Nanofluidic Size Exclusion Chromatograph for In Site Macromolecular Analyses

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ABSTRACT

We are developing a nanofluidic size exclusion chromatograph (nano-SEC) system that can be incorporated into miniature lab-on-a-chip devices which will provide detailed information on the geochemical and biological history of soil, ice, and water samples by identifying and qualifying a broad range of macromolecular polar and nonpolar organic compounds. Molecules traveling through a microcolumn containing nanometer-scale features will have characteristic elution times that directly correlate to molecular weight. Advantages of our nano-SEC over conventional SEC are expected to include the following: 1) column reproducibility is greatly aided by the microfabrication process; 2) column packing is not required; 3) minute sample quantities with sub-nl injection volumes can be analyzed; 4) greater control over size exclusion separation parameters can be achieved; 5) the complete nano-SEC system is expected to be portable, robust, miniature, and operate on low voltages and low powers.

Keywords: nanofluidics, size exclusion chromatograph, lab-on-a-chip, macromolecules.

1 INTRODUCTION

Size exclusion chromatography is a subset of high-pressure liquid chromatography (HPLC) in which molecules are separated based on their retention time in a size exclusion column consisting of small (~10 μm) closely packed silica or polymer beads with uniform nanopores ranging from 10 to 100 nm [1]. Macromolecules larger than the exclusion limit are unable to enter the nanopores and remain in the liquid mobile phase existing the column first. Molecules smaller than the permeation limit can completely penetrate the nanopores, are retained in the stationary phase, and elute last. Fractionation takes place between intermediate sized molecules as the ratio of analyte concentration in the stationary phase to that in the mobile phase decreases with increasing molecular size. Thus molecules elute in order of decreasing size and can be detected with a variety of methods, including laser-induced fluorescence system. The resulting chromatogram leads to molecular identification based on peak elution times and molecular concentrations that can be inferred from the peak size.

2 FABRICATION OF NANO-SEC DEVICE

The nano-SEC's nano-fabricated features, analogous to the traditional SEC's bead nanopores, consist of size-exclusion gaps defined in the z-direction over a matrix of microchannels in the x-y plane, similar to the interstices between the beads as shown in Figure 1.

Figure 1. 3-D and cross-sectional views of nano-SEC schematic.

The fabrication sequence to create a sealed nano-SEC column device consists of the following steps as shown in Figure 2. (A) First, a 100 nm thermal oxide (SiO_2) layer is grown on the entire wafer. (B) Optical lithography and a subsequent CF_4-based reactive ion etching (RIE) process are then used to form 100 nm high SiO_2 post structures, with the thermal oxide layer as a mask material. These structures define the height of the size-exclusion gaps. (C) Subsequently, another optical lithography step along with an SF_6-based RIE process is performed to define 3-5μm wide/500 nm deep Si channels in the wafer. (D) A 10-20 nm thick thermal oxide layer is produced over the entire wafer in order to create a chemically uniform surface (critical for minimizing analyte/surface interactions). (E) Finally, fluid interface ports are created (e.g. by diamond drilling) and a Pyrex cover is anodically bonded to the channel wafer in order to form the device roof. Images of our nano-SEC devices (top-views and cross-sectional views) are shown in Figure 3.

3 THEORETICAL MODELING

We have developed a theoretical model for the nano-SEC based on Hele-Shaw type fluid in parallel plates. In this model, analyte separation is achieved by diffusion of the analyte between a "mobile phase" (eluent flow through the large gap regions) and a "stationary phase" (due to a flow rate considerations, the eluent is essentially at rest in the nano-gap) [2]. In our Hele-Shaw model, assuming a constant pressure across the separation channel, the fluid

flow rate varies as the square of the height of the gap through which the fluid is flowing. Thus, the flow rate differs by two orders of magnitude between the 100 nm gap regions and the 1 μm gap regions. Each fluid region is modeled as a series of bundled capillary tubes of varying diameters (100 nm diameter tubes in the narrow gap regions; 1 μm gap tubes in the wider channel regions.).

Figure 2. Fabrication of a sealed nano-SEC column device. (A) Growth of a 100 nm thick oxide layer on Si. (B) Definition of 100 nm tall post structure by CF_4-RIE. (C) Channel patterning by SF_6-RIE. (D) Growth of 10 nm thick thermal oxide layer. (E) Anodic bonding of a Pyrex cover wafers onto a channel wafer for sealing.

Figure 3. Top and cross-sectional views of the sealed nano- SEC device: (A) SEM image of a top view of the nano-SEC channels. (B) SEM image of a cross-sectional view of the sealed nano-SEC device. (C) Optical microscope image of a top view of the sealed nano-SEC wafer. (D) SEM image of a cross-sectional view of the sealed nano-SEC wafer. A 100 nm deep gap can be seen.

Figure 4(A) shows that the transit times through tubes of various diameters are different. This variation is responsible for separation in time of fluids that come through different capillaries, and hence through different gap regions in the nano-SEC device. For illustrative purposes, Figure 4(A) shows where the fluid fronts are at time t = 1 s. Figure 4(B) shows the separation of a model analyte consisting of nanospheres with varying dimensions. Surface effects are considered negligible, and we assumed a total flow rate through the prototype nano-SEC column geometry of 0.0015 nL/min at a pressure of 55 psi. The theoretically predicted sample separation is highly dependent on molecular diameter (which determines molecular diffusion rates between the 100 nm gap and 1 μm gap regions), leading to efficient size exclusion separation [3].

Figure 4. (A) Distance traveled in cm at time t = 1 s. This figure shows that the transit times through tubes of varying diameters are different (here the tube diameter is denoted by " i " where " i " ranges from 600 nm down to 100 nm in 100 nm increments). (B) Modeled chromatogram, similar to that obtained by conventional SEC, of the separation of an analyte mixture of nanospheres in the nano-SEC. It shows separation of a model analyte consisting of nanospheres with varying dimensions.

4 CONCLUSIONS

We developed a novel nano-fabricated size exclusion chromatograph and have completed initial fabrication and modeling. Development of the nano-SEC will continue with a constant flow rate, nanofluidic injection system consisting of a coupled-syringe pump driven by AFM-positioning motors. With its relatively simple fabrication process incorporating precise control over nanometer-scale features in a chemically uniform, easily modified SiO2 surface, our nano-SEC may offer highly sensitive separation of a variety of analyses, including lipids, proteins, and peptides, in an integrated hand-held device.

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