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Transport and reaction of nanoliter samples in a microfluidic reactor using electro-osmotic flow

Kaushik Arumbuliyur Comandur\textsuperscript{1,3}, Ali Asgar S Bhagat\textsuperscript{2,3}, Subhashish Dasgupta\textsuperscript{1}, Ian Papautsky\textsuperscript{2,4} and Rupak K Banerjee\textsuperscript{1,4,5}

\textsuperscript{1} Department of Mechanical, Industrial and Nuclear Engineering, University of Cincinnati, Cincinnati, OH 45221, USA
\textsuperscript{2} Department of Electrical and Computer Engineering, University of Cincinnati, Cincinnati, OH 45221, USA
E-mail: rupak.banerjee@uc.edu

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Abstract
The primary focus of the paper is to establish both numerical and experimental methods to control the concentration of samples in a microreactor well. The concentration of the reacting samples is controlled by varying the initial sample size and electric field. Further, the paper numerically investigates the feasibility of mixing and reacting nanoliter samples with a wide variation in reaction rates in the microreactor driven by electro-osmotic pumping. Two discrete samples are measured and transported to the microreactor simultaneously by electro-osmotic pinching and switching. The transported samples are mixed in the microreactor and floated for 4.5 s for reaction to occur. It is seen that the normalized concentration of the product increases from 0.25 to 0.45 during that period. Also the effects of sample size and applied electric field on sample concentration during the switching process are studied. It is found that the normalized final sample concentration increases from 0.03 to 0.11 with an increase in sample size from 60 to 150 $\mu$m, at a constant electric field. Further, by increasing the electric field from 100 to 1000 V cm$^{-1}$, at a constant sample size, there is a significant decrease in the final concentration of the sample from 0.14 to 0.04. Our studies also show that the normalized product concentration depends on the reaction rate and increases from 0.28 to 0.48 as the reaction rate increases from 10 L mol$^{-1}$ s$^{-1}$ to 10\textsuperscript{5} L mol$^{-1}$ s$^{-1}$. However, the increase in the reaction rate beyond 10\textsuperscript{5} L mol$^{-1}$ s$^{-1}$ does not influence the product concentration for the present design of the microreactor. Our microreactor with improved mixing can be used for assessing reactions of biological samples. The optimized sample size along with a controlled electric field for sample injection forms the basis for developing a prototype of a microreactor device for high throughput drug screening.

1. Introduction

The study of fluid flow in microchannels is of significant interest due to applications in a wide variety of fields ranging from microscale flow injectors and microelectronic cooling systems to microreactors for bio-chemical and drug discovery systems [1]. The key requirement in these applications is the need for precise movement of micro- and nano-scale samples through the microchannel system, while having the unique advantages of dispensing low sample volumes and high throughput. Low sample volume is of particular importance in drug discovery due to the high costs and also, at times, the limited availability of the sample. Further, the samples sometimes have to be tested against large libraries of biological materials. This requires faster throughput which can be
achieved by cascading the microfluidic devices to ensure parallel processing.

1.1. Electro-osmotic pinching and switching methods

Although a number of methods exist for transporting and mixing precise microscale volumes of samples in microchannels and microreactors, the simplest method is the electrokinetic one that includes electro-osmotic pinching and switching. Electro-osmotic flow (EOF) occurs when the electrical double layer on the microchannel wall is imparted a momentum by the application of an external electric field. Electro-osmotic pinching and switching to control the movement of discrete sample volumes in microchannels were first demonstrated by Harrison et al [2]. Several experimental and numerical studies have been performed to characterize electro-osmotic flows in PDMS channels [3–5]. The processes of pinching and switching have also been evaluated [6, 7]. In our numerical and experimental studies, electro-osmotic pinching and switching techniques are employed to transport and mix precise quantities of samples in the reaction well of the microreactor [8]. The effects of sample mobility, buffer conductivity and sample diffusion coefficient on sample pinching and injection in cross channels have been studied numerically [9, 10]. Dasgupta et al [11] showed that electro-osmotic velocity varied nonlinearly with the applied electric field and wetted perimeter. These studies indicate that EOF is very sensitive to applied electric fields, channel dimensions and buffer conductivity. However, these studies do not provide a specific trend as to how sample concentration varies during electro-osmotic sample transport.

The necessity to study the variation of sample concentration under the influence of various factors arises from the need to obtain precise sample quantities and concentration in a microreactor well. To ensure proper sample delivery, the effects of the initial sample size and applied electric field on sample transport are studied here. In this work, the phenomena of electro-osmotic pinching and switching are used to precisely transport nanoliter quantities of reactants in the microfluidic reactor for mixing, reaction and product formation.

1.2. Mixing and reaction of samples in microreactors

A number of studies have been carried out to characterize chemical kinetics in microreactors [12–16]. These studies however carry out sample mixing and reaction under continuous flow conditions requiring larger sample volumes than in discrete flow. In our microreactor model, we present injection and mixing of precise quantities and concentrations of samples through the processes of pinching and switching. Our microreactor is capable of carrying out microscale reactions between bio-samples.

1.3. Biological reaction rates

Monoclonal antibodies (mAb) and their fragments continue to serve as important components of protocols for the targeted delivery of diagnostics and therapeutics to tumor cells [17, 18]. For example, they can be conjugated to a chelated radionuclide or subunits of bacterial toxins (direct targeting) or rendered bifunctional by conjugation to biotin, streptavidin or an enzyme (pretargeting). In a simplest mAb-based protocol, a mAb labeled directly with a radionuclide can be targeted for reaction with an antigen, Ag. The concentration of the product, mAb–Ag complex, is governed by rate constants, $k_f (\sim 1 \times 10^4 \text{ L mol}^{-1} \text{ s}^{-1})$ and $k_r (\sim 1 \times 10^{-6} \text{ L mol}^{-1} \text{ s}^{-1})$, for the forward and backward reactions, respectively. Compared to the slower reaction rate between Ag and mAb, a higher reaction rate of around $7 \times 10^7 \text{ L mol}^{-1} \text{ s}^{-1}$ is typical for a reaction between streptavidin, sAv, and biotin, b [19], which can be used in the detection of biomolecules during drug targeting. Thus, the rate constants can vary significantly, depending upon the selection of the reactants and their products. Hence, a wide range of reaction rates, $10 \text{ L mol}^{-1} \text{ s}^{-1}$ to $1 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$, is considered in our study. This encompasses typical reaction rates observed in biological molecular reactions. The theoretical reaction model considered by Fletcher et al [20] is applied in our study for calculating the reaction between the biological samples in the microfluidic device for drug screening and targeting.

1.4. Control of sample concentration in microreactor

In our studies, the sample concentration at the reaction well is controlled by adjusting the initial sample size and applied electric field. The samples are injected into the microreactor and the timing of sample delivery to the microreactor well is controlled by applying suitable electric fields. By studying the effects of the electric field on sample dispersion, the injection process can be optimized and adequate sample concentration available for reaction in the microreactor is ensured. The effect of sample size on sample concentration is also investigated in order to determine the suitable pinching conditions for sample transport to the microreactor. Experimental verification of the model is carried out in microchannels fabricated using PDMS/glass hybrid. PDMS offers a number of significant advantages, such as ease of fabrication of complex geometries [21, 22] and high replication fidelity [23].

2. Methods

Samples are pinched and injected towards the microreactor by applying suitable potentials at the reservoirs. The microchannel geometry, governing equations and boundary conditions used in the computational method are described below. An electrical analog model is developed to calculate potentials for sample pinching and switching. The experiments are performed in PDMS/glass hybrid microfluidic devices to validate the numerical results.

2.1. Computational methodology

Calculations are performed to solve the velocity, applied electric field and concentrations of the reacting samples and the product in the flow domain.
2.1.1. Geometry. The schematic diagram of the microchannel design is shown in figure 1(A). Samples are simultaneously injected by two pairs of intersecting microchannels and mixed in a microreactor. The height and width of each channel are fixed at 50 μm. The length of the injection arm is 15 mm, while the lengths of the other arms are fixed at 5 mm. The radius of the microreactor is 100 μm. A 2D computational geometry was modeled using CFD-GEOM (ESI-CFD Inc., Huntsville, AL). Structured meshing was used with 91 000 cells with an aspect ratio of 1.

2.1.2. Governing equations. While solving the governing equations, the assumptions made by Krishnamoorthy et al [24] are considered. Electro-osmotic flow is governed by the continuity equation (1) and Navier–Stokes equation (2) [25]:

\[ \nabla \cdot \mathbf{u} = 0 \quad (1) \]
\[ \rho \left( \frac{du}{dt} + \mathbf{u} \cdot \nabla \mathbf{u} \right) = \mu \nabla^2 \mathbf{u} - \nabla p + f_e \quad (2) \]

where \( u \) is the velocity (m s\(^{-1}\)), \( \rho \) is the buffer density (kg m\(^{-3}\)), \( \mu \) is the dynamic viscosity (N s m\(^{-2}\)), \( p \) is the pressure (N m\(^{-2}\)) and \( f_e \) is the electro-osmotic or coulomb force term due to the interaction of the wall charge with the applied electric field. Electro-osmotic force \( f_e \) is given by equation (3):

\[ f_e = \rho_e \cdot E \quad (3) \]

where \( \rho_e \) is the bulk charge density (C m\(^{-3}\)) and \( E \) is the applied electric field (V m\(^{-1}\)). The net electric potential has two components, the potential due to the charge on the wall \( \psi_y \), which decreases exponentially away from the wall (equation (4)), and the applied external electric potential \( \phi \). If the Debye layer thickness is small, the Debye–Huckel approximation is valid and the potential distribution at the wall is governed by the Laplacian equation (6). The potential due to the external field satisfies the Laplacian equation (6). The operator, \( \nabla \) for a 2D system in \( x \) and \( y \) plane is given by \( \partial/\partial x + \partial/\partial y \):

\[ \nabla^2 \phi = 0. \quad (6) \]

The electric field which causes electro-osmotic flow to occur is given by equation (7):

\[ E = -\nabla \phi. \quad (7) \]

Substituting equations (5) and (7) in the momentum equation (2), we get

\[ \rho \left( \frac{du}{dt} + \mathbf{u} \cdot \nabla \mathbf{u} \right) = \mu \nabla^2 \mathbf{u} - \nabla p + \varepsilon (\nabla^2 \psi \cdot \nabla \phi). \quad (8) \]

For the chemical reaction in the microreactor, we consider a bimolecular, irreversible reaction (equation (9)) with a forward rate constant \( (k_f) \) and a negligible backward rate constant \( (k_r) \). The governing equation of the chemical reaction between the reactants is given in equation (10):

\[ A + B \rightarrow C \quad (9) \]

\[ \frac{DC_i}{Dt} = D_i \frac{\partial^2 C_i}{\partial x^2} \pm k_f C_A C_B, \quad \text{where} \quad i = A, B, C \quad (10) \]

where \( D_i \) and \( C_i \) are the diffusion coefficients and species concentrations of A, B and C, respectively. The negative sign corresponds to the depletion of reactants A and B, while the positive sign represents the formation of product C. Samples A and B can be replaced by any chemical or biological samples such as biotin and streptavidin.
2.1.3. Boundary conditions. Input voltages are applied at all the seven reservoirs to calculate the electric field. The value for electro-osmotic mobility, \( \mu_{eo} \), for a 50 \( \mu \)m \( \times \) 50 \( \mu \)m microchannel was obtained from our previous study [11]. The zeta potential is calculated from the Helmholtz–Smoluchowski equation (11):

\[
U_{eo} = \frac{-\zeta \varepsilon E}{\mu} = -\mu_{eo} \cdot E \tag{11}
\]

where \( U_{eo} \) is the electro-osmotic velocity (m s\(^{-1}\)), \( \zeta \) is the zeta potential (V) and \( \mu_{eo} \) is the electro-osmotic mobility (m\(^2\) V s\(^{-1}\)). \( \zeta \) is applied at the microchannel walls as a boundary condition to calculate \( \psi_w \) (equation (4)). The potential at the wall, \( \psi_w \), is taken to be approximately equal to \( \zeta \). Concentrations of samples A and B are specified at reservoirs 1. Pressure at the reservoirs is set to atmospheric conditions.

An electrical analog model is used to determine the voltage boundary conditions. To predict the voltages to be applied at the reservoirs, a dc circuit analysis is carried out based on Kirchhoff’s law [26]. Figure 1(B) shows the equivalent dc circuit of the microchannel configuration along with the resistances. The dc circuit consists of seven voltage sources to sequence flows in the two intersecting microchannels. \( V_1-4 \) corresponds to the voltages applied in the reservoirs 1–4, respectively. The equivalent resistances for each arm are calculated by using the following equation:

\[
R = \frac{l}{\sigma \cdot A} \tag{12}
\]

where \( R \) is the resistance (\( \Omega \)), \( l \) is the length of the microchannel (m), \( A \) is the microchannel cross section (m\(^2\)) and \( \sigma \) (S m\(^{-1}\)) is the conductivity of phosphate buffer. The electrical model is solved for the currents in the arms for different values of applied voltages using B\(^2\) Spice (Beige Bag, Ann Arbor, MI). The voltages obtained from calculation for various pinching and switching conditions are shown in tables 1 and 2, respectively.

2.1.4. Material properties. A 10 mM phosphate buffer with a density of 1000 kg m\(^{-3}\), dynamic viscosity of 0.001 N s m\(^{-2}\) and relative permittivity (\( \varepsilon_r \)) of 80 is used in the calculations.

2.1.5. Finite volume method. To solve the governing equations, we make use of a multi-physics finite volume solver. The algebraic multigrid (AMG) solver (ESI-CFD Inc., Huntsville, AL) is used to solve the equations. Sample pinching is considered as a steady state process, as the time required to reach the steady state is in the order of micro seconds. The solution obtained in the pinching step is used as the initial condition for the switching process. The switching process is modeled as a transient process. The convergence criterion is set at 10\(^{-6}\).

2.2. Experimental methodology

2.2.1. Fabrication. The microfluidic channels are fabricated in PDMS using standard soft lithography methods [27]. Briefly, a 50 \( \mu \)m thick layer of SU-8 2075 (MicroChem Corp., MA) photoresist is patterned on a 3" bare silicon wafer using conventional photolithography. The patterned wafer is then treated with Sigma acetone (Cat # SL-2, Sigma Aldrich) to facilitate PDMS mold release. Next, the PDMS (Dow Corning) polymer is cast on the patterned wafer to replicate the microchannel features. Following polymer curing, the PDMS mold is peeled off from the SU-8 master, and holes for sample and buffer reservoirs are punched using a 6 mm (I.D.) hole puncher. The cast PDMS molds are 8 mm thick to increase the volume of sample in the reservoirs and to eliminate additional processing steps for reservoir integration. Next, 1 mm thick glass slides with a 200 nm thick gold (Au) evaporated layer are patterned to define the electrodes. The gold layer is patterned using S1818 photoresist followed by Au etching to define the electrodes. Finally, to complete the microfluidic device, the PDMS molds are bonded to a gold patterned glass slide using a corona tester (Electrotechnic Products Inc.). Figure 2 shows an illustration of the fabricated microfluidic chip.

2.2.2. Characterization. The microfluidic device is tested using an eight-port high voltage power supply (HVS448, Labsmith). Prior to testing, the microchannels are treated with a 1 M NaOH solution for 5 min. All experiments are conducted using 10 mM phosphate buffer (pH 7.5). To verify the numerical models, the device is characterized using a

| Table 1. Voltage conditions at the reservoirs for sample pinching. |
|-------------------|--|--|--|--|
| Pinching condition | \( V_1 \) | \( V_2 \) | \( V_3 \) | \( V_4 \) |
| Strong            | 378 | 378 | Ground | 900 |
| Medium            | 504 | 432 | Ground | 900 |
| Weak              | 900 | 492 | Ground | 615 |

| Table 2. Voltage conditions at the reservoirs for sample switching. |
|-------------------|--|--|--|--|
| Electric field (V cm\(^{-1}\)) | \( V_1 \) | \( V_2 \) | \( V_3 \) | \( V_4 \) |
| 100               | 240 | 480 | 240 | Ground |
| 200               | 480 | 960 | 480 | Ground |
| 300               | 720 | 1440 | 720 | Ground |

| Table 3. List of parameters used in numerical calculations. |
|-------------------|----------|
| Parameters       | Value    |
| Buffer concentration | 10 mM    |
| Buffer density    | 1000 kg m\(^{-3}\) |
| Dynamic viscosity | 0.001 N s m\(^{-2}\) |
| Relative permittivity | 80       |
| Electrical conductivity | 0.17 S m\(^{-1}\) |
| Sample diffusivity  | \( 10^{-10} \) m\(^2\) s\(^{-1}\) |
| Electro-osmotic mobility | \( 4 \times 10^{-4} \) m\(^2\) V s\(^{-1}\) |
| Reaction rates    | \( 10^{-7} \) L mol\(^{-1}\) s\(^{-1}\) |

The electrical conductivity (\( \sigma \)) of the buffer is calculated as 0.17 S m\(^{-1}\) with sample diffusivity set to \( 10^{-10} \) m\(^2\) s\(^{-1}\). Table 3 lists the parameters used in the numerical calculations.
10 μm fluorescein sample solution. To visualize and confirm sample loading and injection during testing, high speed images of the channel are captured at the loading junction and the microreactor using an inverted epi-fluorescence microscope (Olympus IX71) equipped with a 12-bit CCD camera (Retiga EXi, QImaging). To analyze the sample position and concentration, grayscale line scans across the microchannel width of the acquired images are recorded using ImageJ®. The fluorescent intensity peaks from the line scans are then used to determine the sample position downstream for different loading and switching conditions. The line scans are also used to compare the numerical results indicating the sample dispersion and diffusion at varying downstream positions.

3. Results and discussion

Results of numerical simulations and experiments for pinching, switching and reacting micro samples in the microreactor by electro-osmotic pumping are analyzed. Electro-osmotic sample pinching and switching are very briefly discussed followed by the effects of sample size and applied electric field on sample concentration. Finally, sample mixing and reaction at different reaction rates in the microreactor are shown. The sample concentration in each case is normalized with respect to the concentration of the sample at the channel intersection. The numerical and experimental normalized peak concentrations for the given cases agree within 13% 

\[
\frac{0.34 - 0.3}{0.3} \text{ at } 300 \text{ V cm}^{-1}
\]

The positions of the samples after 1 s are 400, 800 and 1200 μm at electric fields 100, 200 and 300 V cm\(^{-1}\) respectively, which indicates a linear increase in velocity (table 4) with an electric field as per the Helmholtz–Smoluchowski equation (11). The experimental and numerical velocities as shown in table 4 agree within about 6% 

\[
\frac{0.04 - 0.0376}{0.04}
\]

The numerically obtained sample lengths after 1 s are 250, 450 and 500 μm for electric fields 100, 200 and 300 V cm\(^{-1}\). The error in axial lengths of the sample between the numerical and experimental data is within 15% 

\[
\frac{500 - 425}{500} \text{ at } 300 \text{ V cm}^{-1}
\]

These results indicate an acceptable agreement between the numerical and experimental data.
3.2. Effect of sample size on sample concentration

The effect of sample size or plug length on the final sample concentration in the microreactor is investigated both numerically and experimentally. In this study, the electric field for the injection process is kept constant at 200 V cm\(^{-1}\). The sample lengths are varied from 60 μm to 150 μm. The maximum size of the plug is fixed at 150 μm as longer axial length plugs are difficult to maintain and control. Longer plugs are found to be significantly affected by even small pressure differences between the reservoirs [28].

The contours of the sample at three different time intervals 0.05, 0.15 and 0.5 s after the switching step is shown in figure 4 at 200 V cm\(^{-1}\). The dispersion of the sample can be attributed to three reasons. Firstly, the semicircular sample at the start of switching (\(t = 0\) s) undergoes a deformation as it tries to attain a plug-shaped velocity profile and this results in dispersion of the sample. Secondly, the nonlinear forces caused by the gradient of voltage at the center of the cross channel causes the sample to disperse. Thirdly, it is seen that high concentration gradients between the plug and the leading and trailing buffer result in a significant decrease in initial concentration. Once the sample moves further downstream, the sample dilution takes place solely due to molecular diffusion.

Figure 4. Numerical and experimental sample positions at different times illustrating the deformation of the sample shape during the switching process.

We are interested in the final concentration of the sample in the microreactor at different initial sample sizes. Figure 5(B) shows numerical and experimental results of the effect of sample length on the final concentration of the sample at the inlet of the microreactor. It is found that the final concentration of the sample increases linearly with increasing initial sample length. As the initial length of the sample increases from 60 μm to 150 μm, the experimentally obtained final concentration increases from 0.03 to 0.11. A

![Figure 5](image-url)

Figure 5. (A) Numerical results indicating normalized sample concentration along the injection arm as a function of sample size at a fixed electric field of 200 V cm\(^{-1}\). (B) Numerical and experimental results indicating the effect of sample size on the final concentration of the sample in the microreactor. The samples are injected at a fixed electric field of 200 V cm\(^{-1}\).

The plot shows that there is a significant drop in initial sample concentration within a few millimeters downstream of the microchannel after which there is a gradual decrease in concentration. As shown in figure 5(A), the normalized concentration for a 150 μm wide plug reduces from 1.0 to 0.4, whereas for a 60 μm wide plug the normalized concentration reduces to 0.1 within 0.1 cm of the microchannel intersection. Once the sample moves further downstream, the sample dilution takes place solely due to molecular diffusion resulting in a gradual decrease in the concentration of the sample downstream.

Numerical results showing the variation of the concentration of the sample from the center of the intersection to the inlet of the microreactor, at different initial sample size, and constant electric field are depicted in figure 5(A).
similar linear trend is seen in the numerical results shown in figure 5(B) ($R^2 = 0.994$). The numerical and experimental results agree within 10% of each other. For a flow that is primarily governed by an electro-osmosis phenomenon, sample dilution occurs only due to diffusive forces. This is because of the plug-like flow, which has a uniform axial velocity profile. The absence of any gradient in the velocity profile results in reduced dispersion of samples [29]. Crabtree et al [30] have reported anomalies during injection and separation due to pressure gradients. The presence of a linear trend seen in figure 5(B) indicates that the flow occurring in the microchannel is predominantly electro-osmotic with negligible pressure-driven flow.

3.3. Effect of an electric field on sample concentration

Numerical results of the effect of an electric field on final sample concentration in the microreactor. The initial sample size was fixed at 150 μm. The presence of a linear trend seen in figure 5(B) indicates that the flow occurring in the microchannel is predominantly electro-osmotic with negligible pressure-driven flow.

![Numerical results indicating the effect of electric field on sample concentration in the microreactor. The initial sample size was fixed at 150 μm.](image)

Figure 6. Numerical results indicating the effect of electric field on sample concentration in the microreactor. The initial sample size was fixed at 150 μm.

### 3.3.1. Sample mixing and reaction

The final phase of this study was to investigate the feasibility of simultaneous sample injection to the microreactor and visualize the reaction process. The studies on the effect of sample size and electric field on sample concentration helped us to optimize the size of the sample and also the electric field to be used during the injection process. A plug length of 150 μm and an electric field of 200 V cm$^{-1}$ were used. Figures 7(A) and (B) show the numerical and experimental images of two samples arriving together in the microreactor at $t = 18.5$ s. The numerical and experimental concentrations of the samples along the diameter cross-section X–Y of the microreactor are shown in figure 7(C). From the figure it can be seen that samples A and B each fill half of the microreactor. The line scan from the experimental data is smoothed using 10-point averaging, and the experimental and numerical results match within 8% of each other. The concentration of the samples in the microreactor is normalized with the concentration of the sample arriving at the inlet of the microreactor. Mixing of samples can be achieved by simply floating the samples in the microreactor for a sufficient period of time. The arms are floated by grounding the voltages at the reservoirs. If the two samples were reactive by nature, then there would have been a chemical reaction in the microreactor, thereby resulting in product formation.

Numerical plots of sample mixing and reaction between samples A and B at $t = 18.5$ s and $t = 23$ s are shown in figures 8(A) and (B), respectively. The arms are floated for 4.5 s for purposes of mixing and reaction. We can see that as the inlet concentration of the samples decreases from 1 (arrow #1 in figure 8(A)) to 0.8 (arrow #1 in figure 8(B)), there is an increase in the concentration of the product from 0.25 (arrow #2 in figure 8(A)) to 0.45 (arrow #2 in figure 8(B)) in the microreactor at time 23 s. After the samples have reacted they are taken out of the microreactor to the outlet reservoir by...
reapplying the appropriate electric fields. It is seen that there is an almost 100% increase in product formation from 0.25 to 0.45 between times \( t = 18.5 \) s and \( t = 23 \) s. This is one of the several advantages of microscale reactions, namely faster reactions of nanoliter samples due to an increase in interfacial area as compared to macroscale reactions.

Finally, a numerical calculation was performed to study a chemical reaction between two samples in the reaction well, at varying reaction rates. Figure 9 shows the numerically calculated product concentration as a function of reaction rate. The reaction rates ranged from a very low value of \( 10 \text{ L mol}^{-1} \text{ s}^{-1} \) to a high value of \( 1 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1} \), which encompasses typical reaction rates observed in molecular biological reactions. As the reaction rates increase, the normalized product concentration increases from 0.28 at \( 10 \text{ L mol}^{-1} \text{ s}^{-1} \) and saturates at 0.48 for reaction rates \( 1 \times 10^5 \text{ L mol}^{-1} \text{ s}^{-1} \) and beyond. Thus, the product concentration is influenced only for the lower values of the reaction rate. At a reaction rate of \( 1 \times 10^6 \text{ L mol}^{-1} \text{ s}^{-1} \), which is a typical reaction rate between monoclonal antibody, mAb, and antigen, Ag [17], the normalized product (mAb–Ag) concentration is 0.47. In contrast, for a threefold higher reaction rate of \( 7 \times 10^7 \text{ L mol}^{-1} \text{ s}^{-1} \), between streptavidin (sAv) and biotin (b) [19], the product (sAv–b) concentration increases insignificantly to a value of 0.48. It is therefore evident that for the present design of the microreactor, the higher reaction rates have no influence on product concentration. Consequently, the mixing process becomes diffusion dominated; thus, taking a longer duration for product formation.

In the experiments, two non-reacting fluorescent dyes were brought in contact in the microreactor. The aim of our experiments was to study the feasibility of the designed microreactor to transport and mix precise quantities of microscale samples. Due to the intrinsic complexities associated with experimental testing of reactive samples, as a first step both electrohydrodynamic (EHD) flow experimental and numerical models were developed to show the feasibility of the developed system. The EHD numerical models were validated with experimental data, for accurately solving the electric field and the species transport (diffusion) equations. The electric field was evaluated experimentally by testing different sample pinching and switching conditions. The species transport was assessed experimentally by comparing the sample concentration in the microreactor for varying...
initial sample plug sizes and varying electric fields in the switching arm. After establishing good agreement between the models and experiments (<20% variation), models alone were used to simulate biologically relevant reactions in the microreactor. It is apparent that the aspect of chemical reaction and mixing of complex biological samples will need further experimental evaluation in the near future. In future studies, we intend to bring together two reacting samples in the microreactor with sensors incorporated to detect a reaction. Further, by increasing the residence time of the samples in the microreactor, better mixing and reaction can be achieved and controlled depending on the application.

4. Conclusions

In this study, we have reported numerical and experimental results of studying electro-osmotic pinching, switching and reaction of samples in a microreactor under different pinching conditions, applied electric fields and reaction rates. The ability to load and dispense finite volumes of samples to the microreactor simultaneously is demonstrated. The influence of sample size on the final concentration is investigated numerically and experimentally, indicating that the final concentration of the sample increases linearly with sample size under predominant electro-osmotic flow conditions. Longer samples have significantly higher concentrations in the microreactor due to reduced sample dispersion. It is also found that the applied electric field affects the rate of sample dispersion, hence the final concentration in the microreactor. Increasing the applied electric field results in lower sample concentration in the microreactor well. Also, the product concentration exhibits an increasing dependence on lower

Figure 8. Numerical results showing variation of the concentration of reactant samples A and B and product C along the microreactor diameter at (A) $t = 18.5$ s and (B) $t = 23$ s.

Figure 9. Numerical results indicating the product concentration at $t = 23$ s at different reaction rates. The reaction rates, $k$, range from a very low value of $10 \text{ L mol}^{-1} \text{ s}^{-1}$ to a high value of $1 \times 10^{7} \text{ L mol}^{-1} \text{ s}^{-1}$. $k = 1 \times 10^{4}$ corresponds to the reaction between antigen, Ag, and monoclonal antibody, mAb, to produce mAb–Ag. $k = 7 \times 10^{7}$ corresponds to the reaction between streptavidin, sAv, and biotin, b, to produce b/sAv.
reaction rates and saturates at higher reaction rates as the mixing process in the microreactor is predominantly diffusion controlled. Our simple microreactor with improved mixing can be used for assessing reactions of biological samples. The optimized sample size along with a controlled electric field for sample injection forms the basis for developing a prototype of a microreactor device for high throughput drug screening. Further, the design presented herein is planar and easy to fabricate and sample pumping is done using electro-osmotic flow. A low pressure system such as the device for this study can easily be cascaded and integrated with other microfluidic components for micro total analysis or lab-on-a-chip system.

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Appendix

The process of electro-osmotic pinching and subsequently electro-osmotic switching are shown in figures A1 and A2, respectively. Both numerical and experimental results (pseudo colored green) are shown illustrating pinching and switching.
at $t = 1$ s at 100 V cm$^{-1}$. Channel walls are highlighted for visualization.

**Pinching**

In the pinching process, measured quantities of samples are loaded at the inlet reservoirs and brought to the channel intersection by applying suitable voltages at the reservoirs. Both numerical and experimental contours of the sample during the pinching process are shown in figure A1. The size of the loaded sample is controlled by changing the applied electric fields to give strong, medium and weak pinching conditions. The pinching process is repeated multiple times to ensure reproducibility of results. For example, the voltages at reservoirs 1, 2, 3 and 4 (figure 1(A)) for strong pinching are 378 V, 378 V, 0 V and 900 V, respectively, as shown in table 1. For the medium and weak pinching conditions, rows 2 and 3 of table 1 can be referred to. Thus, the fluorescein sample is pinched at the center of the microchannel without floating; in other words, for better control of the plug, voltages are applied at reservoirs 2 and 4 as shown in table 1.

A floating condition, where voltage at reservoirs 2 and 4 are zero, would have resulted in lesser control of the plug. Jin et al. [32] showed that pinching allows better control of sample size than floating. The length of the plug is measured from the base of the channel junctions $J_1$ and $J_2$ to define the sample plug length. Lengths of the pinched samples are $60 \mu$m, $75 \mu$m and $150 \mu$m at strong, medium and weak pinching, respectively. The numerical and experimental lengths agree within 10% of each other. The numerical and experimental images of sample pinching are shown in figure A1 for one pair of intersecting channels; the results are identical for the other pair.

**Switching**

The next step after pinching is to inject the fluorescein sample into the microreactor through the injection arm. This is the sample switching process (figure A2) and is achieved by changing the electric fields in the arms. Figure A2 shows the numerical and experimental contours of the sample being transported from the microchannel intersection to the microreactor, at an electric field 100 V cm$^{-1}$. The voltages applied for sample switching are shown in table 2. The electric field between the center of the cross junctions ($J_1$ and $J_2$), shown in figure 1(A), and the microreactor determines the electro-osmotic velocity with which the samples move in the injection arm. In the present system, movement along each arm is controlled independently. If biological species are introduced in the system, the voltages in the arms can be varied accordingly to compensate for the velocity change due to electrophoresis. For example if sample A has a higher electrophoretic mobility than B, the velocity with which it moves will be lower than sample B. Therefore, by increasing the electric field in the injection arm sample A can be made to move faster to ensure that both the samples arrive at the same time.

**References**


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