Multiphysics Simulation of Isoelectric Point Separation of Proteins Using Non-Gel Microfluidic System

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Abstract: A portable device that can identify protein and peptides real time in complex biological systems such as human bodily fluids reliably and accurately is in high demand to properly diagnose and treat medical conditions. Recently, success in isoelectric point (pI) based protein separation techniques utilizing a microfluidics system has provided significant hope in developing such a device. However, existing systems are cost prohibitive for the large-scale, multiplexed diagnostics required for complex diseases. Lynntech has developed an innovative Polydimethylsiloxane (PDMS) based microfluidics system with a unique design utilizing multi-channel inlets and outlets for isoelectric point (pI) based separation of proteins. The design of the microfluidics chip is optimized by performing numerical simulations using COMSOL Multiphysics. The operating and design parameters of the microfluidics device are optimized to maximize the pH range and resolution in each chip for efficient separation. This work has provided basis for developing a multi-chip configuration that can achieve varying degrees of pI resolution in each chip.

Keywords: Isoelectric focusing, pI based sorting of proteins, Microfluidics, COMSOL Multiphysics, Design of Experiments
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1. Introduction

The measurement of biochemical changes within the human body can provide a means to monitor a person's physiological health. Many of the biochemicals that provide a snapshot of person's health are proteins. For instance, in PTSD, protein biomarkers (soluble tissue factor, von Willebrand factor, soluble P-selection) are being discovered that may prove to be early indicators (Vidovic, Vilibic et al. 2007[1]; von Kanel, Hepp et al. 2008[2]). Another example, in breast cancer, multiple protein biomarkers (HER2, fibulin-2, osteopontin) have been discovered that are indicative of the disease (Whiteaker, Zhang et al. 2007[3]). Other examples are the recent discoveries of a panel of protein biomarkers (tau protein, 14-3-3 protein, cystatin C) reported in multiple sclerosis (Fiorini, Zanusso et al. 2007[4]) and of numerous potential biomarkers (neuron-specific enolase, MAP-LC3, phosphorylated neurofilament, and spectrum are a few examples) in TBI (Anderson, Scheff et al. 2008; Kochanek, Berger et al. 2008[5]; Prieto, Ye et al. 2008[6]; Sadasivan, Dunn et al. 2008[7]). Furthermore, many biological pathogens and toxins which may be used as biowarfare agents can be detected within a sample using protein analysis techniques such as liquid chromatography and mass spectrometry. For example, various types of botulinum toxins (Type A through F) have been identified using mass spectrometry (van Baar, Hulst et al. 2004[8]). The ability to detect and measure proteins indicative of disease, infection or toxic stress using a single sample of bodily fluid has the potential to produce a more accurate analysis of physiological state and enable a more successful prognosis and more rapid treatment. However, one of the major challenges with protein analysis within bodily fluids such as blood is the diverse number of proteins and concentration range that exists (Anderson and Anderson 2002[5]). This complexity sets forth the need to achieve high-resolution protein separation prior to analysis.
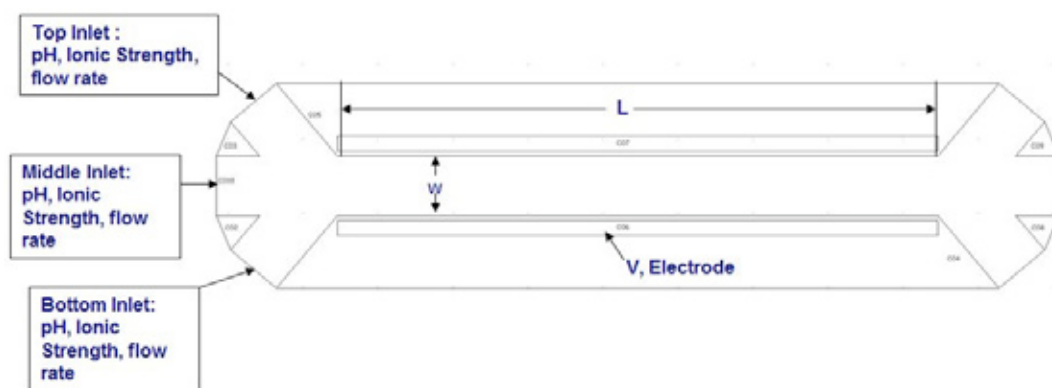
Figure 1: Schematic of single isoelectric focusing chip with three inlets and multiple outlets. This figure shows one of the designs with three outlets.

The ability to quickly detect biomarkers indicative is of utmost importance to the civilians where conventional detection techniques are not readily available. Further, there is approximately 10 orders of magnitude difference in concentration between the most abundant proteins in the blood and those that are fewer in number, which typically are the disease of pathogenic biomarker capture followed by horseradish peroxidase-conjugated antibodies for subsequent colorimetric detection are considered the "gold standard" in protein identification due to their high selectivity and specificity. The drawback of using Enzyme-linked Immunosorbent Assays (ELISAs) is that they are cost prohibitive for the large scale, multiplexed diagnostics required for complex diseases. Further, the ability to field a portable unit based on ELISAs is a challenge due to the various sensitive reagents (i.e. antibodies, enzymes) required for analysis. A second common technique used for protein analysis is two-dimensional gel electrophoresis (2-D gels) where a mixture of proteins is separated in a gel by isoelectric point and mass. Once separated into these two dimensions, the gel is stained enabling the location of each protein to be visualized. However, 2-D gels, by themselves, are limited in providing quantitative data for multiple proteins in a single assay. Additionally, further processing and analysis of 2-D gels is performed using mass spectrometry to achieve accurate protein identification. Finally, the use of 2-D gels for protein analysis is limited due to poor reproducibility, high labor intensity and slow throughput. A microfluidic, non-gel based multidimensional protein separation system has the potential to enable high throughput, high resolution, automated and repeatable protein analysis.

Lynntech has designed a microfluidic, non-gel protein separation microfluidic cartridge that can effectively separate proteins from the bodily fluids. To make the system cost effective, Lynntech has utilized Polydimethylsiloxane (PDMS) material as a substrate, which has the microfluidic architecture stamped into it to generate the flow paths. Molded PDMS can be bounded to electrode-containing substrates through a simple oxygen-plasma treatment and thermal incubation process. As the molded PDMS and selected thermoplastics have elasticity they can simply be coupled to electrode substrates with a simple compression assembly. A schematic of such system is shown in Figure 1. The system operates on Continuous-flow pI-based protein separation (CFPS) principal. CFPS technique is similar to isoelectric focusing (IDF) but does not suffer from the high voltage pitfalls of IEF including dispersion by electroosmotic flow and Joule heating. In CFPS the required electric field for separation is applied across the channel width rather than the length. Potentially, CFPS can be achieved without any applied voltage but by pure diffusion of two different buffer species in situ within a microfluidic channel (Song, Hsu et al. 2006[9]). The following discussion explains our technique of optimizing the design shown in Figure 1 by utilizing COMSOL and Design of Experiments.

Plackett-Burman													
Number	Pattern	Top inlet pH	Top inlet ionic strength	Middle inlet pH	Middle inlet ionic strength	Bottom inlet pH	Bottom inlet ionic strength	L over W ratio	L	W over V	Flow rate	Response	
												Max (pH_A-pH_B)	Max (Mean of dpH/dy-$\sigma_{dpH/dy}$)
1	---+--+-++	Min	Min	Min	Max	Min	Min	Max	Min	Max	Max	-	-
2	--+--+-+++	Min	Min	Max	Min	Min	Max	Min	Max	Max	Max	-	-
3	++----+---	Max	Max	Min	Min	Min	Max	Min	Min	Max	Min	-	-
4	+----++---	Max	Min	Min	Max	Min	Max	Max	Max	Min	Min	-	-
5	-+----+--+	Min	Max	Min	Max	Max	Max	Min	Min	Min	Max	-	-
6	--+--++---	Min	Min	Max	Min	Max	Max	Max	Min	Min	Min	-	-
7	++++++++++	Max	Max	Max	Max	Max	Max	Max	Max	Max	Max	-	-
8	+---+--+-+	Max	Min	Min	Min	Max	Min	Min	Max	Min	Max	-	-
9	-+--+-+++-	Min	Max	Min	Min	Max	Min	Max	Max	Max	Min	-	-
10	++-+--+--+	Max	Max	Max	Min	Min	Min	Max	Min	Min	Max	-	-
11	-+++----+-	Min	Max	Max	Max	Min	Min	Min	Max	Min	Min	-	-
12	+-+++-+---	Max	Min	Max	Max	Max	Min	Min	Min	Max	Min	-	-

Table 1: Plackett-Burman Design with twelve runs of experiments or models can be an effective way to screen most important parameters. Min and Max correspond to the minimum and maximum value of that particular parameter based on physical space constraints and other practical limitations identified during this work.

2. Design of Experiments (DOE)

The goal of the DOE was to maximize the widthwise pH distribution at the outlet of the microfluidic channel with the highest achievable uniformity so that the proteins with their associated pI values can be separated effectively. The design, geometry and the parameters of the isoelectric focusing chip that affect the protein separation were optimized for a broad range of pI values. This was done by performing a Design of Experiment (DOE) using COMSOL Multiphysics and JMP statistical analysis software. The optimization focused on one single chip, with three inlets and three outlets (Figure 1).

Ten individual parameters controlling the pH distribution in the channel were identified. The minimum and maximum values for these parameters based on the requirements of the protein separation chip were defined. For practical considerations and simplicity, L/W was taken as an independent parameter. In a similar manner, electrode voltage (V) was linearly dependent on the channel width; hence W/V was taken as an independent parameter.

Since many parameters affect the pH resolution in the chip and evaluating each one of them experimentally is very time consuming and labor intensive, a Plackett-Burman type of DOE analysis was performed utilizing JMP software to identify how each of the above parameters affected the pH distribution and uniformity at the outlet of the channel. Twelve combinations of the above parameters (Table 1) were identified based on the DOE with the individual responses for each combination in the test matrix being derived from COMSOL Multiphysics simulations. The COMSOL models were set-up using the following modules; Incompressible Navier-Stokes equation which describes ion transport due to convective fluid flow in the channel, Electrostatics equation which describes ion migration due to the external applied potential on the electrodes embedded in the PDMS of the channel and the Nernst-Plank equation that describes ionic transport in incompressible fluids due to diffusion and concentration gradients in the channel. These three modules were coupled with each other and solved in multi-physics mode with the solution from the Navier Stokes equation utilized in the Nernst-Plank equation. There were two separate criteria that were used for the evaluation of the response; maximum and uniform pH distribution
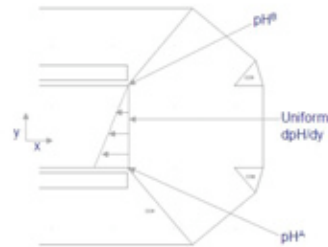

Figure 2: Schematic representation of the response was evaluated using COMSOL Multiphysics. The goal of DOE was to maximize pH^A-pH^B and |Mean of dpH/dy|-$\sigma_{dpH/dy}$; where σ is the standard deviation.
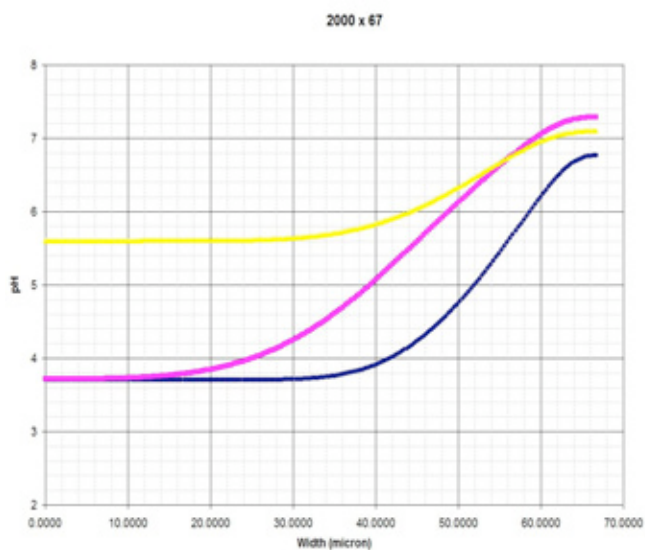
Figure 3: COMSOL analysis results for model 1, 6 and 10 of Table 2 are presented. Microfluidic channel dimension for these three combinations are 2000μmx67μm.

at the channel outlet. These are described using the following constraints: Maximum pH distribution = Max (pH_A-pH_B) and uniform pH gradient = Max (|Mean of dpH/dy|-$\sigma_{dpH/dy}$). This is schematically shown in Figure 2.

3. Result and discussion

Two separate test cases of DOE designs were evaluated using COMSOL; with and without the application of external potential. A representative result from the COMSOL simulation with the width wise pH distribution at the exit of the microfluidic channel with no external potential is shown in Figure 3. PB1, PB6 and PB10 correspond to Plackett Burman design patterns 1, 6 and 10 correspondingly (Table 2). In this figure, three models with similar geometries are grouped together. The left

most side of the curves in Figure 3 corresponds to pH^A and the right most side of the curve corresponds to pH^B. Similar results were obtained for the entire test matrix shown in Table 1.

Analysis of the responses obtained from COMSOL was carried out using JMP software. JMP provided a relationship between the individual parameters, and the variables that are being optimized (maximum values of pH_A-pH_B and |Mean of dpH/dy|-$\sigma_{dpH/dy}$). In JMP, a positive slope for any line pertaining to each parameter represented a positive response of that particular parameter to the response value, indicating a convergence towards maximum pH distribution and uniformity whereas a negative slope for any line pertaining to each parameter represented a negative response of that particular parameter to the response value, indicating a divergence from
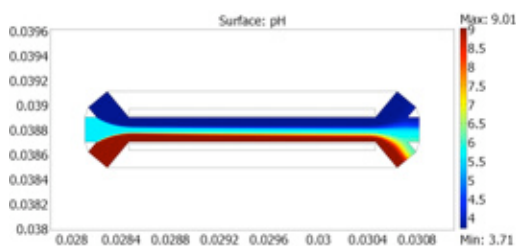

Figure 6: COMSOL model showing pH gradient in the microfluidics channel for the best possible case proposed by DOE analysis; no external potential applied.
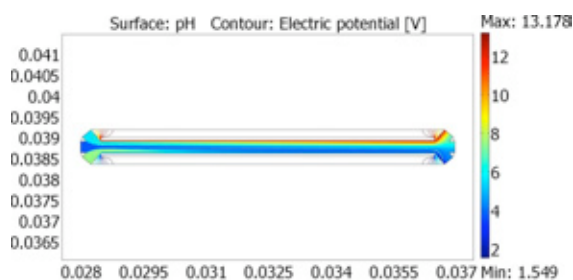

Figure 7: COMSOL model showing pH gradient in the microfluidics channel for the best possible case proposed by DOE analysis; external potential applied.
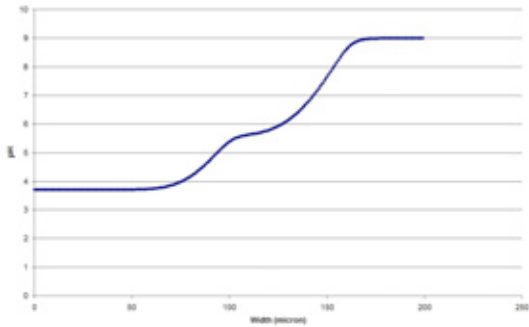
Figure 8: pH distribution at the outlet of the microfluidic channel for the best possible case proposed by DOE analysis when external potential is not applied.
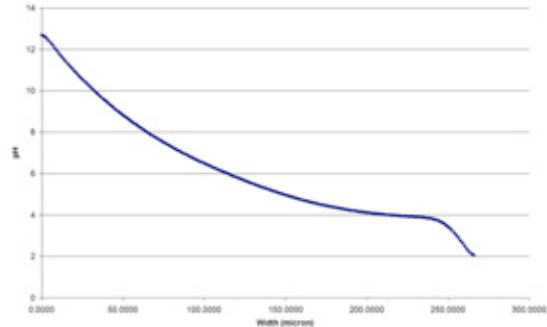

Figure 9: pH distribution at the outlet of the microfluidic channel for the best possible case proposed by DOE analysis when external potential is applied.

maximum pH distribution and uniformity. Combining the results, we were able to achieve maximum pH_A-pH_B, while keeping high uniformity. The actual model and the pH distribution at the outlet of the microfluidic channel with these combinations of parameters are shown in Figure 6 and Figure 8 respectively.

Following the preliminary optimization without any external electric field, a new set of DOEs were performed with applied external potential. The top electrode was at kept at positive potential and bottom electrode was kept at negative potential. Following similar exercise we were able to achieve widthwise maximum pH gradient with highest uniformity. An addition of electric potential as an extra parameter resulted in 10 independent parameters, which were simulated with the Plackett-Burman optimization model explained earlier. With this type of analysis, parameters obtained from DOE optimization were different from the previous case. The results of this analysis are shown in Figure 7 and Figure 9. Due to external electric field effect, hydrogen ion migrated towards negative electrode at the bottom of the microfluidic channel, and formed low pH zone. The lowest pH was noted 1.549; while maximum pH formed at the top most region of the microfluidic channel and its value was 13.178.

4. Conclusions

From the optimization based on numerical simulations, it was observed that a pH gradient in the range of 3.7 to 9.01 can be achieved with one single isoelectric focusing chip in the case of zero voltage applied to the electrode and a pH

gradient in the range of 1.549 to 13.178 can be achieved with the potential applied to the electrodes. These results are critical because they allow Lynntech the flexibility to separate proteins almost over the entire range of pH values. It was also found that application of external voltage enhanced pH distribution in the microfluidic channel. Utilizing DOE technique with the results obtained from COMSOL has proved to be very effective in optimizing microfluidic chip design.

This technique can also prove very effective in optimizing wide range of products at their early stage of development or improvisation of existing ones. It has increased prototyping speed and efficiency along with significantly reducing the cost.

5. References

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6. Acknowledgements