AC electrothermal enhancement of heterogeneous assays in microfluidics

Hope C. Feldman, Marin Sigurdson and Carl D. Meinhart

5.1 Velocity Results

5.1.1 PIV Measurement Inaccuracies.

The particle velocity may differ from fluid velocity if a force on the particles, such as gravity or dielectrophoresis (DEP) is substantial compared with the fluid drag force on the particles [1]. Since DEP scales with particle radius cubed, the 0.7 μm dia. particles will follow the fluid to within approximately 5%, under the current experimental conditions.

Additionally, the measured peak velocity may be lower than the true peak velocity due to averaging inherent in the micro-PIV method. The measurement is averaged in the out-of-plane (y) direction over the depth of focus of the objective lens (here, about 15 μm for our 40X , NA=0.75 lens), and in the x- and z-directions by the size of the interrogation region in the PIV processing (10 μm × 10 μm). Even with these large averaging domains, the reported peak velocity would not be reduced by more than about 10%. This cannot account for the 1.5 order of magnitude discrepancy observed. We conclude, therefore, that the velocity discrepancy is a physical phenomenon and not an artifact of the PIV technique.

5.1.2 Physics

1. Reduced Electric Field. A reduced electric field in the fluid would explain the lower velocity. Because of the 4th power dependence on voltage, a reduction of the effective voltage experienced by the fluid to 38% of the applied voltage would explain the lower velocities measured experimentally (see Fig. 3). Electrode polarization can be responsible for reducing the effective electric field, particularly in highly conductive solutions. The potential drop across the double layer (by which potential drop across the fluid bulk is reduced) can be estimated for an electrode pair with a narrow gap

$$\Delta\phi_{DL} = \frac{V_o}{2 + i\omega\pi(\varepsilon/\sigma)\kappa x} \qquad (9)$$

where $\kappa = \lambda^{-1}$, and $\lambda \approx 1$ nm is the double layer thickness estimated according to [2]. In our case, however, the applied frequency is sufficiently high and the double layer sufficiently thin that the fractional drop of the applied potential over the double layer is only $|\Delta\phi_{DL}/V_o| \approx 0.01$, suggesting

electrode polarization is not a significant factor.

Additionally, if electrode polarization were to reduce significantly the effective voltage, the ohmic current through the fluid would also be reduced. Electrical current measurements do not show a reduced current. Therefore, electrode polarization is not considered to be significant.

2. Competing body forces on fluid. A competing body force on the fluid may reduce the observed velocity. In the current experiment, thermally-generated buoyancy forces act in the opposite direction from electrothermal flow, forcing the warmer fluid between the electrodes up. Through experiments, we have come to realize that buoyancy forces can be important for (1) deeper (~1 mm) channels, and (2) thermally insulating base material (such as glass, rather than silicon). We have eliminated these two conditions as possible sources for the discrepancy in velocity. The characteristic Rayleigh number is $Ra = g\beta\Delta T h^3 (v\alpha)^{-1} \approx 0.04$, and therefore buoyancy forces are not important in for the current operating conditions; where β is the thermal expansion coefficient, h is the cavity height, v is the dynamic viscosity, and α is the thermal diffusivity of the fluid.

3. Reduced thermal gradient. The device is dependent on a steep thermal gradient in the vicinity of the high electric field. This gradient is maintained by (1) the generation of heat in the fluid, and (2) the removal of heat through the base, made possible by the highly conductive silicon substrate, which is thermally coupled to a TEC. A reduction in the thermal gradient, caused by lower material conductivity, lower heat generation, lower heat removal, or competitive heat generation will result in reduced velocity. However, numerical simulations suggest none of these effects are likely to occur or significant, and therefore the discrepancy in velocity does not result from a reduced thermal gradient.

5.2 Binding Results

Temperature Effects on Binding

The Eyring equation describes the temperature dependence of reaction rate:

$$k_{on,off} = \frac{k_B T}{h} \exp(-\Delta G / RT) \qquad (1)$$

where $k_{on,off}$ is the association / dissociation rate, k_B is the Boltzmann constant, T is the absolute temperature, h is Planck's constant, ΔG is the activation energy of dissociation of the protein-ligand system, and R is the universal gas constant [3]. In terms of absolute temperature, the maximum variation in temperature, 11.8 K out of 300 K, is only 4%; the variation in the kinetic

rate constants should be similarly small. For a detailed description, refer to Chilkoti and Stayton's (1995) [4] in depth study on the kinetics of biotin-streptavidin system for temperatures ranging from 10 to 35°C. They suggest that the local temperature increase in our system is not sufficient to denature the protein-ligand and is therefore amenable for immunoassays.

Supplementary References:

1   D. Wang, M. Sigurdson and C.D. Meinhart, *Exp. Fluids,* 2005, **38**, 1-10.
2   R.F. Probstein, *Physio-chemical Hydrodynamics, An Introduction*, 1994, Wiley Interscience, New York, 192.
3   H. Eyring, *J. Chem. Phys.*, 1935, 3, 107-115.
4   A. Chilkoti and P. Stayton, *J. Am. Chem. Soc*., 1995, **117**, 10622-10628.