turning colourless (black) again beyond their thermal range. The process is reversed when the source of heat is removed. Standard products are available covering a range of start temperatures from  $-30\text{ }^{\circ}\text{C}$  to  $+120\text{ }^{\circ}\text{C}$  and a colour bandwidth from  $0.5\text{ }^{\circ}\text{C}$  to  $40\text{ }^{\circ}\text{C}$  allowing either a broad or precise temperature indication over a wide temperature range for the rapid accumulation of experimental data. The parameter used for temperature measurement is based on the wavelength of reflected light, hence the method is not dependent on any absolute intensity level. Previously, TLCs have been utilised for the thermal mapping of a micro-fabricated array for PCR.<sup>16</sup> In this arrangement, the PCR mixture was replaced by TLC beads for studies of thermal transfer between the heating elements and the microarray. However, the experiments detailed in ref. 16 were performed without a reliable data interpretation system for measurements of hue. The authors were reliant on the ability of the human eye to distinguish between shades of colour for temperature determination, which severely limited the accuracy of their method. More recently, TLCs were utilised for the temperature calibration of microheaters in a PCR micro device.<sup>17</sup> Temperatures for a given power input were estimated from photographs of microfluidic channels filled with TLCs. Once the heater calibration was completed, the channels were emptied and then filled with PCR reagents. Hence, the reaction could not be monitored in real time.

In this paper, we report on the development of a microfluidic system, which utilises a simple integrated heating element combined with TLCs interrogated using a miniature spectrometer, to provide the excellent thermal control that is required for the thermal optimisation of a heated synthetic reaction.

## Experimental

The colour spectrum exhibited by the TLCs spans the visible spectrum between 400 and 700 nm. To allow for precise temperature monitoring, the reflectance spectra of the TLCs were acquired using a high sensitivity fibre optic spectrometer (AVS-S2000, Avantes Inc., USA). A 100 micron fibre optic was positioned perpendicular to the TLCs using a micrometer controlled translation stage and reflectance spectra from particular locations were recorded with a resolution of  $\approx 0.5\text{ nm}$  and integrated over 75 ms using 00Ibase2 software (Ocean Optics Inc., USA) running on a personal computer. To obtain a spectrum, a halogen lamp fitted with a light guide (Leica GLS100) was placed above the chip to illuminate the device. Calibration data for temperature *versus* the wavelength of peak reflectance were obtained by recording spectra from a chip containing TLCs which was immersed in an oil bath and heated over a range of temperatures.

Serpentine Nickel–gold thin film resistive heating elements were fabricated on a glass substrate by evaporative deposition, (306A Coating System, Edwards, UK with a custom fabricated brass mask) each layer being approximately 40 nm thick. To map the spatial thermal performance of these microheater

elements, a thin layer of TLC beads (R60C10, Hallcrest, Poole, Dorset, UK) sandwiched between two glass slides was placed onto a microheater. Power was applied to the element, which caused the TLCs to change colour according to local temperature (Fig. 1).

The integration of TLCs into a microfluidic reactor for in-channel temperature measurement was achieved by the addition of a second microfluidic channel, which ran collateral with the reaction channel (Fig. 2D to E). To ensure an even temperature distribution and efficient transfer of heat to the microfluidic channels, the resistive heater element was placed in a separate layer of the device and each turn of the meandering fluidic channels was mirrored by the heating element in such a way that each part of the collateral fluidic network was equidistant from the heating element. With similar dimensions and thermal properties as the reaction channel, the temperature experienced by the TLCs occupying the second channel due to the heat supplied by the heating element, would be identical to that of the corresponding part of the reaction channel, as the characteristic dimension for heat transfer was maintained. Furthermore, by placing the heating element in a separate device layer, the geography of the fluidic channel and the heating element remained independent and the need for any additional electrically insulating material was eliminated. The TLCs were introduced into the second channel as a polymer bead slurry. Encapsulation of the TLC mixture in a polymer shell protects them against the atmosphere and any residual solvents, which may adversely affect their performance. Additionally, use of the beads as an aqueous slurry facilitates easier handling and offers similar thermal conductivity properties to that of the fluid filled reaction channel.

The glass microchip (footprint 30 mm × 20 mm) was made in-house using direct-write laser lithography, wet chemical etching and bonding techniques as previously described.<sup>16</sup> Briefly, soda lime glass substrate precoated with a positive photoresist (AZ 1518) and a low reflective chromium layer (Nanofilm, Westlake Village, California) was exposed using a

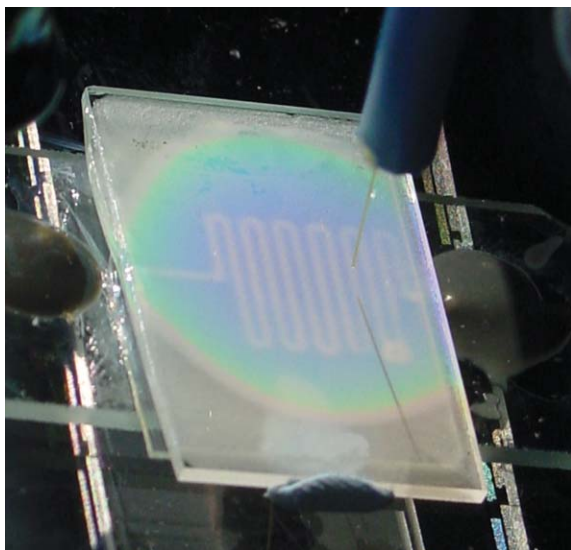


Fig. 1 Thermal mapping of a microheater element using TLCs.

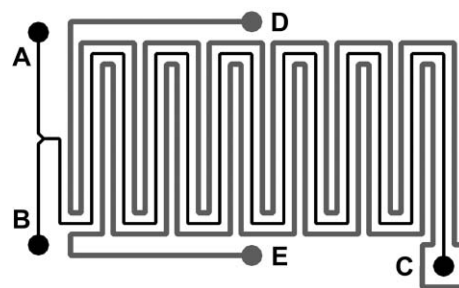


Fig. 2 Schematic of microfluidic reactor chip channel pattern used in all syntheses. Identities of components A–E provided in the text.

DWL system (DWL2.0, Heidelberg Instruments, Heidelberg, Germany) to transfer the channel design. After the photoresist was developed (Microposit 351, Shipley Europe Ltd, Coventry, UK), the channels were etched into the glass substrate using a buffered oxide etching solution (HF/NH<sub>4</sub>F) and external access holes were drilled. To form enclosed channels, a glass cover plate cleaned with concentrated H<sub>2</sub>SO<sub>4</sub> was thermally bonded in a furnace (Heraeus Instruments GmbH, Hanau, Germany) at 858 K. The reactor channel pattern is schematically described in Fig. 2. The chip consisted of a 'T' shaped inlet (A and B), a serpentine reaction sector 140 mm in length and an exhaust (C) at which samples were collected for analysis. The etched channels had an average depth of 50 μm and an average width of 150 μm and a total volume of 1.05 μL. The collaterally running microfluidic channel for the incorporation of the TLC slurry consisted of a single serpentine channel 263 mm in length, 250 μm wide and 50 μm deep and a total volume of 3.3 μL. The encapsulated TLC slurry was introduced into the device by injection into the channel through the inlet (D) until the channel was completely filled; the excess TLCs were collected at the outlet (E). Once this channel was completely filled with TLCs it was capped with a reversible seal.

Electrical power was supplied to the heating element so that an appropriate temperature was indicated by the TLCs. The voltage supplied to the element was then adjusted accordingly as the reaction progressed.

For the continuous-flow formylation of β-naphthol, solutions of the phenol in ethanol (1.25g, 8.7 mmol in 3 mL) and chloroform (1.6g, 13.4 mmol) and 25% sodium hydroxide (2.5g, 62.5 mmol) in water (5 ml) were prepared. These solutions were introduced into inlets A and B respectively of the heated reactor chip using a syringe pump (PHD 2000, Harvard Apparatus, Kent, UK). Reactant flow rates between 5 and 20 μL min<sup>-1</sup> were investigated. The reaction was quenched immediately upon exiting the chip outlet (C) in a continually stirred solution of 12% hydrochloric acid. Once collected, the aqueous and organic layers were separated and product analysis was performed using GC without further purification, by comparison to spiked standardised solutions. GC-MS spectra were also obtained and compared.

## Results and discussion

From the reflectance spectra of the TLCs over a range of temperatures, an exponential relationship between peak

reflectance wavelength and temperature was observed between 60 and 70 °C. Although this temperature range is not large, it was more than adequate to indicate the critical temperature such that reaction reflux could be avoided, whilst allowing sufficient heat to be supplied so that the reaction proceeded at an acceptable rate. Over the range of interest (60 to 65 °C), temperature could be determined to within 0.4 °C (Fig. 3).

Beyond 65 °C, temperature resolution decreases as the rate of wavelength change with temperature decreases. However, each spectrum is composed of a number of overlapping peaks. These peaks could be analysed using a multi-wavelength algorithm as opposed to a single peak maximum, leading to improved temperature resolution across a broader temperature range. Although feedback control for these experiments was performed manually, the use of a miniaturised spectrometer allows for the facile integration of a computer regulated feedback loop for automated temperature control.<sup>11</sup>

Inclusion of the TLC slurry into a second microfluidic channel allows different types of TLC to be injected and removed repeatedly without significant loss. In this way a range of liquid crystals with differing thermal properties could be incorporated, enabling different temperature regimes to be studied. Alternatively, the TLCs could be encapsulated within chemically inert polymers such as PTFE. This would allow the TLC beads to be included within a reaction mixture. Hence, temperatures experienced in the reactor channel could be monitored during a reaction, for direct temperature determination *in situ*. This would be ideal for devices of complicated channel geometry where the use of a collaterally running channel would be restricted and reactions such as PCR,<sup>9,16</sup> where it is important to be able to measure in-channel temperature with a high degree of accuracy.

The effectiveness of TLCs for microfluidic applications was clearly illustrated by the thermal mapping of the microheater elements. From Fig. 1 it is possible to identify several different temperature zones. The use of the fibre optic spectrometer enabled the collection of accurate temperature profile data. An example of this for a lateral profile is given in Fig. 4. The fibre optic probe was positioned 2 mm above the centre of the device shown in Fig. 1 and spectra were collected at 0.5 mm intervals outwards from the centre. It is possible to see that the temperature begins to decrease rapidly beyond the point 2 mm

from the centre, until at 4.5 mm, the temperature has decreased to a value that is below the lowest required for this type of TLC to change colour. By scanning the probe in this manner, distributed measurements could be made. For real time, distributed measurements, multiple probes could be used to eliminate the need for scanning. Alternatively, a CCD camera system connected to a computer with image processing software could also be used.

On the bulk scale, yields from the Reimer–Tiemann reaction are frequently low, typical yields of 10 to 20% are not unusual.<sup>4</sup> Biphasic reaction systems such as the Reimer–Tiemann are often mass transfer limited as the reaction is restricted to the interface between the two immiscible phases. Mechanical agitation can be used in bulk scale reactors to increase surface area by creating smaller droplets. However, these droplets rapidly coalesce back into larger droplets with smaller surface to volume ratios. In a microfluidic mixer, the inherent high surface area to volume ratio provides a large interface boundary for efficient mixing. Friction between the solvent slugs and the channel walls causes internal circulation, continually replenishing the liquid/liquid interface and thus leading to further increased mixing.<sup>18</sup> Additionally, operating in a continual flow regime prevents coalescence of the two phases thus maintaining the interface boundary. In this way microreactors may be utilised to obtain increased reaction yields over bulk scale processes. Although yields of only 10% were obtained during these experiments, in these initial studies, a substantially lower concentration of aqueous base was used compared to traditional bulk syntheses to reduce the viscosity and hence, reduce back pressure. Consequently, the ultimate yields were relatively low when compared with those obtained from bulk scale systems.

To study the effect of temperature on the Reimer–Tiemann reaction in a continuous flow microfluidic device, the formylation of  $\beta$ -naphthol was performed (Scheme 1) at set flow rates over a range of temperatures. The transparency of the glass microreactor and the highly coloured nature of the intermediate (blue) and the product (red) enabled facile reaction progress monitoring by visual observation. Heat was supplied by the thin film resistive elements. Temperature sensing was performed by spectral TLC observation and product yields were obtained from GC analysis. The effect of

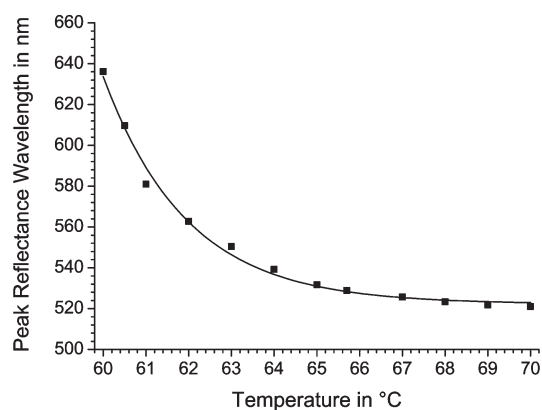


Fig. 3 The variation in the wavelength of maximum reflectance of the TLCs with increasing temperature.

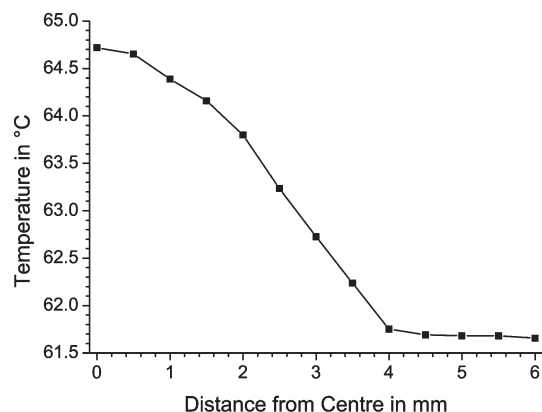
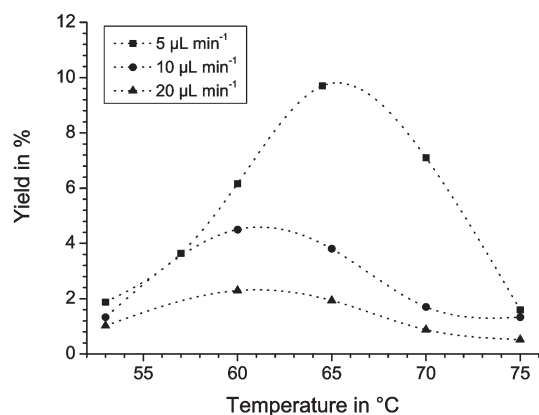


Fig. 4 The temperature profile across the device as a function of distance from the centre.





**Fig. 5** The effect of temperature on reaction yield in an enclosed microchannel. Temperature readings were taken from the centre of the chip.

increasing temperature in the microreactor for a given flow rate is demonstrated in Fig. 5, which represents the observed yield variation with temperature.

As expected, upon increasing the reaction temperature inside the microreactor, a corresponding increase in reaction rate was observed. However, once the boiling point of the solution was exceeded, thermal expansion and boiling led to severely decreased residence times. The use of TLCs combined with microheating elements made it possible to hold the temperature in the microreactor device at an optimal temperature such that the reactant mixture was kept just below its boiling point. Without the level of control demonstrated in this work, the reaction would have to be performed at a much lower temperature to avoid reflux and, as a consequence, the yield obtained would be significantly lower.

TLCs are ideal for microfluidic applications, as they enable the temperature of small microenvironments such as fluidic channels to be monitored, the size of which is only limited in this example by the bead size and the optical detection coupling. Reducing the size of the beads would allow smaller channels to be monitored and improving the collection optics would increase the spatial temperature resolution. Additionally, non-encapsulated TLC oils could be used to monitor the temperature of sub-micron channels.

Although the collateral device used in this work has been shown to be extremely useful, there are some disadvantages to this design. For microreactor applications, the temperature in the reaction channel will differ slightly from the temperature in the collateral channel due to the heat produced by the reaction itself. If the TLCs can be encapsulated within an inert polymeric material, for example, PTFE, then such beads could be introduced directly into the reaction channel and this would provide an *in situ* means for the measurement of channel temperature.

## Conclusions

The utility of thermochromic liquid crystals for accurate on-chip, distributed temperature determination and control in microfluidic devices has been demonstrated. TLCs also offer potential for *in situ* direct temperature determination of the reaction mixture by encapsulation in a suitably inert polymeric shell. In these initial experiments, a resolution of better than 0.4 °C was obtained over a relatively small temperature range using a manually controlled system. Automated temperature control may be achieved with improved resolution across a broader temperature range by utilising alternative TLC materials and applying a multi-wavelength detection algorithm with a computer regulated feedback loop. Such a system would be ideal for on-chip, continuous PCR, where several individual heated zones are required and temperature has a significant effect on the efficacy of the process.

**Alexander Iles,<sup>a</sup> Robin Fortt<sup>b</sup> and Andrew J. de Mello<sup>c</sup>**

<sup>a</sup>National Institute for Materials Science (NIMS), International Centre for Young Scientists (ICYS), 1-1, Namiki, Tsukuba, Ibaraki 305-0044, Japan. Fax: +81 (0)29 860 4706.

<sup>b</sup>Central Research Laboratories Ltd, Dawley Road, Hayes, Middlesex, UB3 1HH, UK. Fax: +44 (0) 20 8848 6565.

<sup>c</sup>Imperial College London, Exhibition Road, South Kensington, London, SW7 2AY, UK. Fax: +44 (0) 20 7594 5833; Tel: +44 (0) 20 7594 5820.

## References

- 1 K. F. Jensen, *Chem. Eng. Sci.*, 2001, **56**, 293.
- 2 H. Lowe and W. Ehrfeld, *Electrochim. Acta*, 1999, **44**, 3679.
- 3 A. d. Mello and R. C. R. Wootton, *Lab Chip*, 2002, **2**, 7N.
- 4 H. Wynberg and E. W. Meijer, *Org. React.*, 1982, **28**, 1.
- 5 O. Woz, K. Jackel, T. Richter and A. Wolf, *Chem. Eng. Technol.*, 2001, **24**, 138.
- 6 G. Veser, *Chem. Eng. Sci.*, 2001, **56**, 1265.
- 7 E. Garcia-Egido, S. Y. F. Wong and B. H. Warrington, *Lab Chip*, 2002, **2**, 31.
- 8 S. K. Ajmera, C. Delattre, M. A. Schmidt and K. F. Jensen, *Sens. Actuators, B—Chemical*, 2002, **82**, 297.
- 9 M. A. Northrup, B. Bennett, D. Hadley, P. Landre, S. Lehw, J. Richards and P. Stratton, *Anal. Chem.*, 1998, **70**, 918.
- 10 J. C. T. Eijkel, A. Prak, S. Cowen, D. H. Craston and A. Manz, *J. Chromatogr. A*, 1988, **815**, 265.
- 11 I. M. Hsing, R. Srinivasan, M. P. Harold, K. F. Jensen and M. A. Schmidt, *Chem. Eng. Sci.*, 2000, **55**, 3.
- 12 I. Schneegaß, R. Bräutigam and J. M. Köhler, *Lab Chip*, 2001, **1**, 42.
- 13 D. R. Ross, M. Gaitan and L. Locascio, *Anal. Chem.*, 2001, **73**, 4117.
- 14 A. Dodge, G. Turcatti, I. Lawrence, N. F. deRooy and E. Verpoort, *Anal. Chem.*, 2004, **76**, 1778.
- 15 K. Swinny and D. J. Bornhop, *Electrophoresis*, 2001, **22**, 2032.
- 16 A. M. Chaudhari, T. M. Woudenberg, M. Albin and K. E. Goodson, *J. Microelectromech. Syst.*, 1998, **7**, 345.
- 17 J. Liu, M. Enzelburger and S. Quake, *Electrophoresis*, 2002, **23**, 1531.
- 18 J. R. Burns and C. Ramshaw, *Lab Chip*, 2001, **1**, 10.