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Development and Characterization of Microfluidic Channels for Chromatography-on-a-Chip Applications

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Summary:

In this work, we designed and fabricated a microfluidic device to perform chromatographic separations. The device consists of a column filled with silica microbeads between smaller height channels working as a frit. To characterize the column efficiency, the breakthrough method was used with a solution of fluorescein isothiocyanate (FITC) in ethanol and by measuring the fluorescence signal. Several mobile phase velocities were tested to obtain retention times and plate heights.

Keywords: Microfluidics, Liquid Chromatography, Cyclic Olefin Copolymer, Breakthrough Curves, Silica Microbeads

Background

Liquid chromatography is a separation technique widely used for the detection of molecules present in complex solutions [1], but it requires trained users in a laboratory and consumes a large quantity of reagents. On the other hand, microfluidics is a technology that has the potential to significantly improve biological and chemical analysis by being inexpensive, portable, and fast [2]. The miniaturization of liquid chromatography using microfluidics combines both technologies but has not been studied in detail. This work aims to develop microfluidic channels for liquid chromatography that can have separation performances comparable to those obtained at macroscale.

Methodology

Computer numerical control (CNC) milling was used directly in cyclic olefin copolymer (COC) plates with a flat endmill with 0.4 mm diameter. The feed rate was maintained at 100 mm/min at 12000 rpm. The device sealing and connections are described elsewhere [2]. Briefly, the machined COC is drilled and sealed against the same type of COC by thermal bonding. After the sealing, PEEK connectors are glued to the device. Silica (SiO_2) microbeads with a diameter between 45 and 75 µm diluted in PEG 8000 30% (w/w) are loaded into the device with the help of a syringe pump. The design used (see Fig. 1) consists of three 1 cm long channels with 100 µm height, and between them are 20 µm height channels to trap the microbeads inside the middle channel.

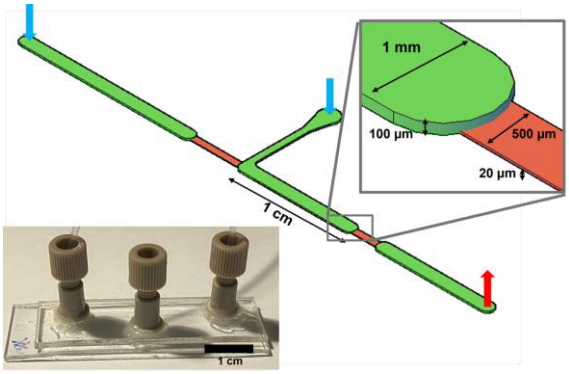

Fig. 1. Schematics of the chip design and a fabricated device in-use.

For breakthrough curve experiments, a solution of 12 µg/mL of fluorescein isothiocyanate (FITC) in ethanol was used, and the signal was acquired by fluorescence and quantified using ImageJ. The solution is flowed into the device at the desired flow, and time zero is taken when the fluorescence solution reaches the beginning of the microbeads. Several flow rates of the mobile phase were tested between 0.5 µL/min and 5 µL/min.

Results

In this device, we have a column of 1 cm in length packed with SiO_2 beads. A simple way to assess the column performance is by using the breakthrough curve method (or frontal chromatography). By performing breakthrough curves, we are observing the concentration of adsorbed analyte as a function of time, expecting the system to be fully saturated at the end of the experiment. In this case, our analyte was the fluorophore FITC, so the increasing adsorption was observed using a fluorescence microscope. In Fig. 2A are represented the breakthrough curves obtained for each flowrate tested (0.5 µL/min, 1 µL/min, 2.5 µL/min, and 5 µL/min).

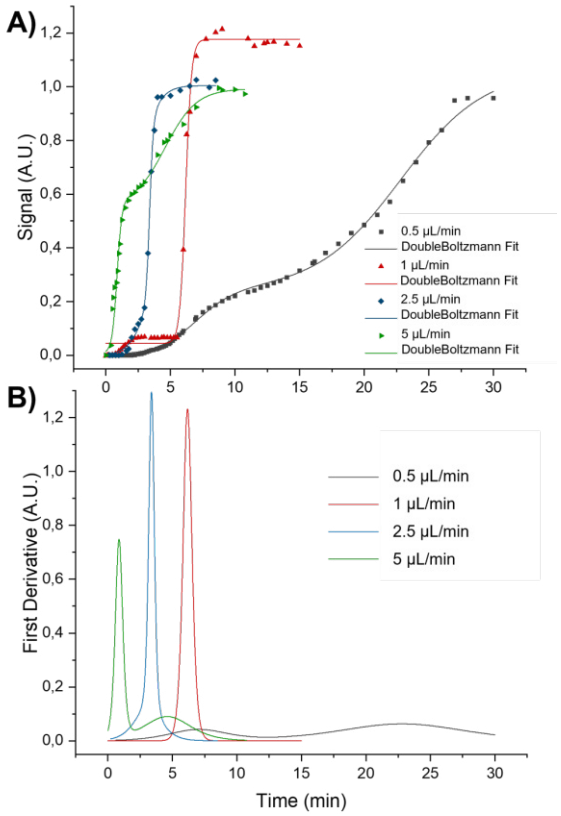

Fig. 2. A) Breakthrough curves and B) First Derivative plotted as a function of time for each flowrate.

To analyze the breakthrough data, the first derivative is calculated to obtain a pulse function, as seen in Fig. 1B. For all flowrates except for the 0.5 µL/min, a clear peak is observed, and as expected, the higher the flowrate, the closer to the origin is the peak. This system shows no retention of the FITC due to chemical interactions with SiO_2 beads, but the flow path is still interrupted by the beads, hence the retention times obtained. As for the 0.5 µL/min condition, the observed behavior may possibly be due to the B term of the van Deemter equation, the axial diffusion. Since the flow rate is lower, the residence time is longer, allowing the FITC to have more time to diffuse longitudinally and perhaps also within the pores. From the peak analysis it is possible to obtain the retention time (R_t) and the full width at half maximum (*FWHM*), and with these values to calculate the number of plates (N) and the plate height (H). At 1 µL/min we obtained 286 plates with a R_t of 6.17 min, resulting in a plate height of 34.99 µm. As expected, the retention time decreased for the other flow rates, being 3.37 min and 0.86 min for 2.5 µL/min and 5 µL/min, respectively, resulting in a decrease of the number of plates and increase of the plate height. For 2.5 µL/min we obtained 180 plates and H = 55.49 µm for 5 µL/min we obtained 9 plates and H = 1081.44 µm.

According to these results, using our microfluidic device we achieved the lowest plate height at 1 µL/min. By normalizing the number of plates, we obtained 28580 plates/m which is close to the range needed for HPLC [3].

Conclusions

Using our microfluidic device with a 1 cm long column and silica microbeads with a diameter between 45 and 75 µm we were able to achieve separation performances near the HPLC range. Increasing column length and decreasing microbead diameter to 5 µm is expected to drastically improve our separation efficiency, allowing us to obtain a device directly comparable to macroscale liquid chromatographic systems.

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