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Measurement of Translational Diffusivity by Microchannel Multiphase Flow*

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Abstract

A method is proposed for the rapid measurement of translational diffusion coefficient by employing the unique multiphase flow of perfectly miscible incompressible fluids in microchannels. The molecule of interest is injected, as a bolus or continuously, into the middle phase of a three phase flow system. Subsequently, the phase or stream is depleted of molecule by diffusion into the adjacent streams through the common interface. By monitoring the elution profile of the molecule, one may obtain estimates of the diffusion coefficient under fixed experimental conditions. Transport of molecule in the microchannel is described by a transient convection-diffusion-dispersion model. Analytical and numerical solutions are provided to illustrate the change of elution profile with stream velocity and other design parameters. The diffusion coefficient is estimated from a graphical plot. It is shown that diffusion coefficient may be measured without prior knowledge of dispersion. Experimental data is presented for the proteins, fibrinogen and insulin. A number of advantages over the T-sensor and Taylor-Aris methods are highlighted.

Key words: Multiphase Flow, Microchannel, Diffusion Coefficient, Protein, Transport Model

1. Introduction

Measurement of the orientation averaged translational diffusion coefficient of molecules and macromolecules is important in describing and understanding numerous chemical and biochemical processes. Some well established methods for determining diffusion coefficient are dynamic light scattering, ultracentrifugation, spin-echo NMR, Taylor-Aris dispersion, and fluorescence recovery after photobleaching (FRAP)[1]. The Taylor-Aris method is often preferred for its simplicity and cost as compared to the other methods [2]. However, this method suffers from limitation of long residence times on the order of hours. More recently, the use of narrow bore glass capillaries (diameter of 50 to 100 μm) has reduced the residence time considerably although it still remains high for larger sized solutes [3-5]. Moreover, a number of non ideal experimental conditions, such as unsteady flow, can give rise to large errors in the measured diffusivity [6].

An alternate method that overcomes the Taylor-Aris long time asymptotic limit has been proposed recently and is known as the T-sensor [7]. The T-sensor employs two phase flow of two perfectly miscible aqueous fluids in a microchannel to make rapid measurements of molecular diffusion. Fluorescently labeled protein diffusion across the common interface of the two streams was measured by epi-illumination microscopy to yield diffusivities within 2.4% of literature values [7]. Some limitations of this method include

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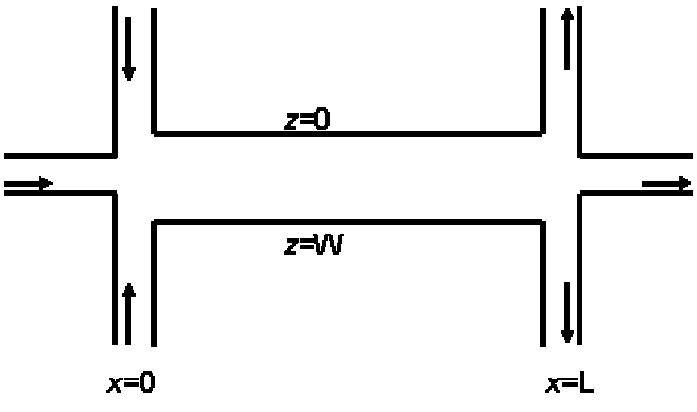
requirements of fluorescence tagging and detailed microscopic imaging. In this system, variable scaling law (one third instead of one half) for diffusive broadening of the interface near and parallel to the channel walls, has been reported [8] and found to revert to the one half law at large distances downstream [9].

Measurement of biomolecule diffusivity presents a number of additional difficulties, the two most ubiquitous ones being adsorption onto surfaces and molecular aggregation. The former is especially prevalent in microchannels due to the high surface area to volume ratios. Thus it is mandatory to employ a coating or blocking strategy, to prevent adsorption, in order to make accurate measurements of biomolecular transport in solution [10]. Aggregation of biomolecules may be alleviated by dilution, which predicates the use of a sensitive detection method.

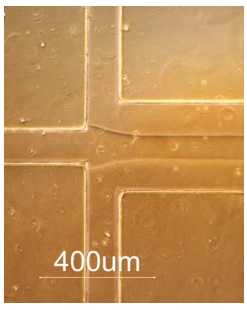
A theoretical and experimental study is presented here, to demonstrate diffusivity measurement using three phase flow in a microchannel. A theoretical description of microchannel transport of the diffusing molecule is given first. Transient analysis of a bolus as well as steady state elution of a continuously infused stream is discussed. The general method for determining the translational diffusion coefficient is elucidated. Experimental results are presented for two proteins, fibrinogen and insulin. The advantages and potential limitations of this method are also discussed.

2. Theory

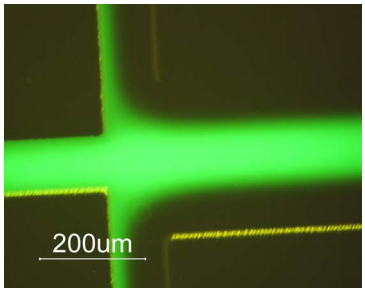
The physical system depicted in Figure 1(a), comprises of three inlet streams merging and flowing through a length of microchannel before exiting into three outlets. Owing to the special nature of microscale flow, the three merging incompressible fluid streams give rise to three phase flow and molecular transport across the two common interfaces occurs by diffusion only (Figs. 1(b) and 1(c)). A mathematical description of solute transport in the microchannel is presented for two cases, a bolus of solute, and continuous solute infusion.



(a)



(b)



(c)

Fig. 1. (a) Schematic representation of the three inlet and outlet microchannel with arrows denoting the direction of fluid flow, (b) brightfield image (100x) of the microchannel inlet, (c) Fluorescence image of fibrinogen at the microchannel outlet showing the protein diffusing into adjacent streams.

The governing mass balance equation for solute of concentration (C) and applicable to both the cases is

$$\frac{\partial C}{\partial t}+U\frac{\partial C}{\partial x}-D_L\frac{\partial^2 C}{\partial x^2}-D_m\frac{\partial^2 C}{\partial z^2}=0 \tag{1}$$

Where, the average solute (assumed equal to fluid) velocity is U. For the analysis presented here, the viscosity of the three streams is assumed nearly equal and constant, so that the pressure driven flow resembles Poiseuille flow. The longitudinal dispersion coefficient is D_L and the molecular or translational diffusion coefficient is denoted by D_m. For the case of a bolus of solute, the initial and boundary conditions are given as

$$C(t=0,x,z): \quad C=0 \tag{2}$$

$$C(0<t\le t_1,x=0,z_1\le z\le z_2): \quad UC-D_L\frac{\partial C}{\partial x}=UC_0;$$

$$C(t>t_1,x=0,z_1\le z\le z_2): \quad UC-D_L\frac{\partial C}{\partial x}=0;$$

$$C(t,x=0,0\le z\le z_1): \quad UC-D_L\frac{\partial C}{\partial x}=0; \tag{3a-d}$$

$$C(t,x=0,z_2\le z\le W): \quad UC-D_L\frac{\partial C}{\partial x}=0;$$

$$C(t,x=L,z): \quad \frac{\partial C}{\partial x}=0;$$

$$C(t,x,z=0): \quad \frac{\partial C}{\partial z}=0; \tag{4a-c}$$

$$C(t,x,z=W): \quad \frac{\partial C}{\partial z}=0.$$

The three phases extend from (0, z_1), (z_1, z_2) and (z_2, W) in the microchannel. W and L are the width and length of the main microchannel respectively. A bolus of length b will result in t_1 = b/U. The case of continuous solute infusion may be analyzed as a transient or steady state problem. The steady state problem is presented here and describes the experiments discussed below. The transient term as well as the dispersion term may be removed from Eq. (1), simplifying it considerably, and making possible an easily derived analytical solution. The boundary conditions for this case is

$$C(x=0,z_1\le z\le z_2): \quad C=C_0;$$

$$C(x=0,0\le z<z_1): \quad C=0; \tag{5a-c}$$

$$C(x=0,z_2<z\le W): \quad C=0;$$

$$C(x,z=0): \quad \frac{\partial C}{\partial z}=0; \tag{6a-b}$$

$$C(x,z=W): \quad \frac{\partial C}{\partial z}=0$$

The solution is given as

$$\frac{C(x,z)}{C_0}=\frac{(z_2-z_1)}{W}+\sum_{n=1}^{\infty}\left(\frac{2}{n\pi}\right)\left[\sin(\frac{n\pi z_2}{W})-\sin(\frac{n\pi z_1}{W})\right]\cos(\frac{n\pi z}{W})\exp\left[-\frac{n^2\pi^2 D_m x}{UW^2}\right] \quad (7)$$

The analytical solution for bolus transport is obtained by solving Eq. (1) without the dispersion term. The solution is given by Eq. (7) with the right hand side multiplied by ($\bar{U}(t\text{-}x)$-$\bar{U}(t\text{-}t_1\text{-}x)$), where $\bar{U}$ is the unit step function. For continuous infusion, the average solute concentration in the outlet middle stream is estimated from

$$C_e=\frac{\int_{z_1}^{z_2}C(L,z)dz}{(z_2-z_1)} \quad (8)$$

A finite difference scheme was implemented to solve Eqs. (1)~(4). Errors arising from the convection term are overcome by quadratic upstream interpolation. Stepping in time was done by simple Euler algorithm. The numerical code was verified with the analytical solution given by Eq. (7) and published results for the case of transient longitudinal dispersion [10]. All computations were carried out on a desktop PC with a 2.4GHz Intel processor and 1GB of RAM.

3. Experimental procedure

Biomolecules. Proteins, insulin and fibrinogen, were purchased from Sigma-Aldrich (USA) and Fluka Buchs (Switzerland) respectively. The proteins were reacted with fluorescein succinimidyl ester (Molecular probes, USA) and the reaction mix was separated with a G25 column (Alltech, IL, USA). The labeling was verified by dual wavelength HPLC (Waters, USA) on an Alltech Macrosphere GPC column. Protein concentrations were measured by the BCA assay (Pierce, USA) and UV absorption at 280nm.

Microchannel fabrication. Poly(dimethylsiloxane) or PDMS (Sylgard 184, Dow Corning, USA) was the polymer of choice for fabricating the microchannels using the soft lithography method. Briefly, a master was first produced with a suitable photoresist and photomask design of the microchannels. PDMS was then micromolded against the master template and cured at 65°C to yield three sides of the microchannels. The fourth side was a glass slide sealed against the other sides with an adapter. Main channel dimensions were 300 μm(W) x 50 μm(H) x 2 cm(L), while the inlet and outlet channels were all identical at 110 μm(W) x 50 μm(H). Inlets and outlets were punched in the PDMS and tubing inserted to complete connections with external pumps and sensors. Microchannels were coated with bovine serum albumin to prevent adsorption.

Diffusion experiments. A syringe pump (model: A-74901-15, Cole Parmer, USA) was connected to the three inlet ports of the microchannel via PEEK tubing. The left and right inlet ports connect to syringes (Becton Dickinson, USA) containing phosphate buffer, pH 7.4 with 0.05% azide. The middle inlet port connects to a syringe containing the biomolecule of interest in phosphate buffer with azide and also passes through a flow cell to measure the inlet concentration. The three outlet ports lead to waste collection tubes, however, the central outlet port passes through a flow cell with an online fiber optic fluorescence sensor. This sensor monitors the outlet concentration of biomolecule in the middle stream. In principle, any type of detector, such as absorbance, refractive index and mass spectrometer, may be employed. The experiment was initiated by first ensuring a

bubble free microchannel and connecting tubing. A flow rate was set on the syringe pump and the sensor starts monitoring the fluorescence signal at the outlet. The outlet signal was monitored until a new steady state value, i.e. no change in fluorescence intensity for 20 residence times (=L/U), was attained. The flow rate was subsequently changed and the data recording process was repeated. The data recorded to a file was then analyzed and the biomolecule concentrations estimated from a previously determined calibration curve. Calibration curves were generated to establish the linearity ($R^2 > 0.9995$) of the fluorescence versus concentration data over the working range of the experiments. Additionally, a two point (buffer and inlet protein concentration) calibration was conducted at the beginning and end of every experiment.

4. Results and discussion

A stable three phase flow of miscible liquids through a microchannel can be easily established experimentally and constitutes a simple, low volume and rapid method for determining the effective translational diffusivity of a molecule or macromolecule. The microchannel dimensions are the main design parameters that may be determined from the solution of the transport equations discussed below. Once the appropriate channel dimensions are fixed, the main operating parameter becomes the flow rate or velocity of each phase. Although the viscosity of each phase may be changed independently, the translational diffusivity at infinite dilution is best determined with identical solvent viscosities of the three phases. Thus, all theoretical and experimental results presented here involve aqueous solvent at room temperature.

Bolus method. Measurement of the diffusion coefficient may be carried out by injecting a bolus of the molecule into the middle stream. As the bolus travels through the

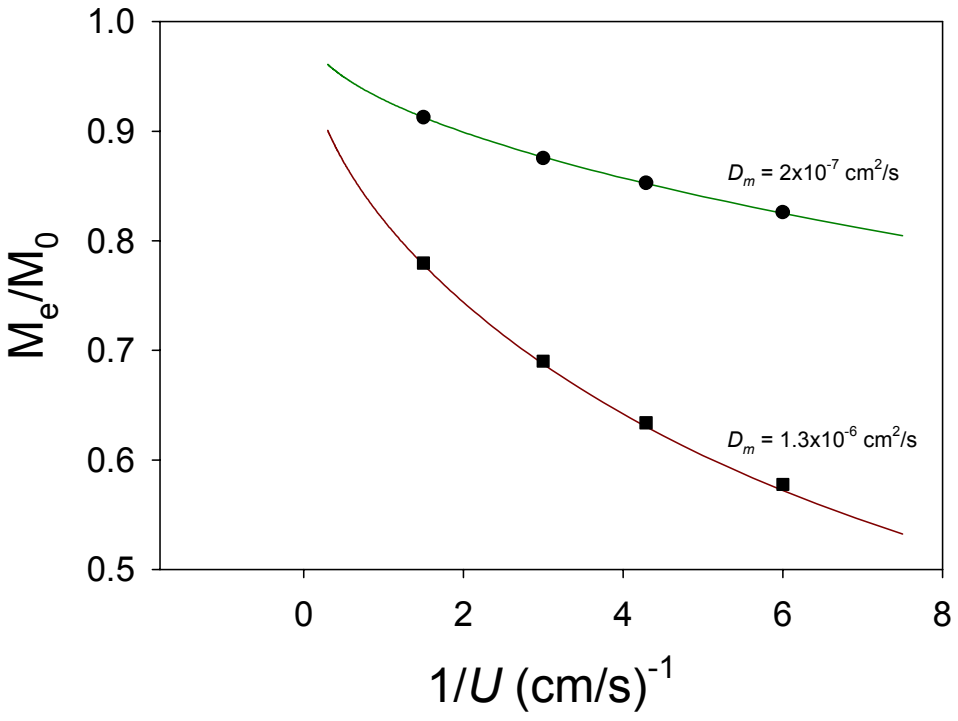


Fig. 2. Plot of the bolus outlet to inlet cumulative mass ratio (M_e/M_0) versus inverse average velocity (1/U) for different values of diffusivity, D_m. Solid lines are the analytical solution without dispersion. Squares and circles are numerical results with dispersion. Channel dimensions are as given before.

common length of microchannel, the molecule diffuses into the adjacent streams through the common interface and is thereby depleted from the middle stream. By plotting the ratio of outlet to inlet cumulative mass (i.e. integrated over t and z) versus inverse average velocity, an estimate of the diffusivity is obtained (see Fig. 2). A mathematical description of the molecular transport is given by Eq. (1) subject to initial and boundary conditions as expressed by Eqs. (2)~(4). An important simplification of Eq. (1) may be achieved by removing the dispersion term and deriving an analytical solution of the resulting transient convection diffusion problem. To evaluate the effect of ignoring dispersion, numerical simulations of Eq. (1) with experimentally determined Taylor-Aris dispersivity of fibrinogen and insulin [10] were compared with the results of the analytical solution. As seen in Fig. 2, the numerical and analytical solutions are in close agreement over a wide range of velocities. This means that the simpler analytical solution can be fitted to the experimental data to establish the diffusion coefficient of the molecular species under study. From Fig. 2, it is apparent that diffusion coefficients can be obtained with greater resolution at lower velocities.

Continuous infusion method. An alternate method is proposed here, where by, the diffusing solute molecule is infused continuously into the middle stream. A steady state elution concentration is attained in the middle stream due to continuous diffusion into the two adjacent streams. A plot of the steady state outlet to inlet concentration ratio of the diffusing solute as a function of the inverse average velocity will yield an estimate of the diffusivity. The theoretical description of this microscale transport is given by Eq. (1) without the transient and dispersion terms, subject to the conditions prescribed by Eqs. (5) and (6). The solution, Eq. (7), may be averaged, as in Eq. (8), to obtain the eluting concentration in the middle stream.

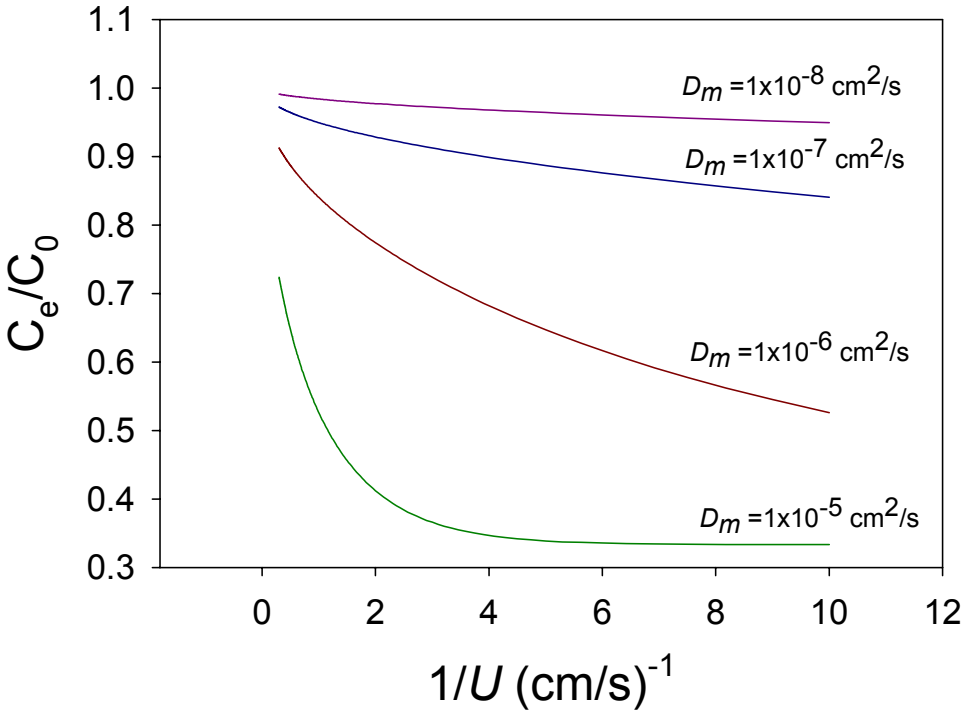


Fig. 3. Continuous infusion method. Outlet to inlet concentration ratio versus inverse average velocity (1/U) for different values of diffusivity, D_m. Channel dimensions are as given in the main text.

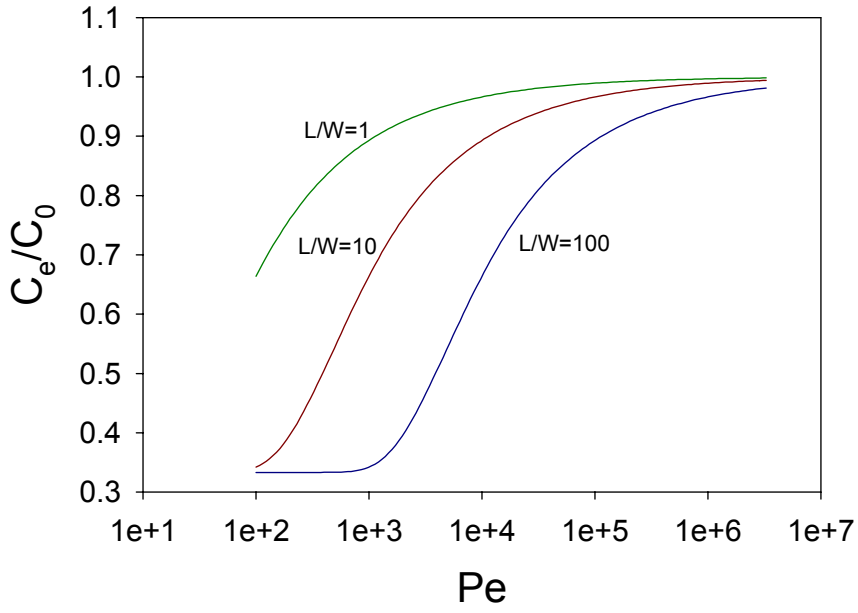


Fig. 4. Semi-log plot of dimensionless concentration ratio (outlet/inlet) as a function of Péclet number (Pe = UW/D_m) at different L/W ratios.

Figure 3 shows a family of curves describing solute diffusivities that differ by over four orders of magnitude. As in the case of bolus data, the diffusivities may be resolved most accurately at lower average stream velocities. Figure 4 presents the solution of Eqs. (7) and (8) as a function of Péclet number (Pe = UW/D_m) for different values of L/W. This graph is useful for determining the microchannel dimensions and establishing a suitable range of flow rates. It is important to note that microchannel height H, does not occur in Eq. (7) but may nevertheless influence the outlet concentration as H/W ratio changes. A qualitative understanding of this may be obtained by replacing L with H in Fig. 4.

Experimental protein diffusivities. To evaluate the general validity of the proposed method, the diffusion coefficients of two proteins were measured by the continuous infusion method. The two proteins, fibrinogen (MW 330000) and insulin (MW 5808), have widely varying size, shape and diffusion coefficients. In order to detect low concentrations, the proteins were labeled with a fluorescent probe. Although this labeling may potentially alter the diffusivity, the change is expected to be negligibly small due to the small size of the fluorescent molecule. For fibrinogen, concentration was measured by two different methods, (a) fluorescence imaging with an 8-bit ccd camera attached to a Nikon TE2000 microscope, and (b) fluorescence intensity recorded via fiber optic linked photomultiplier tube (PMT). For insulin, only the fiber optic detector was employed. The advantage of using the fluorescence microscope lies in the ability to monitor the microchannel flow continuously. However, the dynamic range of intensity measurement is low in 8 bit imaging. Alternately, the photon counting PMT based fiber optic detector offers higher resolution data. As seen in Fig. 5(a), the experimental data for fibrinogen via the imaging method lies below the theoretically predicted curve. This means that the diffusion of fibrinogen is greater than the literature reported value of 2×10^{-7} cm^2/s at 20^{o}C in water [11]. Since the experiment was conducted at room temperature or ~22^{o}C, the difference is not due to temperature. The buffer pH was 7.4 and it is reasonable to expect some changes in protein mobility due to change of configuration with solution pH. Data from the fiber probe (Fig. 5(a)) has somewhat higher resolution and follows the same quantitative trend. Data

for insulin in Fig. 5(b), obtained by the fiber probe, shows reasonable agreement with the assumed literature value of 12.9×10^{-7} cm^2/s (solid line in Fig. 5(b)), albeit with low resolution due to the limited data.

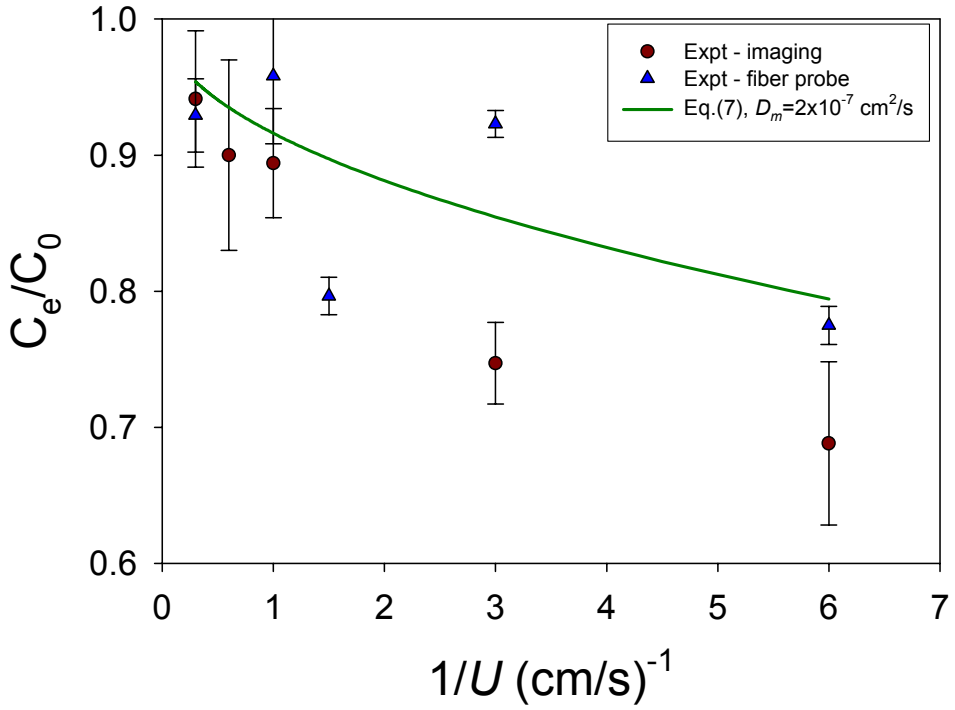


Fig. 5 (a). Comparison of experimental and analytical results for fibrinogen (C_0 = 100 nM) diffusivity in phosphate buffer (pH7.4, room temperature).

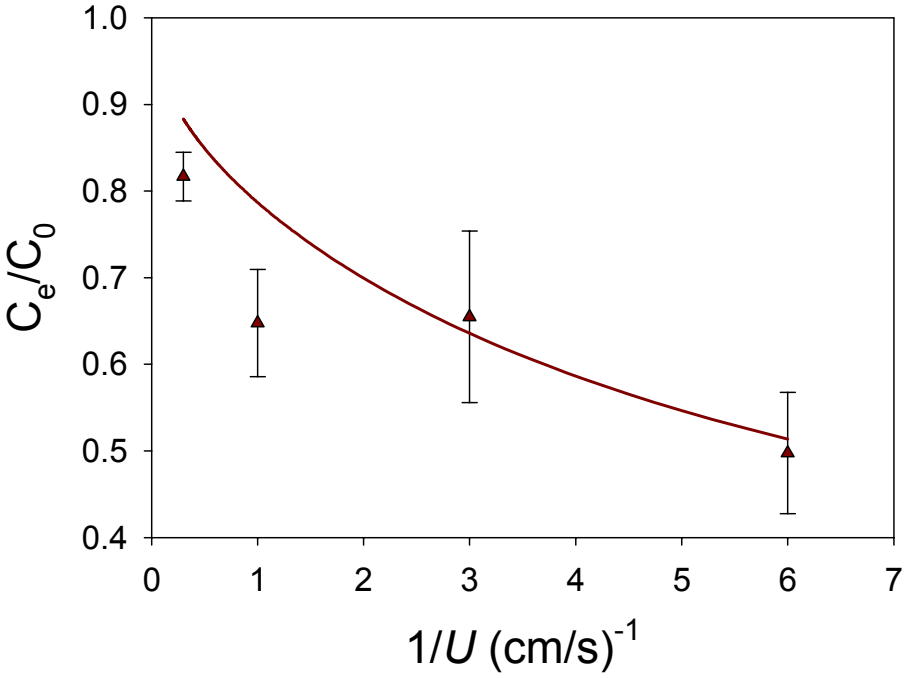


Fig. 5 (b). Comparison of experimental (triangles) and analytical (solid line) results for insulin (C_0 = 500 nM) diffusivity in phosphate buffer (pH7.4, room temperature).

Detailed modeling of microchannel transport of a diffusing molecule must include the velocity profile since this will impact the molecule distribution between phases. The author's previous research (unpublished theoretical and experimental results) has found that for high aspect ratio (Width/Height > 5) channels, the velocity profile along the width is nearly plug flow except for the near wall region. The velocity profile along the channel depth remains parabolic and dispersion arises due to this flow non-uniformity. Thus, there is very little difference between the average molecule velocity at the two interfaces and the average velocity of the phase. For high aspect ratio channels therefore, the analytical solution based on average velocity is a good approximation of solute transport (the C_e/C_0 ratio is slightly underestimated).

In principle, the proposed method and accompanying model can be implemented for two or three phase microchannel flows. The author feels that the three phase format is preferable, mainly due to doubling of the diffusion interface and reduction of adsorption, leading to greater experimental accuracy. Also, this method has a number of advantages over the T-sensor such as, (1) it allows any detection method and is not restricted to fluorescently labeled molecules, (2) increases the effective diffusion area and reduces the overall length for slowly diffusing molecules, (3) reduces the microchannel surface area available for molecular adsorption. Additionally, when one considers the bolus method, it is not practical in the T-sensor format. A bolus of 100 nM protein of volume 10 nL will contain about 1 femtomoles resulting in major savings of expensive biomolecules. Moreover, unlike the Taylor-Aris method, this method does not suffer from the limitation of residence time (L/U) scaling with the inverse of the diffusion coefficient (D_m). Thus, for a molecule of $D_m = 1\text{x}10^{-8}$ cm^2/s, studied in a microchannel of height 50 μm, the Taylor-Aris residence time may be more than 45 mins, as compared to few tens of seconds for this method.

Sources of measurement inaccuracy. The measured diffusion coefficient may differ from literature values due to artifacts arising from the microchannel system proposed here. Most importantly, this flow system gives rise to a Lévêque like effect that, results in the scaling law for diffusive displacement near and parallel to microchannel surfaces to follow one-third power instead of one-half power. This phenomenon was studied by Kamholz and Yager [9] and shown to prevail only near the inlet region. Based on their study, one may estimate the error introduced in diffusivity measurement, as less than 5% for microchannel dimensions used in this study. This error may be further reduced by increasing the length of the microchannel. Another factor that affects the measurement is adsorption, which is present in all systems and may be overcome by adopting a suitable blocking method. Variations in flow rate and interfacial diffusion area due to partial blockage of the microchannel must be considered and prevented, since they impact the quality of data obtained. Orientational bias of rigid nonspherical molecules [12] occurs in all flow based diffusivity measurements and may give rise to small differences in the average translational diffusivity measured and compared to non-flowing methods.

5. Conclusions

The ease of establishing three phase flow in microfluidic channels using identical aqueous solvents yields a rapid method for measuring diffusivity of bio/macro molecules. The method is developed with the help of a mathematical model of microchannel transport, showing how diffusivity is estimated from experimental measurements of inlet and outlet concentrations as a function of inverse average velocity. A major simplification is achieved for high aspect ratio channels by showing that diffusivity can be measured,

without knowledge of dispersion, via an analytical result. Rapid advancement in detector miniaturization and integration with microchannels will improve the adoption of this method. Further development of this method is expected to allow various measurements such as mutual diffusion coefficients in binary solutions and temperature and concentration dependent diffusivities.

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