ELECTROKINETIC PRECONCENTRATION OF PROTEINS ON THIN PDMS MEMBRANES

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ABSTRACT

On-chip protein preconcentration at the head of a separation column affords substantial improvements in the limit of detection for low concentration analytes. In this work we demonstrate 40-fold preconcentration of FITC-BSA at the head of a CE column by applying high voltage across a thin PDMS wall, and injection of the concentrated sample into a CE column. This method offers very simple fabrication and complements efforts by Singh et al. using nanoporous silica beads [1], by Khandurina et al. using a spin-on glass membrane [2], and Yu et al [3] using adsorption on a polymer monolith.

KEYWORDS: Preconcentration, Proteins, Electrophoresis

INTRODUCTION

On-chip protein preconcentration at the head of a separation column affords substantial improvements in the limit of detection for low concentration analytes. Recent efforts by various groups using electrokinetic means include Singh et al., who employed nanoporous silica beads [1], by Khandurina et al. using a spin-on glass membrane [2], and Yu et al [3] using adsorption on a polymer monolith. The mechanism for preconcentration in each of these cases is apparently somewhat different for each case. In this work we demonstrate 40-fold preconcentration of two proteins at the head of a CE column by applying high voltage across a thin PDMS wall, and injection of the concentrated sample into a CE column. The experimental results imply a combination of dielectric breakdown and electrokinetic preconcentration mechanisms. This method offers very simple fabrication method; however, run to run repeatability is still an issue.

EXPERIMENTAL RESULTS

A PDMS microchannel network, shown in Figure 1, was fabricated by casting various thicknesses of PDMS (3.4mm, 2.9mm, and 1.7mm) over an SU-8 mold on a
Figure 1. Chip Layout and voltage program for preconcentration (top). A 20 micron thick membrane of PDMS isolates the top chevron-shaped channel from the bottom channels/separation column. Fluorescence time sequence shows preconcentration near the membrane.

Three possible preconcentration mechanisms have been hypothesized. The first is that the thin PDMS membrane acts as a simple steric sieve; electroosmotic flow (EOF) and electrophoresis through the channels & membrane transports the silicon substrate. Channels are approximately 20μm wide and 10μm deep. The PDMS slab is then placed over a glass microscope slide, and ports are punched through to form reservoirs. The network consists of two flow-through loading ports, one of which is connected to the top of a chromatographic column.

A dilute solutions of protein in various buffers (10mM phosphate buffer pH 7.2, phosphate buffer saline, with a variety of dilutions into carbonate buffer) is loaded into port 1 (port 2 is a vent). 10μM FITC-labeled BSA in the same buffer is loaded through port 3 (port 4 is a vent); note that this means that the CE column is also filled with the relatively weak analyte solution.

To preconcentrate the protein, ports 1 and 2 are grounded while voltages ranging from 20V up to 1kV is applied at ports 3 & 4 (the channels are about 2.2 cm from port-to-port). A time-lapse sequence of fluorescence images shows preconcentration near the membrane. Profiles of the concentration profile versus time (Fig. 2) suggest that FITC-BSA is concentrated by a factor of about 40, in less than 30 seconds (the observed saturation characteristic is due to a lack of dynamic range in the camera). The protein is then injected into the CE column as shown in Figure 3. Interestingly, the preconcentrated bolus "recoils" once the power configuration in Fig. 1 is removed (e.g. before the voltage program in Figure 3 has been applied!). This suggests that there is some form of stored energy (such as a pressurized region, or a charge capacitance) at the membrane during preconcentration, and this may be an important clue regarding the preconcentration mechanism.

**DISCUSSION**

Three possible preconcentration mechanisms have been hypothesized. The first is that the thin PDMS membrane acts as a simple steric sieve; electroosmotic flow (EOF) and electrophoresis through the channels & membrane transports the...
proteins until they become trapped against very small nanopores in the PDMS. While one indeed might expect ionic transport to be possible through a very thin PDMS membrane, one would expect EOF to be quite minimal. Three strange observations appear to deny this explanation: (1) the negatively-charged proteins collect on the anode side of the membrane, (2) the membrane’s resistance varied with thickness of the PDMS slab (constant membrane dimensions) (285MΩ for the 3.4 mm-thick chip; 145MΩ for the 2.9 mm-thick chip, 33MΩ for the 1.72mm chip), and the preconcentration was faster when the PDMS slab was thinner, and (3) In Figure 3, after the ports are suddenly floated, a portion of the sample “recoils” away from the intersection for a moment and then ceases, suggestive of some form of stored energy being discharged.

A second preconcentration mechanism hypothesized was that dielectric breakdown through the PDMS-glass interface was somehow responsible for the observed behavior. This hypothesis was supported by two observations, namely (4) occasional “shooting” of protein across the membrane, and erratic currents, found at high voltages. To explore this, we constructed PDMS/PDMS chips that (mixed slightly off-stoichiometric in the manner of Unger et al [4]), achieved a much stronger bond between chip halves. Indeed, the observed preconcentration, which is observed for voltages as low as 20V in the case of PDMS/glass chips, was then not observed until applied voltages exceeded 200V. When it did occur in the PDMS/PDMS chips, it was also immediately accompanied with an irreversible jump in the current and bubble formation, which is strongly suggestive of dielectric breakdown. While this strongly suggests that the PDMS/glass interface plays a crucial role, it was also observed that the dramatic, erratic, “shooting” of protein across the membrane (suggestive of dielectric breakdown) was not a prerequisite for preconcentration behavior, and so dielectric breakdown does not appear to be a complete explanation, either.

However, these observations suggest that a hydrated narrow gap (at most a few nanometers thick) between the glass and PDMS exists, which is capable of significant ionic transport. Indeed, calculations suggest that a 1-5 nm thick gap, acting as a single nanopore with a 25mV surface zeta potential, would adequately account for the membrane resistances observed. Thus the important part of the “membrane” may simply be a nanometers-thin gap between the glass and the PDMS. In this way, observation (1)
is explained, as EOF is indeed expected to dominate over electrophoresis in this situation; observations (2) & (3) can be explained as resulting from slight flexure of the PDMS slab, perhaps as a result of electrokinetic pressure generation [5], and then the recoiling of the slab after the potential difference is removed. This would only be slight in the 10µm-deep channels here (about 1 kPa at 100V), but perhaps sufficient to explain 1-5 nm of deflection.

CONCLUSIONS
At least 40-fold rapid preconcentration of labeled ovalbumin and BSA has been achieved using simple PDMS/glass chips. The mechanism at present appears to be a straightforward combination of steric and electrokinetic effects, although our elucidation of the mechanism is not totally conclusive. The existence of a small nm-sized hydrated gap between PDMS and glass would explain most of the behavior observed. It appears that a slight electrokinetic pressure developed near the membrane interface may be responsible for a sudden “recoiling” behavior, wherein the preconcentrated analyte suddenly is thrust away from the preconcentration site once the applied voltage is removed. This behavior can be used to advantage to cleanly inject analyte into a separation column.

REFERENCES