Microwell Mixing Via Jet Injection and Aspiration
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Objectives

This project had two main objectives. The primary objective was to develop a Femlab computer model for mixing in a small volume via the injection and subsequent aspiration of a high concentration fluid. The secondary objective was to compare the mixing time predicted by this model with published experimental data (Nealon 2006)[1].

Problem

The particular model in question involved the addition of 90 μL of a concentrated fluid through a pipette tip into a flat-bottomed cylindrical microwell containing 200 μL of stagnant liquid. This was to be followed by the removal of 90 μL through the same pipette tip (Fig 1).

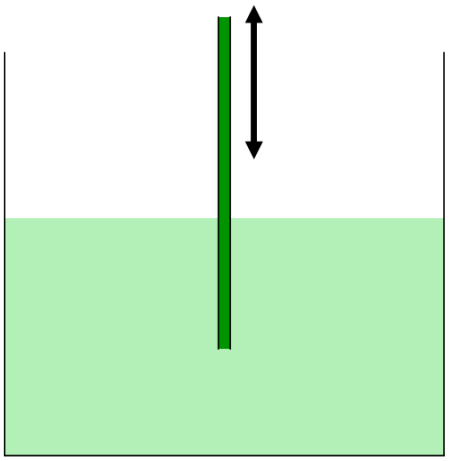


Figure 1: Not-to-scale diagram of microwell mixing problem. 90 _L of high concentrated fluid is injected and then aspirated from a microwell initially containing 200 _L.

This problem proved to be difficult to solve not only because a convection/diffusion problem had to be evaluated simultaneously with a fluid dynamics problem, but also because the model had to account for a moving, deformed boundary at the surface of the fluid in the microwell.

[1] Nealon, Anthony J., Ronan D. O'Kennedy, Nigel J. Titchener-Hooker, Gary J. Lee. "Quantification and prediction of jet macro-mixing times in static microwell plates." Chemical Engineering Science 61(2006): 4860-4870.

Procedure

Equation System

There are two primary equation systems that must be solved simultaneously in this problem. The first is a momentum balance represented by the incompressible Navier Stokes equation as shown in Fig 2.

$$\rho \frac{Dv}{Dt} = \frac{\partial v}{\partial t} + (v \cdot \nabla)v = -\nabla p + \mu \nabla^2 + \rho g$$

Figure 2: The incompressible Navier-Stokes equation.

The second equation is a mass transfer equation accounting for convective and diffusive driving forces as shown in Fig 3.

$$\frac{\partial c}{\partial t} + \nabla \cdot (-D \nabla c) = -v \nabla c$$

Figure 3: The mass transfer equation used to account for convection/diffusion.

These two equation systems would be evaluated using the Comsol Multiphysics package of Femlab.

Parameters

The parameters defining the fluid properties were approximated using common values for water at standard temperature and pressure. In particular, the model used a fluid density of 998 kg/m^3 and a dynamic viscosity of 0.001 Pa_s. The diffusion coefficient defining the rate of mass transfer of the "solute" in the model was set at 10^{-5} m^2/s. This coefficient, which is determined by the chemical properties of both the solute and solvent (water in this case). Because the solute in this case is not specified, a "predictable" or "common" value for the diffusion coefficient was assumed to be 10^{-9} m^2/s. Hence, the model solution presented in this report is greater than this common value by four orders of magnitude. As will be explained later, the reason for this decision was that a convergent solution could not be obtained when modeling with such a low value for the diffusion coefficient.

Domain Geometry

The geometry of this problem was modeled in two dimensions, assuming symmetry about the center axis (axisymmetricgeometry). In this fashion, we assume that the fluid velocity vector into and out of the page is zero at all times. Similarly, we assume that particle diffusion within the fluid does not take place in the directions into and/or out of the page. Although this may not generate truly accurate results, modeling

the entire vessel geometry in three dimensions would be extremely time consuming, and could prove to be too stressful a model for the computing power that we had at hand.

The microwell diameter as well as the pipette tip diameter were obtained from the Nealon article, allowing the calculation of the initial height of the domain representing the initial fluid in the microwell. The pipette tip geometry was extended well above the surface of the fluid in the microwell, allowing the flow within the tip to become fully developed by the time the fluid exits the pipette tip. It was assumed that the pipette tip extends 0.001 m below the surface of the liquid in the microwell. This geometry can be observed in Fig 4, where all given dimensions are in meters.

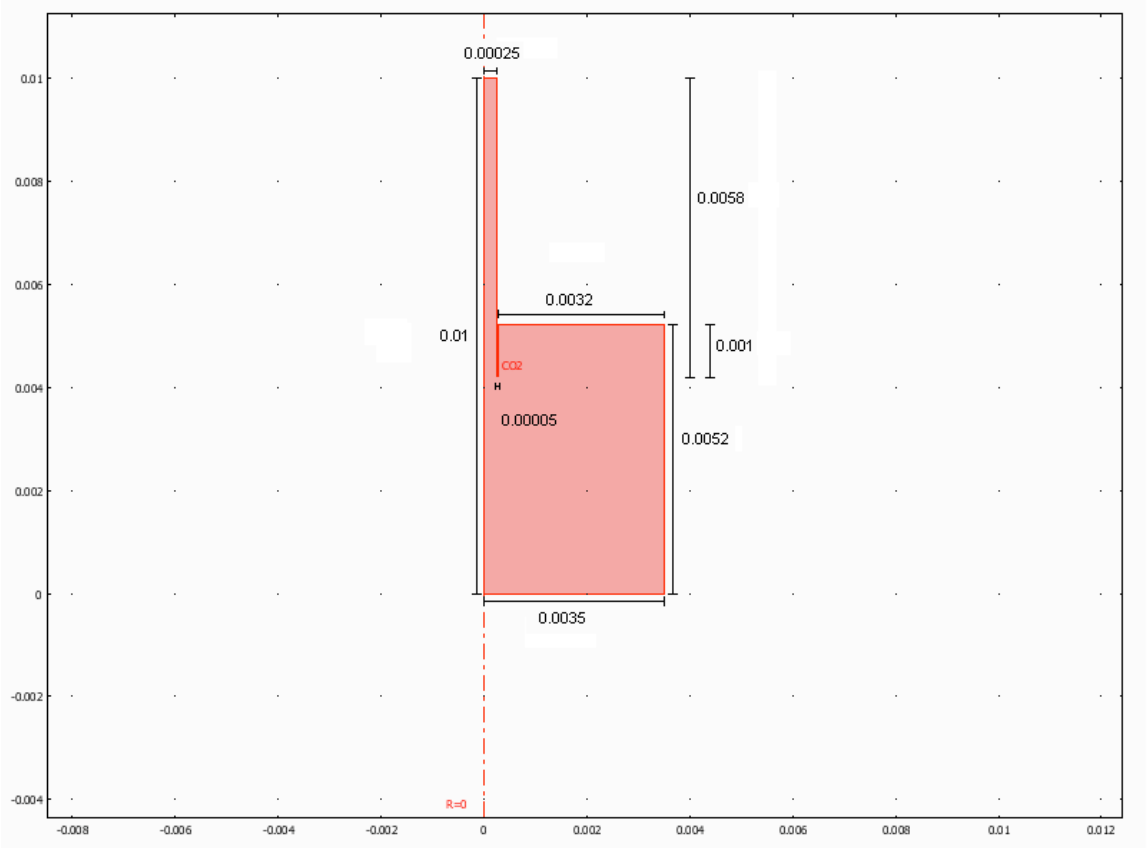
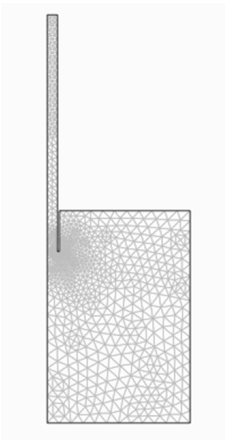


Figure 4: Domain geometry of the microwell mixing model. All dimensions are in meters. The domain is 2-dimensional and is axis-symmetric about R=0 m.

Domain Mesh



In order to solve this problem computationally, the domain is divided into several smaller geometries using a mesh. In this way, each mesh element is assumed to have uniform properties, including velocity, concentration, etc. The refined mesh used to generate the solution given in this report is shown in Fig 5. Note that near the finer features of the domain geometry, mesh elements decrease substantially in size, and increase substantially in number. The mesh

in this model contains 1,812 individual elements, 154 boundary elements, generating 20,438 degrees of freedom.

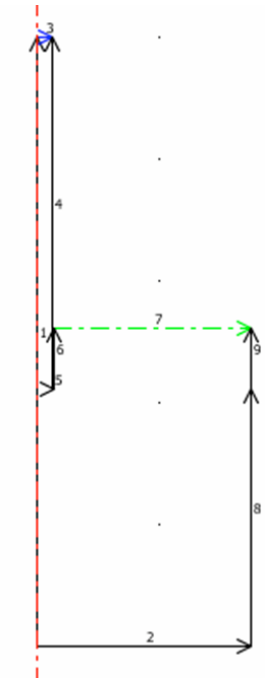
Figure 5: Refined domain mesh containing 1,812 elements.

Boundary Conditions

In addition to specifying the domain geometry and the appropriate fluid properties, we must also specify the boundary conditions for each equation model applied to the system. Although there are only two equation systems that we are solving for in this problem, there are really three different models applied to the system. For each boundary in the domain, we must specify conditions for the fluid dynamics model, the convection/diffusion model, and the moving-mesh model that allows for deformation and displacement of the free fluid surface (the surface at the top of the fluid in the microwell). Each boundary of the domain is assigned a number as shown in Fig 6. The specified conditions for each of these domains in each of the three models are described in Table 1.

#	Description	Fluid Dynamics	Conv/Diff	Moving Mesh
1	Center Axis	Axial Symmetry	Insulation	Displacement=0 m
2	Well Bottom	No Slip	Insulation	Displacement=0 m
3	Inlet	Inflow Velocity	C= 1 mol/L	Displacement=0 m
4	Pipette Wall	No Slip	Insulation	Displacement=0 m
5	Pipette Edge	Slip Symmetry	Insulation	Displacement=0 m
6	Inner Well Wall (near surface)	Slip Symmetry	Insulation	Displacement=0 m
7	Fluid Surface	Neutral	Convective Flux	Velocity= u*nr+v*nz
8	Well Wall	No Slip	Insulation	Displacement=0 m
9	Outer Well Wall (near surface)	Slip Symmetry	Insulation	Displacement=0 m

Table 1: Boundary conditions.



ain boundaries.

Fluid Dynamics Boundaries

- No Slip: fluid touching the boundary does not move.
- Slip Symmetry: fluid is allowed to move parallel to the boundary.
- Axial Symmetry: fluid is allowed to move in the direction of the axis only.

Conv/Diff Boundaries

- Insulation: material can not diffuse into or out of the boundary.
- C = 1 mol/L: an arbitrary concentration set at the inlet. Initial concentration of the rest of the domain is at 0 mol/L.
- Convective Flux: material is allowed to move with the flow.

Moving Mesh Boundaries

- Displacement = 0 m: the mesh boundary can not move.
- Velocity = u*nr+v*nz: allows the mesh along the boundary to move and deform according to the radial velocity of each element (u), the axial velocity of each element (v) and the respective vectors nr and nz.

Entrance Velocity

The entrance velocity (velocity at boundary 3) is key parameter for controlling the addition and subsequent removal of fluid from the microwell. The experimental data on which this model is based injected fluid through the pipette tip at a Reynolds number of 3000. Unfortunately, a computer solution could not be achieved with a Reynolds number this high. The highest Reynolds number for which we obtained results was 162. This is obviously a significant difference, and as such, our results could not be correlated to experimental data. However, our model was able to generate a predicted mixing time and concentration profile for the parameters used.

Furthermore, it became necessary to control the velocity such that after 90 μL had been added, the inlet velocity changed sign such that fluid was removed. Although this could be done with a simple Boolean expression, such a discontinuous function generated serious complications when attempting to compute a solution. The way around this problem was to establish a continuous function of velocity over time. A periodic function was calculated using the equations shown in Fig 7. Figure 8 shows how the specific velocity function used was calculated.

$$v = A\sin(Bt + C)$$

$$\int_{t_0}^{t_{f,injection}} Q \cdot dt = A_{cross} \int_{t_0}^{t_{f,injection}} v \cdot dt$$

$$\int_{t_0}^{t_{f,injection}} v \cdot dt = A_{cross} \frac{A}{B}\left[\cos(Bt_0 + C) - \cos(Bt_{f,injection} + C)\right]$$

Figure 7: Equations for generating a periodic velocity function.

- Re = 162

$$\mathrm{Re} = \frac{\rho < v > L}{\mu}$$

$<v>_{max}$	**0.300**	**m/s**
ρ	998	kg/m^3
μ	0.001	kg/ms
L	0.00054	m
Re	162	

A_{cross}	2.29E-07	m^2
Q	6.87E-08	m^3/s
V_{added}	9E-08	m^3
t_{final}	1.947542	s
$A_{cross}\int v^*dt$	**9.E-08**	**m^3**
2^*t_{final}	**3.8951**	**s**

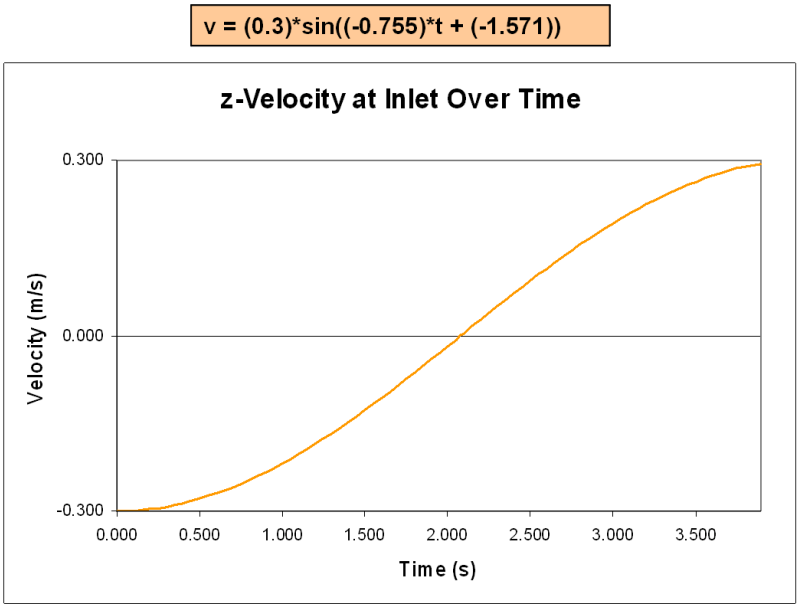


Figure 8: Calculations and plot of the velocity function used in the computer model.

Results

Once a convergent solution was obtained, the resulting model could be displayed graphically in an number of different ways, depicting the concentration gradient, velocity gradient, streamlines, and many other parameters at several time points. An example is shown in Fig 9, where we can observe a specific band of concentration at an intermediate time, as the well is still being filled with fluid. In this figure, it is easy to see the diffusion of the "solute" through the fluid as well as the deformation of the fluid surface.

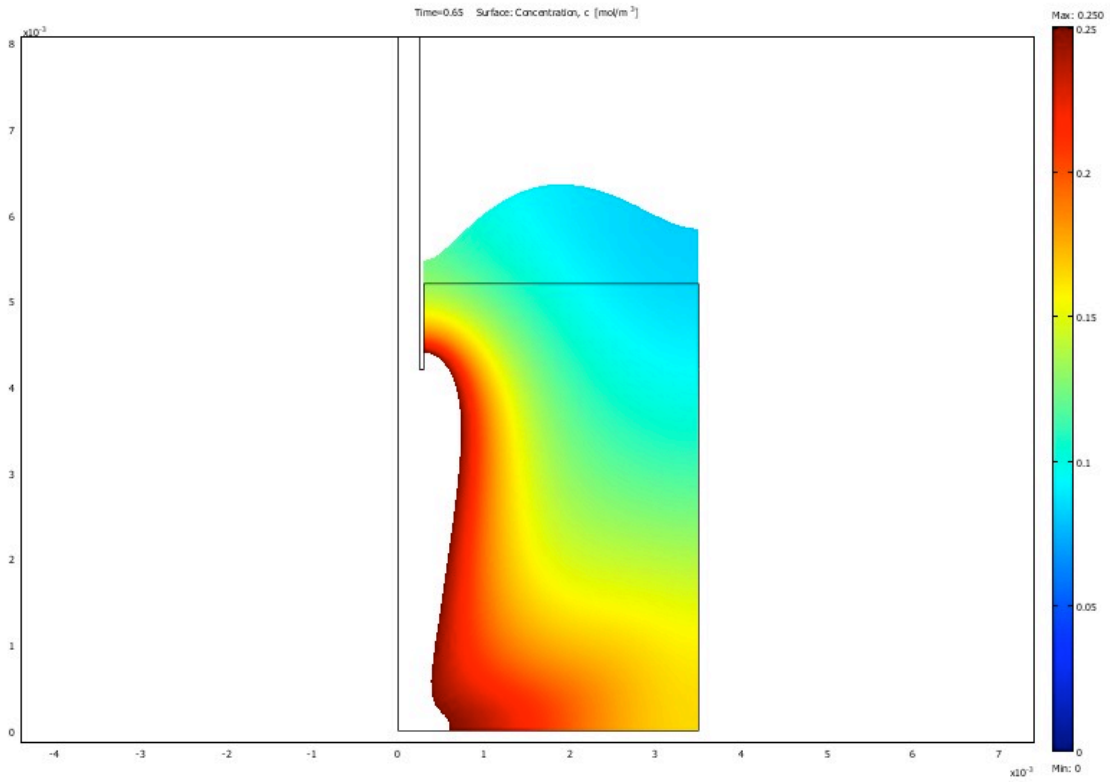


Figure 9: Concentration gradient from 0 to 0.25 mol/L at time t = 0.65 s.

We can also observe streamlines at each time point, which represent the path of individual particles in the fluid. This can be useful for demonstrating flow patterns within the microwell. In Fig 10, we notice that the fluid circulates in the lower half of the well, while in the upper half, at t = 2.3 s (at time at which fluid is being aspirated out of the well) the fluid is falling back towards its initial position in relatively straight "streams."

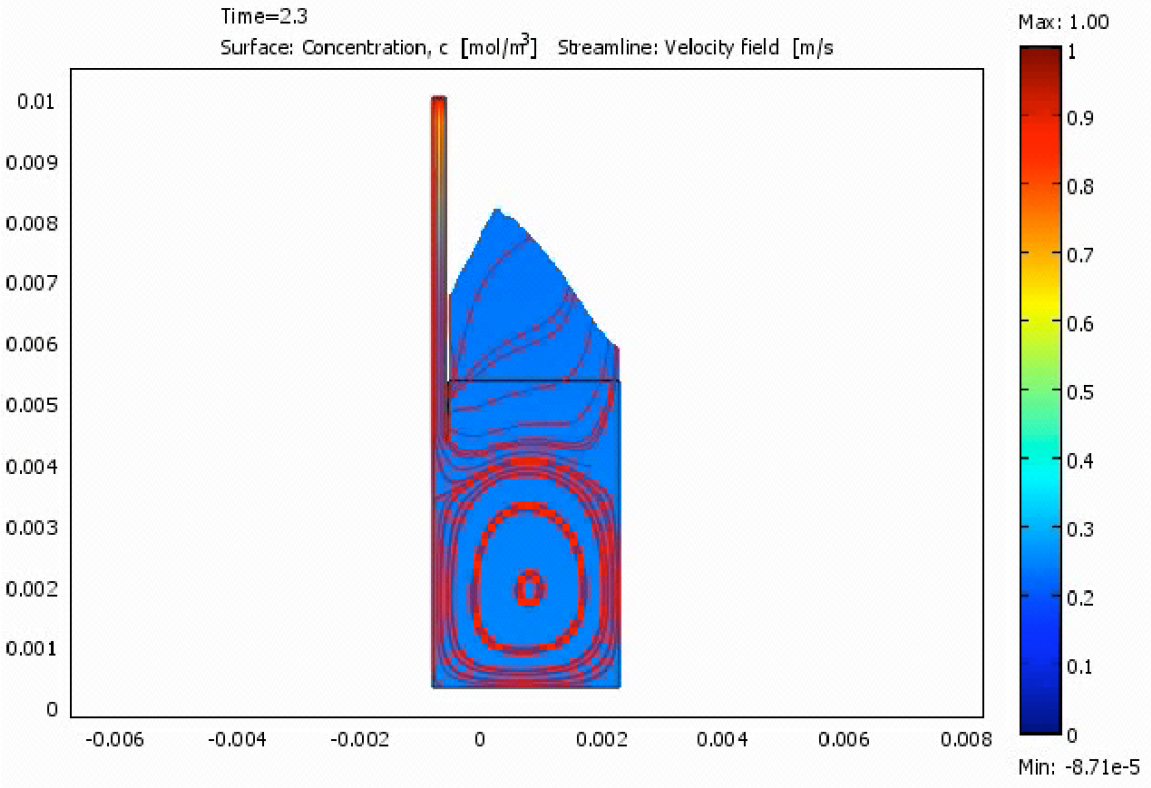


Figure 10: Streamlines depicting flow patterns of individual particles through the fluid at t = 2.3 s. At this time point, fluid is being aspirated out of the microwell.

These graphs show clearly that this particular problem can be modeled using computational fluid dynamics. However, in order to generate a prediction for the "mixing time" (time at which the fluid is "well mixed"), we must perform statistical analysis on the model at each time point. In order to determine the time at which the fluid becomes well mixed, we will look at the variance in concentration at each time point. The concentration variance can be calculated with the equations shown in Fig 11.

$$c_{avg} = \frac{\int c \cdot dV}{\int dV}$$

$$\sigma^2 = \frac{\int \left(c - c_{avg}\right)^2 dV}{\int dV} \left[=\right] \frac{mol^2}{L^2}$$

Figure 11: Equations used to calculate the concentration variance at each timepoint.

Using these equations, we can calculate the variance and standard deviation in the concentration over the whole domain at each time point. These data points were exported to a spreadsheet and the graph shown in Fig 12 was prepared.

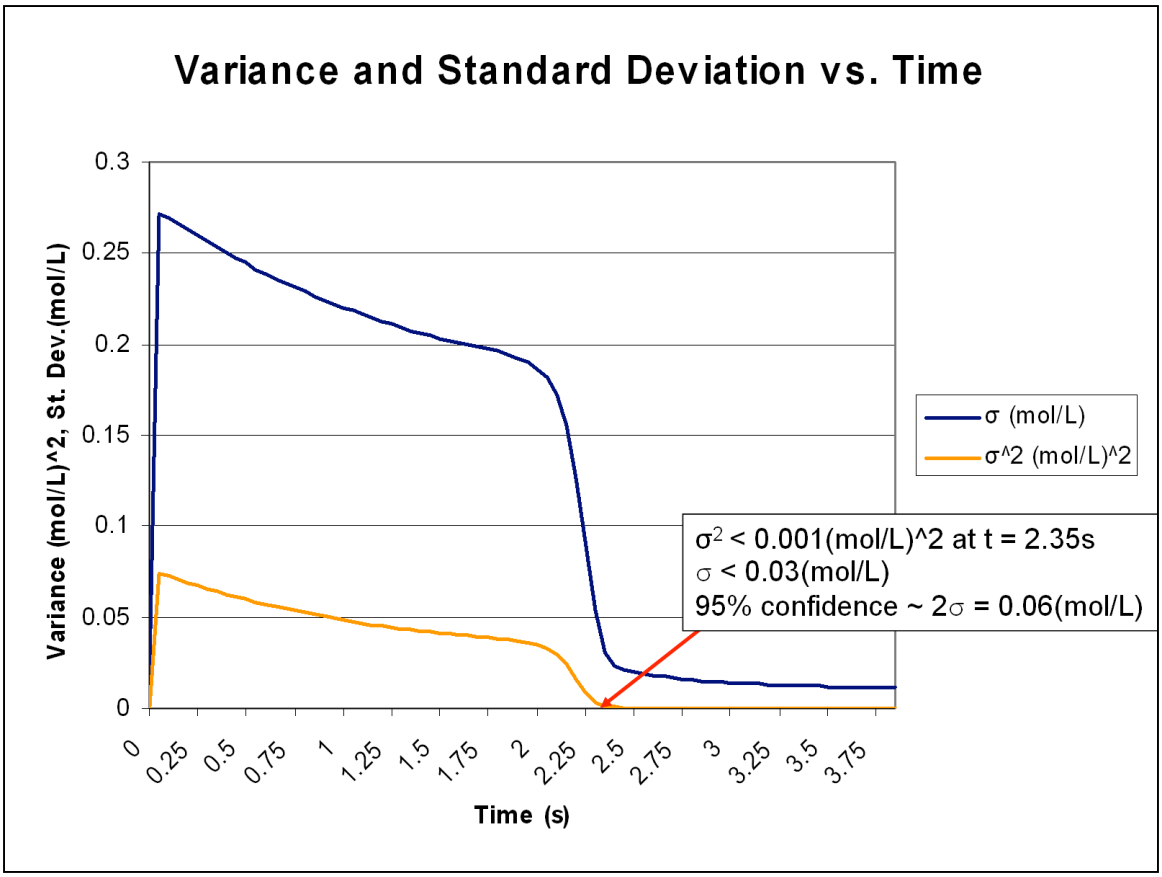


Figure 12: Variance and Standard Deviation of concentration over time. The fluid becomes well mixed at time t = 2.35s.

In Fig12, we can see clearly that the initial variance in concentration is 0 mol^2/L^2, meaning the entire domain has a uniform concentration (at 0 mol/L). Upon adding concentrated fluid, the variance immediately spikes, indicating that the solution is not mixed (i.e. there are regions at very high concentration and regions at very low concentration). The variance then gradually reduces over time as the fluid becomes mixed, and drops off even more rapidly as the extremely concentrated portions of the fluid are aspirated out of the microwell. The variance then reduces to near zero values as the solution concentration becomes nearly uniform.

We will assume that the fluid becomes well mixed when 95% of the fluid falls within two standard deviations of the mean concentration (95% confidence). Based on an average final concentration value of approximately 0.247, the mixing time value is approximately 2.35 seconds.

Conclusion

The results of this project indicate clearly that difficult fluid dynamics problems involving moving interfaces can be modeled using computers. Such computer analysis also provides the ability to analyze results from a number of perspectives (concentration, velocity, etc) and at a number of different time points. However, such computer modeling has severe limitations. Often, coercing such computer models into producing a

convergent solution is as much art as it is science; several iterative techniques must be used in order to generate results. Furthermore, computer modeling is often an "all or nothing" approach. Testing a small change in the simulation parameters can mean hours of wasted time as the computer reaches a time step where a solution can not be found. Obviously choice of hardware and software plays a key factor in determining these sorts of limitations.