Electroosmotic Pumping of Organic Solvents and Reagents in Microfabricated Reactor Chips

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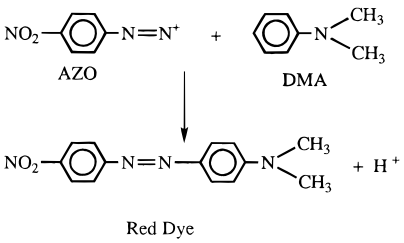
The ability to use electrokinetic effects to control fluid flow in a valveless microchip system has proven to be very powerful for analytical applications in aqueous solvents;[1−5] its extension to manipulating the flow of organic solvents for the purposes of on-chip organic synthesis could become equally useful. A complex network of channels within a microchip that uses electric fields to control the flow of organic solvents instead of valves, could control the sequential delivery of a variety of chemical reagents.[1−3,5] Such a system could be programmed to perform combinatorial syntheses.[6−9] This report demonstrates the first requirement for such an organic microreactor: electrokinetic control of the flow of some common organic solvents within a glass chip, as evidenced by controlled reagent mixing with a following chemical reaction.

Here we demonstrate an organic phase reaction of *p*-nitrobenzenediazonium tetrafluoroborate (AZO) and *N*,*N*-dimethylaniline (DMA) in a microfabricated glass chip, with fluid



Red Dye

pumping and control driven by electroosmotic flow (EOF). For illustration purposes, we selected an organic reaction that is relatively fast (a few seconds), proceeds in neutral or weakly acidic media, and generates a product we could monitor using absorbance detection.[10] Electroosmotic pumping and control of the delivery of the AZO and DMA reagents was shown in both protic (methanol) and aprotic (acetonitrile) solvents. Observation of EOF in glass chips is consistent with results in conventional fused silica capillaries, in which electroosmotic flow and electrophoretic separation in polar organic solvents

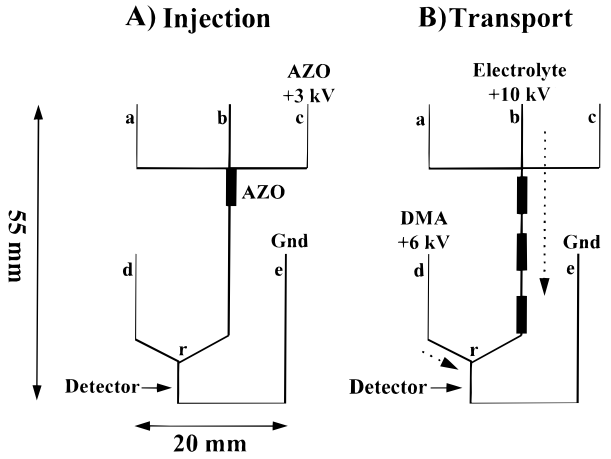


Figure 1. Schematic of PCRD2 microchip and the potential programs used. The channel lengths are approximately to scale. Reservoirs a, b, and e were filled with organic electrolyte solution. Reservoirs c and d contained 0.1% (m/v) *p*-nitrobezenediazonium tetrafluoroborate and 2% (v/v) *N*,*N*-dimethylaniline in electrolyte, respectively. Loading step: a, b, and d floating; +3 kV and ground connected to c and e, respectively. Transport step: a and c floating; +10 kV, +6 kV, and ground connected to b, d, and e, respectively.

with added electrolyte are known.[11−15] While there are a limited number of reports, most very recent, using nonaqueous solvents in CE,[11−15] there has been considerable use of polar organic solvents in conventional electrophoresis.[16,17]

The reactions were performed in a Corning Pyrex 7740 glass device with the same layout as one reported previously, called PCRD2.[3a] The layout is illustrated in Figure 1. The overall device size is indicated in the figure, and the channel lengths are approximately to scale. The exact lengths of each channel have been reported in detail elsewhere.[3a] However, the channels were etched to a 90 μm depth, to give a sufficient path length across the channel's depth for absorbance detection. Isotropic etching of the glass gave a channel width of about 190 μm at the top of the channel. Product formation was monitored by absorbance at 488 nm, using an Ar-ion laser source. After attenuation with a neutral density filter, the laser beam was focused with a 15 cm focal length lens onto the channel and directed with a mirror through the underside of the chip, so that it traversed the 90 μm depth of the channel. A 7× Rolyn Optical objective lens (0.2 numerical aperture, 12.5 mm working distance) was located above the chip to collect the transmitted light, directing it through a 400 μm diameter pinhole, through a 488 ± 1.5 nm laser line filter, and onto a photomultiplier tube. Further details on the detector are described elsewhere.[18]

The potential program for delivering and mixing the reagents under electroosmotic control is illustrated as two steps in Figure 1. A small plug of AZO was injected into the main channel

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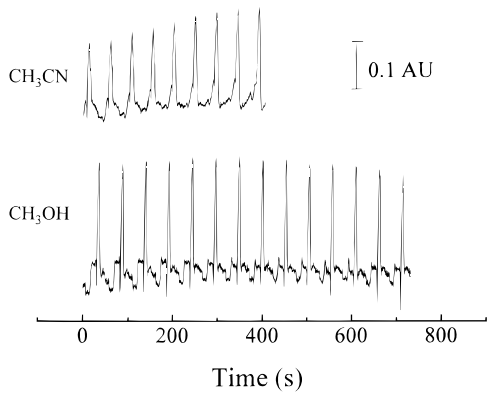

Figure 2. Plots of absorbance (AU) versus time at the detector, obtained by computer-controlled alternation between the loading and transport steps shown in Figure 1 to form dye product in methanol or acetonitrile solvent with 0.001 M TEAP electrolyte.

from the reagent reservoir (Figure 1a), then both AZO and DMA were driven toward the reaction point *r* (Figure 1b). Alternately switching the potentials under computer control between the two states in Figure 1 created a train of reagent plugs, which migrated toward the reaction point r. The AZO velocity with 10 kV between points b and e was 0.080 cm/s in CH_3CN and 0.047 cm/s in CH_3OH, giving apparent (i.e., overall) mobilities of 8.8 and 5.2 $\times$ 10^{-5} $cm^2/(V\cdot s)$, respectively.

The reagent plugs react with the electroosmotically driven flow of DMA downstream of the mixing point. Figure 2 shows that product formation in either acetonitrile or methanol resulted in an increase in absorbance. In either solvent the reaction could be consistently performed in a repetitive fashion, by alternately switching between the states a and b shown in Figure 1. Some noise was observed in the base around each reaction peak. Control experiments with no AZO present show that this effect arises in part from a difference in the refractive index of the solvent in the presence and absence of DMA, which is not driven by the detector during injection step a. Index changes were also caused by Joule heating effects arising from the applied potentials, accounting for the observed baseline drift. When corrected for baseline drift, the mean peak heights and areas for the product in methanol were 0.277 $\pm$ 0.008 (SD) and 1.31 $\pm$ 0.06, respectively. The equivalent results in acetonitrile were a mean height of 0.18 $\pm$ 0.01 and area of 1.08 $\pm$ 0.05. The data show the ability to control delivery of reagents in polar organic solvents in a programmable manner, with a precision of 3−6%.

The reaction efficiency was evaluated by mixing the same solutions off-chip and allowing them to react for 10 min, then determining the absorbance with a conventional spectrometer after 10-fold dilution, using 1 mm path length cells. Normalized to path length, the relative reaction efficiency on-chip was 22% in acetonitrile and 37% in methanol. The detector was located 5 mm downstream of the mixing point, allowing about 2.4 or 4.1 s for reaction in acetonitrile or methanol, respectively. The reduced reaction efficiencies on-chip likely indicate greater reaction time was needed. This suggestion is supported by the greater extent of reaction in the solvent with the lower flow rate; however, we did not move the position of the detector further downstream during this initial study.

Electrolysis at the electrodes can produce reactive products in organic solvents, and will change the local apparent pH,[13a] just as occurs in water. We employed large, 1 mL solvent reservoirs to reduce the effect of electrolysis products[13] and to prevent problems with solvent evaporation.[14] The use of organic phase buffers has been shown to prevent variation in the flow rates of 10−20% over a 10 h period, which are seen in absence of a buffer.[13a] However, the dye forming reaction studied is known to be pH sensitive, occurring only in neutral or very weakly acidic media.[10] Consistent with this, we found no reaction when tetraethylammonium (TEA) hydroxide was used as electrolyte. When an acidic mix of TEA acetate/acetic acid was used as electrolyte and buffer, a steady decline in product formation was seen with time, due to decomposition of the AZO reagent.[10] Consequently, 0.001 M TEA perchlorate (TEAP) was the preferred choice with this reaction. The data illustrated in Figure 2 could be obtained reproducibly for at least 1 h in our system, even without a buffer present. The use of 1 mM electrolyte ensured high electroosmotic flow rates. EOF increases with decreasing ionic strength in organic solvents,[13a] just as it does in water. Additionally, currents were held to less than 50 μA with this dilute electrolyte, which kept Joule heating effects relatively small (45 μA in acetonitrile, 20 μA in methanol).

Demonstration of the ability to control the flow of organic solvents greatly extends the possible range of reactions available to electrokinetically pumped systems for on-chip synthesis. Utilizing electroosmotic pumping to drive and control fluid flow within a manifold of integrated capillaries simplifies the fabrication of microfluidic devices, as no micropumps or valves are required and the chips can be made of chemically resistant materials.[1] Clearly, the final goal is to develop combinatorial synthesis on a microchip using a large number of parallel channels, driving different reagents to perform the required synthesis, all automated on a microscale.[6,7,9] Large quantities of material are not required in such syntheses when the goal is screening for drug leads, particularly if the biochemical assays to be used could ultimately be integrated within the same chip.[4] While many other integrated system elements will be required to achieve this, this study illustrates that one of the major requirements, valveless control of organic fluids within a microchip, is available.

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