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TWO-DIMENSIONAL PROTEIN SEPARATION IN A PLASTIC DEVICE WITH A MICROVALVE ARRAY

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ABSTRACT

Two-dimensional (2D) protein separation is demonstrated in a plastic microfluidic device by integrating isoelectric focusing (IEF) in 1 channel and polyacrylamide gel electrophoresis (PAGE) in 29 parallel channels that are orthogonal to the IEF channel. An array of microfluidic valves is developed for introducing different separation media into two dimensions and for transferring proteins from the first to the second dimension. Fabrication of valves is achieved by *in situ*, photo-initiated gel polymerization. The total separation time is less than 10 minutes, which is favorable when compared to 1-2 days for conventional 2D slab gel electrophoresis.

Keywords: Two-dimensional separation, microvalves, electrophoresis, proteins

1. INTRODUCTION

Among the approaches being developed for proteomics, two-dimensional gel electrophoresis (2DGE) is an essential tool [1]. 2DGE consists of isoelectric focusing (IEF) as the first dimension and polyacrylamide gel electrophoresis (PAGE) as the second dimension. One major advantage of 2DGE is its enormous separation resolution, whereas its key limitations include poor reproducibility and time-consuming procedure. This work is carried out to address the limitations.

2. EXPERIMENTAL

Cyclic olefin copolymer (Topas) is used to fabricate 2D separation devices using compression molding. **Figure 1** shows the device design; it consists of one horizontal channel (AB) for IEF and 29 vertical channels (CD) for PAGE. The size of the device is 1" x 3", about the size of a microscope slide. All channels are 110 µm wide and 40 µm deep. The distance between channels (center-to-center) is 360 µm. An exploded view of a part of the device is shown in the middle of Figure 1. On the right is the interface between IEF and PAGE, illustrating proteins in each pI band being transferred from the first to the second dimension and then separated. The

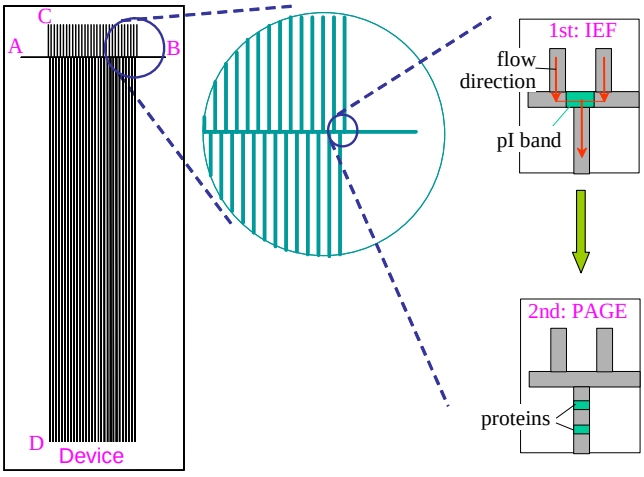

***Figure 1**. Design of a microfluidic device for 2DGE.*

arrows indicate the flow direction.

Acrylamide monomer, 1-hydroxycyclohexylphenylketone (HCPK), proteins are purchased from Sigma-Aldrich (St. Louis, MO) while carrier ampholytes are from Bio-Rad Laboratories (Hercules, CA). The detection is carried out using a laser-induced fluorescence, whole-channel imaging system [2].

3. RESULTS AND DISCUSSION

The device in Figure 1 is different from those using two orthogonal separation channels, in which capillary electrophoresis (CE) is coupled with either micellar electrokinetic chromatography [3, 4] or IEF [5]. The key difference is the use of multiple channels, rather than a single channel, for the second dimension to match the separation capacity of the slab in 2DGE. This work is also different from the existing work on miniaturized 2DGE [6, 7]. The key advances are twofold: (1) an array of microfluidic valves allows the seamless introduction of two different separation media; and (2) a fluidic network with densely-packed channels enables the appropriate transfer of proteins from the first to the second dimension.

The valve array is created by *in situ* gel polymerization. To make gel valves at a precise location, acrylamide monomer solution containing HCPK is first filled in all channels. HCPK is a photosensitive agent to initiate gel polymerization, replacing chemical initiators. After the AB channel of the device in Figure 1 is covered by a mask, photopolymerization is conducted by exposure to UV light. The solution in the exposed region (CD channels) polymerizes to form gel, whereas the solution in the AB channel is blocked by the mask and do not polymerize. **Figure 2** shows the gel valve array. These gels function as "closed" valves when an IEF separation medium is introduced into the AB channel without flowing into the CD channels. After IEF, they function as "open" valves when an electric field is applied to the CD channels. The focused proteins can be transferred from the first into the second dimension, because proteins can be electrokinetically injected through gels.

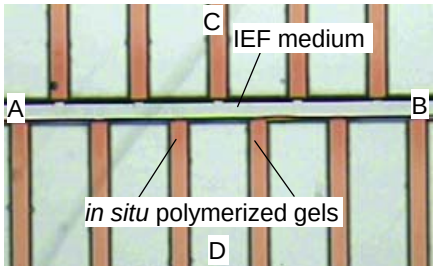

Figure 2. *Micrograph of the valve array. Polymerized gels are dyed for easy visualization.*

In the presence of microvalves, IEF separation of proteins is implemented in the device as shown in **Figure 3**. The pH gradient is established using carrier ampholytes with a pH gradient of 3-10. It should be noted that the IEF analysis time is typically in 2–5 minutes, much shorter than hours needed in the traditional IEF strip format. Three fluorescently-labeled proteins, bovine serum albumin (BSA, pI = 4.6, MW = 66 KDa), ovalbumin (4.6, 44 KDa), and parvalbumin (4.0, 12 KDa), are used in this experiments. BSA and ovalbumin have the similar isoelectric point (pI), thus focused in the same pI segment. However, both of them are separated from parvalbumin. The broad peak of parvalbumin is due to heterogeneity in labeling, which is confirmed by IEF of the individual protein.

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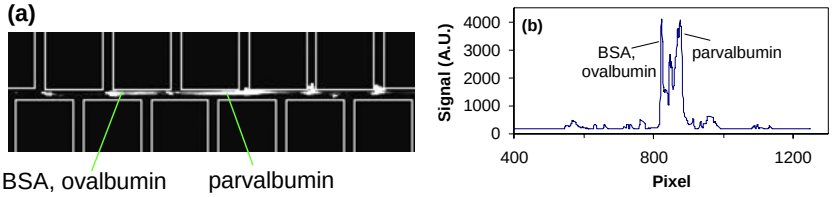

Figure 3. *(a) IEF of fluorescently-labeled BSA, ovalbumin, and parvalbumin in the device. Lines are added to show the channel locations. (b) The corresponding electropherogram of (a). Peaks of proteins are indicated. Each pixel in x axis approximately corresponds to 20 µm.*

BSA and ovalbumin can be separated in the second dimension as shown in **Figure 4**, in which we also used carbonic anhydrase (5.9, 31 KDa) and hemoglobin (6.8, 65 KDa). Electropherograms in all vertical CD channels (#1 to 29) are illustrated together to form the 2D map. The channel number is related to pI while migration time corresponds to the molecular size. The total analysis time is less than 10 minutes. The result suggests that 2DGE can be implemented in a plastic device with a microvalve array.

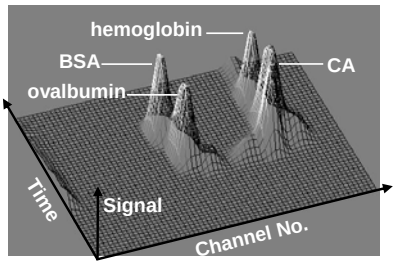

Figure 4. *2D map of BSA, ovalbumin, hemoglobin and carbonic anhydrase (CA) obtained in the 2D electrophoresis device.*

4. CONCLUSION

We demonstrated the integration of IEF and multi-channel PAGE in a plastic microfluidic device. The key element that enables 2D electrophoresis in the device is an array of microfluidic valves, which is created by in situ, photo-initiated gel polymerization. The gel valves allow the introduction of different separation media into two dimensions and the transfer of proteins from the first to the second dimension.

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