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Microchip Liquid Chromatography: Future of Chromatography and Separation Techniques

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Separation is the Art and Science to Maximize Differential Transport and Minimize Dispersive Transport

To date, Giddings' above-mentioned statement [1] hold true even after the advent of microfluidics and miniaturized chromatographic systems and that's why it is followed as guiding principle for designing and developing separation systems on microchips. Since the inception of liquid chromatography, it has successfully established itself as the most powerful separation techniques which can be widely used in various fields. However, necessity of faster analysis, higher separation efficiency and sample volume reduction has given birth to the development of microchip liquid chromatography (μC-LC).

In the initial phase of the development of μC-LC, analytical scientists found a less than satisfying separation performance because of serious design flaw, applying wrong operating conditions, or difficulty in understanding of some of the basics of separation science in the miniaturized systems. Besides, there is another critical query raised in their mind whether a given separation problem is indeed best tackled by a μC-LC system. Nevertheless, they continued their research in order to find the solution of whether new μC-LC method could be successful replacement of tradition LC method or not.

In 1999, it was found theoretically that microfabricated perfectly ordered pillar array columns could lead to higher separation efficiency as compared to traditionally best packed column [2]. However, report of Ocvirk et al. is considered as first experimental demonstration of μC-LC system [3], where silicon-glass fluidic network was microfabricated through incorporation of split-injection tee, a channel packed with 5 pm reversed phase particles adjacent to a series of smaller channels that could act as a particle-retaining frit, and an optical detection flow cell. That microchip was placed inside a clamping device and enabled connection of fused silica capillary to an external pump and injector. Nevertheless, the efficiency of this first μC-LC column was very poor and it shown the seed of μC-LC systems. Later on, over the last two decades, microfluidics-based research has covered a wide area of miniaturized chemical analysis systems often considered as Lab-on-Chip Systems [4]. Analytical chemists have pushed themselves to develop miniaturized sensors, detectors and hence led to μC-LC separation systems. The practical use of μC-LC requires a longer column length as it provides a higher theoretical plate number. Thus, in order to fabricate longer column length on a chip the separation channel has to be folded several times which results into turn-induced dispersion through multiple channel curves. Thus, longer column length costs degradation in separation performance. To overcome this, Griffiths et al. introduced "low-dispersion channel curve" in microchannel systems [5] which was further used successfully with μC-LC [6]. We have now entered a phase of pruning, improving, fine-tuning, making practical, commercializing, and introducing miniaturized separation systems into larger workflows.

Inception and development of μC-LC relies upon (i) materials used, its fabrication and geometrics involved; (ii) pumps involved having low/high pressure; (iii) injection and other related connections; (iv) involved stationary phase support; (v) and various detection methods involved like optical, electrochemical, mass spectrometry-based detections. Research in these mentioned sections is required in order to get an advanced μC-LC. Since an ideal μC-LC should be able to work even at 10,000 bar pressure and be compatible with a wide range of chromatographic mobile phase [7], and these conditions are beyond the limit of robustness of μC-LC, materials used, and their fabrication processes play the key role to overcome these. So far, silicon, more refined glass variants such as quartz and fused silica, polymers such polydimethylsiloxane, poly (methyl methacrylate), poly(styrene), and cyclic olefin copolymer, polyimide and their several modifications have been used during the journey of the development of μC-LC. Nowadays, three-dimensional (3D) printing is being used frequently for the fabrication purpose. During the development of pumps involved in μC-LC, initially, off-chip pumps were used. Later on, on-chip pumps as electroosmotic, magnetohydrodynamic or electrochemical flow pumps were used [8]. However, careful design of injection, connection fittings, tubing turns and detectors etc. is required to get optimized performance of μC-LC system [9]. Hydrostatic pressure, acupuncture and electro kinetic injections were checked and utilized for this purpose. On the other hand, since the first use of open-tabular μC-LC, it has created its own space due to elimination of eddy diffusion and possession of the highest column permeability [10]. But, nowadays, most column technology studies have focused upon packed-bed, monolithic, and microfabricated columns. Without discussing the adopted detection techniques, study of μC-LC, can't be completed. In comparison to conventional HPLC, it needs higher sensitivity and faster responding capacity from μC-LC detection. Optical techniques such as UV/visible absorption detection, laser-induced fluorescence, and chemiluminescence have been used for this purpose. However, as electrochemical detection approaches conductivity, amperometry, and potentiometry are being used in μC-LC devices. Additionally, different ion sources of mass spectrometry such as electrospray ionization, matrix-assisted laser desorption/ionization, atmospheric pressure chemical ionization, photoionization are being used as interface with μC-LC chips.

Eventually, I must say that μC-LC is the future of chromatography and separation techniques as it clearly a big step towards lower flow liquid chromatographic systems, reducing solvent/sample consumption and hence finally, commensurating smaller carbon footprint. This will lead us to a portable, field deployable

instrumentation through fuel miniaturized column, pumping, detection and μC-LC development.

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