Joint Event
9th World Congress on Chromatography | 24th International meet on Pharmaceutical Biotechnology
May 13-14, 2019 | Paris, France

Narrow open tubular liquid chromatography (NOTLC): A new paradigm for ultra-high-resolution and ultra-fast separation

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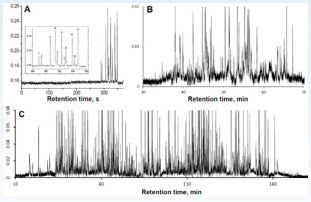

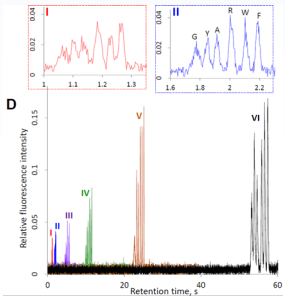

In this work, we demonstrate narrow open tubular liquid chromatography (NOTLC) for ultra-high efficiency and ultra-fast separations. Theoretical studies has predicted that open-tubular columns would offer the best means of achieving high separation efficiencies for liquid chromatography and that the optimal i.d. of the open tubular column would be in the range of 1 to 2 um. However, NOTLC has never been systematically tested using columns in this i.d. regime due to the intrinsic challenges of utilizing such narrow columns, and high-efficiency NOTLC has remained only an idea for decades. Here we use 2-um-i.d. capillaries and obtain ultra-high efficiency and ultra-fast results for amino acid and peptide separations. The narrow open tubular (NOT) column was coated with trimethoxy(octadecyl) silane (C18). An Agilent 1200 HPLC pump coupled with a flow splitter served as a gradient pump. A 6-port valve was used for sample injection. A laser-induced fluorescence (LIF) detector was employed to monitor the separation process. Fig. 1A presents the results for amino acid separation; an expanded view is shown in the inset for clear presentation. Many of the peaks have full widths at half maxima (FWHM) of 0.3-0.5 s. Because of the use of a highly mass-permissive open-tubular column, the elution pressure was low (600 psi) and the separation was complete with 6 min. Fig. 1B presents a chromatogram for separating trypsin-digested cytochrome C and we estimated a peak capacity of 810 within 54 min. Fig. 2C presents a separation of pepsin/trypsin digested E. coli lysate, and we estimated a peak capacity of 1870 within 174 min. Fig. 2D presents six fast NOTLC separations. Under an elution pressure of ~3000 psi, the separation was complete within ~1.3 s (see chromatogram I) and all six amino acids were resolved within 300 ms. The above efficiency, peak capacity, and separation speed are all extraordinary or record numbers.

Biography

Shaorong Liu, Director of the Center for Bioanalysis and Professor in the Department of Chemistry and Biochemistry at University of Oklahoma in Norman, Oklahoma, USA. Dr. Liu's group is focused on developing enabling technologies for biotech research, and more specifically miniaturized analytical systems for ultra-high speed and ultra-high resolution separations of biomolecules. The miniaturized systems include capillary-based apparatus, microchip-based devices instruments and capillary-microchip hybrid devices, while the biomolecules consist of DNA, protein, peptides, steroids and other small molecules. Recently in Dr. Liu's lab, they have demonstrated a record-high resolution for separating an enzyme-digested E. coli lysate and obtained a peak capacity of close to 1800 in 3-h gradient time using a narrow open tubular (NOT) column. Using a short NOT column, they have also demonstrated sub-second separation of 6 amino acids; all six amino acids were resolved within less than 500 milliseconds. He is the author of more than 120 scientific papers, and inventor of seven patents.

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