Design and simulation of fluid flow in paper based microfluidic platforms

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Abstract. Computational simulation for the fluid flow in a paper based microfluidic system was performed and was experimentally validated in this work. Comsol Multiphysics based simulation was performed and the module used was species transport in porous medium. Two separate simulation for the reagent distribution and the interaction of sample with the immobilized reagent in paper based microfluidics devices were carried out. This was performed by varying the parameters such as adsorption constant, diffusivity and average velocity of fluid in the porous medium for finding the concentration profile. Finer mesh were used for the simulation which gives more accurate results with less computational time. The reagent distribution was experimentally validated by dropping methylorange indicator over Whatman filter paper.

1. Introduction

Microfluidics find application in many areas of science and technology and molecular analysis is one among them. There are different types of substrates used for microfluidic device fabrication which include glass or silicon, polymer, paper and thread [1]. The first two substrates are well established while the latter are in its nascent stage. Paper based microfluidic devices have many advantages over other microfluidic platforms because of its easiness of fabrication and disposal, distribution of reagent, capillary driven flow, good distribution of reagent, inexpensive, availability of raw materials, fluid flow in x, y and z direction [2]. Paper based microfluidic devices for sensing application are widely used, which include liver function test [3], HIV test [4], and pregnancy test [5].

Very few reports are available on the study of fluid flow in paper based systems so far. Elizalde et al. proposed an analytical model for the controlled fluid flow in paper by varying the geometry of the paper substrate[6]. Moving mesh simulations were carried out to study the absorption of fluid in the cellulose fibre networks of paper substrate [7]. Studies were also performed to find out the permeability of air in cellulose fibres and derived different empirical relations to find out the relative permeability [8]. From the experimental studies carried out by Chiou and Smith, it was found that the adsorption constant of material is different for different solutes and also it varies with the grade of paper used for making the device [9]. Two types of paper based devices are mainly used, lateral flow devices and 3D devices. Of this lateral flow based systems are extensively studied and are applied in commercial products. To the best of our knowledge, the simulation studies for finding the concentration profile of reagent over the lateral flow paper based devices have not been studied so far.

A major problem faced in paper based microfluidics for sensing application is the distribution of reagent after the sampling [10]. It was observed that the immobilised reagents swept away by the sample solution and which leads the non-uniformity or reagent over the substrate. Hence it is difficult to locate the exact position of the substrate where the maximum concentration of reagent occurs. A simulation can be performed to find the maximum concentration zone in the substrate after sampling so that the values for further analysis can be obtained from that particular location of the...
substrate. In this work simulation of fluid flow in porous substrate like paper was carried out using Comsol Multiphysics software. Various physical parameters like adsorption constant, diffusivity and average velocity of the fluid which affect the flow were identified and varied to study the concentration profile on the paper substrate during the injection of reagent and after the sampling.

2. Experimental:
2.1 Materials and softwares used
Methyl orange solution and Whatman Chromatography paper (grade 1) was used for experimental validation. Comsol Multiphysics 4.3 for simulation, AutoCad 2012 for designing and Image processing toolbox of MATLAB R2015a from MathWorks for image processing were used in this study.

2.2 Simulation
The physics selected in Comsol Multiphysics for finding the concentration profile in paper based substrate is porous and subsurface flow because since paper is a porous medium made of cellulosic fibre and the flow happens in all the direction inside a paper. In this physics various parameters like adsorption constant, diffusivity, velocity and porosity were altered and studied.

2.2.1 Geometry

![Figure 1: Geometry of the paper substrate](image)

The inner circle represents the reagent injection zone where the reagents are dropped over the substrate and the zone is where the reagent get distributed through capillary forces. All the dimensions are in mm.

2.2.2 Equations
Following equations were solved in porous and subsurface flow in comsol multiphysics inorder to find the concentration profile of the solute which distributes in the media.

\[ P_{1i} \frac{\partial c_i}{\partial t} + P_{2i} + \nabla \cdot \hat{\Gamma}_i + u \cdot \nabla c_i = R_i + S_i \]  
\[ P_{1i} = \epsilon + \rho_b k_{pi} \]  
\[ P_{2i} = (c_i - c_{pi}) \rho_p \frac{\partial \epsilon}{\partial t} \]  
\[ \rho_p = \frac{\rho_b}{1-\epsilon} \]  
\[ N_i = \hat{\Gamma}_i + u c_i = -(D_{di} + D_{ei}) \nabla c_i + u c_i \]
Where, \( c_i \) is the surface concentration, \( R_i \) is the reaction source, \( S_i \) is the Species source, \( u \) is the velocity vector, \( \rho_p \) is the density of the porous media, \( \rho_b \) is the density of the solid, \( D_d \) and \( D_e \) represent diffusivity value for bulk diffusion and eddy diffusion respectively, \( \varepsilon \) is the porosity, \( K_p \) is the adsorption constant of solute on substrate and \( t \) is the time.

The possibility of modelling a reaction and also the species entering source were included in the equations. For the present study, only the species source was given assuming that there was no reaction happening inside the system. Since there was inclusion of time, both steady state as well as time depended studies can be performed. Since we are more interested in understanding the concentration profile rather than concentration itself from the simulation at different time, the time dependent study was performed.

### 2.2.3. Mesh

![Meshed geometry for (a) reagent distribution (b) sampling after reagent distribution](image)

Figure 2: Meshed geometry for (a) reagent distribution (b) sampling after reagent distribution

Figure 2 shows the mesh used for the simulation in this work. Even though there is possibility of using user controlled mesh in Comsol Multiphysics, the present study used the inbuilt meshes available in software. There are seven different meshes available in Comsol Multiphysics which starts from extremely fine to extremely coarse mesh. Extremely fine gives more accurate answers but it requires high computational time whereas the reverse is true for extremely coarse mesh. Finer mesh is chosen as the appropriate mesh for all the simulations as it takes less computational time with least effect on the accurateness of the answer. Also, from the above diagram it is clear that mesh used is triangular in shape where it will improve the results rather than going for tetragonal mesh.

### 2.3 Fabrication and testing

The design was drawn in AutoCad and printed into Whatman filter paper using wax printer. 15 µl of methyl orange indicator was dropped at the centre of the paper and allowed to dry for 15 min. A photograph of methyl orange distributed paper was taken and the image was processed using Image processing toolbox in MATLAB.

### 3. Results and discussions:

Two separate simulations were carried out to find the concentration profile of the reagent that distributed over the paper substrate before and after sampling.

#### 3.1 Reagent immobilisation

Figure 3 shows the distribution of reagent over the paper substrate. The green colour (Figure 3A) represents higher concentration and on moving to yellow the concentration decreases. It is evident from the colour profile that the higher concentration is at the centre of the substrate where the
reagent is being dropped (species source point) and it decreases on moving to the periphery. In order to quantify the same, concentration was plotted against the distance from the centre where it is being measured. It was found that the reagent concentration varies on the paper with order two as moving from the centre of the paper to the outer region (Figure 3B). The surface concentration is normalised with the concentration at the centre in order to compare various physical parameters of the paper substrate (Figure 3C).

Figure 3: (A) Reagent concentration when simulated with porosity value 0.4, adsorption constant 0.5 m³/kg and diffusivity 0.01 m²/s at time step of 50 s, (B) Concentration $C_A$ with respect to distance from the reagent injection zone and (C) Normalised concentration profile with respect to distance from reagent injection zone.
The main parameters which affect the surface concentration are adsorption constant and diffusivity of reagent, which thereby affects the velocity of liquid through the porous substrate. The uniformity was estimated graphically, plotting the normalised concentration with respect to distance from centre for same geometry, the concentration is more uniform throughout the paper if the concentration at the centre is almost equal to that at the extreme point.

Figure 4A, represents the variation of concentration profile with respect to change in diffusivity. Higher the diffusivity, the concentration is uniform throughout the substrate. This is because as the diffusivity increases the overall mass transfer coefficient of the system increases thereby achieving the uniformity in concentration throughout the substrate. Also it was observed that there is no change in the concentration profile if the diffusivity is less than $10^{-4}$ m$^2$/s. Also, if the reagent is largely made of water and the concentration of solute in it is very less, it can be treated as water itself. The diffusivity value of water-air system is in the range of $10^{-9}$ m$^2$/s. So it can be concluded that diffusivity is not a direct parameter which affect the concentration profile in real systems.

Figure 4B gives a good idea in understanding how the adsorption constant affects the concentration profile in a porous system. Higher the adsorption constant of the system, the more reagent gets attached to the substrate and thereby reducing the uniformity throughout the substrate.

3.1.1 Experimental validation:
Experiments were carried out with colour reagents (methyl orange) immobilized on paper to validate the simulation results for reagent immobilisation. Methyl orange indicator was dropped at the centre of the paper substrate. It was dried and images were obtained using mobile phone camera. In order to have the same ambient light conditions and height of the camera from the substrate, a single photograph with all four samples were obtained.

The obtained image is being processed using Matlab image processing toolbox and the blue intensity is found out. This is done because blue colour is complimentary to yellow colour. Figure 5 shows how the reagent get distributed when dropped into paper substrate. Concentration of the reagent is
directly proportional to the blue intensity. As obtained in the simulation the reagent distribution in paper substrate follow as polynomial distribution with order two. All the values of four samples were varying with each other within 5% range. The regression value is found to be 0.983 which shows that the polynomial fit is appropriate and is similar to that obtained from simulation with regression value 0.998.

3.2 Two dimensional devices
The concentration profile obtained from the first simulation was fed as the initial value for the second simulation. The rectangle with centre at (-10, 0) is taken as the sample injection zone and flows to the extreme points of the circular region with centre (0, 0) and radius 10 units (specified as the outlet in simulation) (Figure 6). The concentration profile of the reagent on the paper substrate after sampling is shown in Figure 6. The image resembles the real situation on how the reagent gets swept away as the sample is introduced into the reagent immobilised paper. Majorly, the Whatman filter paper is used for the fabrication of paper based microfluidic devices. The velocity of fluid in the substrate varies with different grades of filter paper as the porosity value is different for different grades of Whatman filter paper. So in order to find the uniformity of the sample over different paper substrates the velocity of the fluid on the substrate can be found out and fed into simulation.

![Figure 6: Concentration profile of reagent after sampling in lateral flow device](image)

Porosity of different Whatman filter paper (Grade 1, 2, 3 and 4) is calculated using the equation (6) [11]. In this equation $\epsilon$ represents porosity value, $D_f$ and $d$ represents fibre thickness and pore size respectively.

$$1 - \epsilon = \frac{3\pi D_f^2}{4(d+D_f)^2}$$  \hspace{1cm} (6)

The capillary pressure drop over the paper substrate is driving force for the flow inside paper. The capillary pressure drop is calculated using the equation.

$$P_c = \frac{2\gamma \cos \theta}{d}$$  \hspace{1cm} (7)

Assuming $\gamma$ of air-water system as 0.07286 N/m and contact angle to be 0º. The pore size of different kinds of paper is denoted as $d$.

With all the above parameters the linear flow rate of fluid in paper substrate can be calculated with the equation below.

$$\frac{dl}{dt} = \frac{v^2 P_c}{8\mu l}$$  \hspace{1cm} (8)

Where $P_c$ is capillary pressure drop, $\mu$ is the dynamic viscosity of the fluid, $r$ is the radius of the pore size, $l$, the length of substrate taken as 1 cm for the calculation. The velocity is calculated as

$$v = \frac{dl}{\epsilon}$$  \hspace{1cm} (9)
The following table shows how the porosity, capillary pressure drop, permeability and velocity and standard deviation in the concentration of various positions of different grades of Whatman filter paper.

From the table it is observed that the standard deviation in the concentration with respect to distance is least in the case of Whatman Grade 2 and Whatman Grade 3. So these kinds of paper will be suitable for the fabrication of lateral flow paper based microfluidic devices for point of care application.

| PAPER No. | Pore diameter (m) | Porosity | Pc (Pa)   | Velocity (m/s) | Standard Deviation in Conc. |
|-----------|-------------------|----------|-----------|----------------|-----------------------------|
| Grade 1   | 1.10E-05          | 7.07E-01 | 1.75E+04  | 1.40E-04       | 3.3114E-09                 |
| Grade 2   | 8.00E-06          | 5.67E-01 | 2.40E+04  | 1.27E-04       | 3.0778E-09                 |
| Grade 3   | 6.00E-06          | 4.11E-01 | 3.20E+04  | 1.31E-04       | 3.0778E-09                 |
| Grade 4   | 2.25E-05          | 8.96E-01 | 8.53E+03  | 2.26E-04       | 5.20140E-09                |

3.3 Three dimensional devices (Sampling at the middle of the substrate)
In order to study the reagent profile in a 3D device after the sampling, the sample was introduced at the centre of the paper substrate (Innermost circle in Fig.7 with centre (0, 0)). In these set of simulations too, for different grade of Whatman filter paper, the velocity values are estimated as shown in Table 1, which is fed into the simulation. The concentration profile of the reagent after sampling in 3D device is shown in Figure 7.

![Figure 7: Concentration profile of the substrate after sampling in 3D device](image)

From the simulation it was found that all the points in the paper substrate is having the same concentration value. This is confirmed by taking the standard deviation of concentration at various points in the substrate and was found to be zero. Also, the simulation is repeated for all kind of
Whatman filter paper (Grade 1-4) and it was found that all got the same concentration profile. So, irrespective of grades of Whatman filter paper, all have got uniform colour distribution and thereby any grade can be used for fabricating 3D devices.

4. Conclusion:
From the simulation and experimental studies it was found that the reagent does not immobilised uniformly in paper substrate and it follows a nonlinear variation in concentration with respect to the distance from the point of injection. This was experimentally validated using the distribution of methylorange indicator over paper substrate. The concentration profile of the substrate after sampling is studied to identify the grade of Whatman filter paper which gives maximum uniformity in reagent distribution after sampling. It was found that Whatman filter paper grade 2 and 3 have got maximum uniformity in the concentration of the reagent after sampling and thereby it can be used for the fabrication of paper based microfluidic devices. Irrespective of all grades of filter paper, 3D devices showed uniform colour distribution and thereby 3D devices are best suited for paper based microfluidics for point of care application devices.

5. References
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