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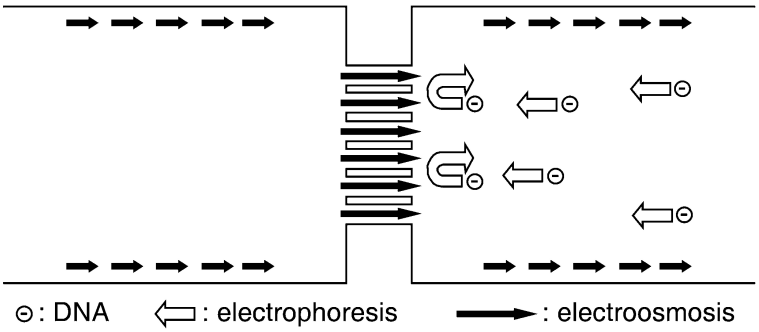
J. Am. Chem. Soc., **2003**, 125 (43), 13026-13027• DOI: 10.1021/ja0374776 • Publication Date (Web): 04 October 2003



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Published on Web 10/04/2003

Electrokinetic Trapping and Concentration Enrichment of DNA in a Microfluidic Channel

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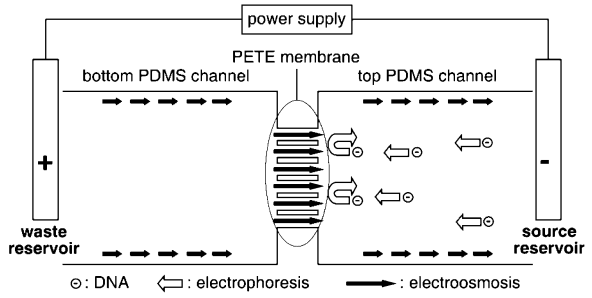
We report a simple and efficient method for concentrating analytes within microfluidic channels. Proof of concept is demonstrated here using DNA, but the approach is versatile and should be applicable to any charged molecule or object. The method relies on exerting spatial control over the electrokinetic velocity of the analyte. Specifically, the electroosmotic (eo) velocity of the buffer solution in one region of a microfluidic system opposes the electrophoretic (ep) velocity of the analyte in a second region (Scheme 1). This results in ep transport of DNA to a location where the ep and eo velocities are equal and opposite, and DNA concentrates at this location. This enrichment method is conceptually distinct from field-amplification stacking,[1,2] isotachophoresis,[3,4] micelle sweeping,[5,6] size exclusion,[7,8] and solid-phase extraction,[9,10] but it is conceptually linked to a recent report in which micrometer-scale beads were trapped within a microfluidic channel by opposing pressure-driven flow and eo flow.[11] Our new approach requires no complex microfabricated structures and no special manipulation of the solvent, and the concentrated analyte remains in solution rather than being captured on a solid support.

Scheme 1



Scheme 1 illustrates the principle of operation of this microfluidic concentrator (design details are provided in the Supporting Information). The three-dimensional microfluidic system consists of a nanoporous polyester membrane[12] (Osmonics, PETE, 200 nm pore diameter, 10 μm thick, and 3×10^8 pores/cm^2) sandwiched between two poly(dimethylsiloxane) (PDMS) blocks, each containing a single fluidic channel (100 μm wide, 25 μm deep, and 5 mm long) connected to a reservoir (3 mm diameter). This design is similar to previously reported arrangements,[13−16] but the function is fundamentally different. Specifically, Whitesides' group used this design to facilitate diffusive transport but suppress convective transport,[13] and Bohn and Sweedler principally used this design to direct the transfer of analytes between PDMS channels.[14−16]

The channels and reservoirs were filled with 1× TBE buffer (89 mM TRIS base + 89 mM boric acid + 2 mM EDTA, pH 8.4) at reduced pressure and conditioned by applying 100 V between the reservoirs until the current stabilized. Then the buffer solution in the source reservoir was replaced with 10 μg/mL DNA (a 20mer ssDNA, 5′-labeled with fluorescein, from IDT, Coralville, IA) in

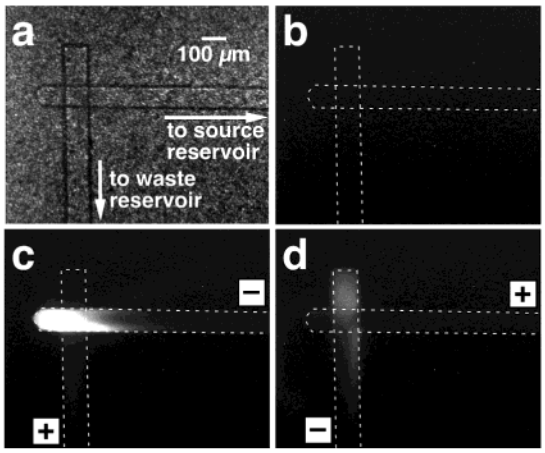


Figure 1. (a) Optical micrograph of the microfluidic system used for DNA concentration enrichment. The uneven background results from light scattering off the porous membrane. The distance between the electrodes in the source and waste reservoirs is 1 cm. (b) Fluorescence micrograph of the region shown in (a) before application of a bias voltage. (c) Fluorescence micrograph obtained after applying a 100 V forward bias for 68 s. Immediately after the micrograph in (c) was obtained, the bias was reversed, and 36 s later the fluorescence micrograph in (d) was obtained. Before applying the bias, the channels and waste reservoir were filled with 1× TBE buffer, and the source reservoir was filled with 1× TBE buffer containing 10 μg/mL DNA.

1× TBE. After taking optical and fluorescence micrographs (Figure 1, a and b, respectively), a forward bias (negative potential in the source reservoir) was applied between the electrodes, and the resulting motion of DNA was recorded using an inverted fluorescence microscope (TE300, Nikon, Japan) equipped with an imaging CCD camera (SenSys 1401E, Photometrics, Tucson, AZ). Under these conditions, concentration of DNA is apparent within 30 s and reaches an enrichment factor of 11 within 68 s, as shown in Figure 1c (a movie is available in the Supporting Information). When the bias is reversed (Figure 1d), DNA immediately transports through the PETE membrane, indicating that concentration is not a consequence of physical blocking or size exclusion, and is trapped in the left channel (Scheme 1) by the same balance of ep and eo velocities that were initially responsible for concentration in the right channel.

As shown in Scheme 1, the first requirement for concentration of DNA is that the ep velocity of DNA must be larger than the fluid velocity of the buffer solution in the right PDMS channel. This requirement is met because DNA is transported from the source reservoir toward the PETE membrane when the channel is initially filled with buffer (Figure 1, b and c). The second requirement for DNA enrichment is that the local velocity of the electroosmotic jets emanating from the pores within the PETE membrane should be larger than the ep velocity of DNA. However, this requirement can be achieved only in the absence of similitude between the velocity and electric fields.[17] Here, similitude simply means that

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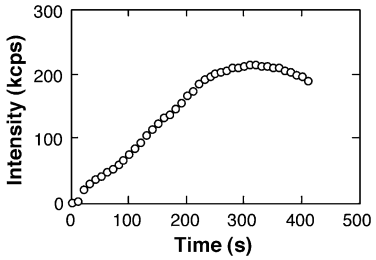

Figure 2. Fluorescence intensity integrated over the exposed PETE membrane area (100 μm × 100 μm) as a function of the time allotted for concentration enrichment of DNA. All intensity values were measured in the center of the enriched band and were corrected by subtracting the background value at $t = 0$. The DNA concentration in the source reservoir was 10 μg/mL, and the forward bias voltage (applied at $t = 0$) was 100 V.

the spatial velocity profile has the same shape as the profile of the electric field. Of the conditions required for the existence of similitude,[17] only one is apparently not met: the surface ζ potential of the fluidic duct may not be uniform. That is, the surface of the track-etched, base-hydrolyzed polyester membrane has a higher negative charge density than the native, untreated PDMS surface. Thus, there may exist a region at the junction of the PETE membrane and the PDMS channel where the fluidic velocity vector does not scale with the electric field vector.[18]

Another possible explanation for DNA enrichment is temperature gradient focusing (TGF).[19] This seems reasonable because TBE buffer exhibits a temperature-sensitive ionic conductivity. However, TGF cannot be the dominant mechanism responsible for our observations for the following reasons. First, the field strength used in our experiment is typically around 100 V/cm, which is too small to generate the heat required for a large temperature gradient.[20] Second, such a gradient cannot be formed easily, given the extreme thinness (10 μm) of the PETE membrane. Third, we have observed the same enrichment phenomenon when 89 mM Tris-HCl buffer, which has a very small temperature-dependent conductivity, was used in place of TBE. Imaging of the temperature distribution with a fluorescent dye[21] also indicates a maximum temperature rise of less than 0.5 °C and a nondetectable temperature gradient in the vicinity of the PETE membrane.

As shown in Figure 2, the magnitude of DNA concentration reaches a limiting value within a finite time (t_{CONC}). At $t < t_{CONC}$ the enriched band has a nearly constant length, but when $t > t_{CONC}$, this band begins to expand longitudinally in the direction of the source reservoir. For an initial concentration of 10 μg/mL DNA and a 100 V forward bias, t_{CONC} is about 5 min, and the enrichment factor, calculated from the relative fluorescence intensity, is 30. However, when the DNA concentration in the source reservoir is reduced to 1 and 0.1 μg/mL, the enrichment factors increase to 300 and 800, respectively. Considering the simplicity and compactness of this microfluidic system, these enrichment factors are significant.

To summarize, we have reported a simple and efficient concentration enrichment method for microfluidic applications, and demonstrated its properties using DNA. Two characteristics of this method are noteworthy. First, only a single homogeneous analyte phase is required, and second, enrichment factors exceeding 100 can be achieved using channels that are only 5 mm in length. We want to emphasize that the principle of concentration enrichment described here is general and versatile, and that it should be applicable to any charged molecule or object and to other types of devices. Forthcoming reports will focus on mechanistic studies of the observed phenomemon and coupling preconcentration with other fluidic operations such as sample injection.

Acknowledgment. We gratefully acknowledge financial support from the U.S. Department of Energy, Basic Energy Sciences (Contract No. DE-FG03-01ER15247). We thank Dr. Ali Beskok, Mr. Jungyoon Hahm, and Dr. Jyh-Myng Zen for helpful discussions. We also thank Drs. David Ross and Laurie E. Locascio (NIST) for bringing their temperature gradient focusing studies to our attention.

Supporting Information Available: Design details of the three-dimensional microfluidic device used in this work (PDF) and a movie (AVI format) demonstrating concentration enrichment at forward potential bias and transmembrane transport under reverse bias. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Chien, R.-L.; Burgi, D. S. *Anal. Chem.* **1992**, *64*, 489A−496A.
(2) Lichtenberg, J.; Verpoorte, E.; de Rooij, N. F. *Electrophoresis* **2001**, *22*, 258−271.
(3) Walker, P. A., III; Morris, M. D.; Burns, M. A.; Johnson, B. N. *Anal. Chem.* **1998**, *70*, 3766−3769.
(4) Foret, F.; Szoko, E.; Karger, B. L. *J. Chromatogr.* **1992**, *608*, 3−12.
(5) Sera, Y.; Matsubara, N.; Otsuka, K.; Terabe, S. *Electrophoresis* **2001**, *22*, 3509−3513.
(6) Quirino, J. P.; Terabe, S. *Anal. Chem.* **1999**, *71*, 1638−1644.
(7) Han, J.; Craighead, H. G. *Science* **2000**, *288*, 1026−1029.
(8) Khandurina, J.; Jacobson, S. C.; Waters, L. C.; Foote, R. S.; Ramsey, J. M. *Anal. Chem.* **1999**, *71*, 1815−1819.
(9) Yu, C.; Davey, M. H.; Svec, F.; Fréchet, J. M. J. *Anal. Chem.* **2001**, *73*, 5088−5096.
(10) Fritz, J. S.; Macka, M. *J. Chromatogr., A* **2000**, *902*, 137−166.
(11) Lettieri, G.-L.; Dodge, A.; Boer, G.; de Rooij, N.; Verpoorte, E. *Lab Chip* **2003**, *3*, 34−39.
(12) Martin, C. R. *Science* **1994**, *266*, 1961−1966.
(13) Ismagilov, R. F.; Ng, J. M. K.; Kenis, P. J. A.; Whitesides, G. M. *Anal. Chem.* **2001**, *73*, 5207−5213.
(14) Kuo, T.-C.; Cannon, D. M., Jr.; Chen, Y.; Tulock, J. J.; Shannon, M. A.; Sweedler, J. V.; Bohn, P. W. *Anal. Chem.* **2003**, *75*, 1861−1867.
(15) Kuo, T.-C.; Cannon, D. M. Jr.; Shannon, M. A.; Bohn, P. W.; Sweedler, J. V. *Sens. Actuators, A* **2003**, *102*, 223−233.
(16) Cannon, D. M., Jr.; Kuo, T.-C.; Bohn, P. W.; Sweedler, J. V. *Anal. Chem.* **2003**, *75*, 2224−2230.
(17) Cummings, E. B.; Griffiths, S. K.; Nilson, R. H.; Paul, P. H. *Anal. Chem.* **2002**, *72*, 2526−2532.
(18) Herr, A. E.; Molho, J. I.; Santiago, J. G.; Mungal, M. G.; Kenny, T. W. *Anal. Chem.* **2000**, *72*, 1053−1057.
(19) Ross, D.; Locascio, L. E. *Anal. Chem.* **2002**, *74*, 2556−2564.
(20) Effenhauser, C. S.; Bruin, G. J. M.; Paulus, A.; Ehrat, M. *Anal. Chem.* **1997**, *69*, 3451−3457.
(21) Ross, D.; Gaitan, M.; Locascio, L. E. *Anal. Chem.* **2001**, *73*, 4117−4123.

JA0374776