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On Chip Liquid Chromatography- A Review

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***Abstract*—*Liquid chromatography is applied in biology, medicine, the environment, forensics, and other fields, making it an essential separation method. Liquid chromatography is a technique used to separate and analyze components of a liquid mixture based on their interactions with a stationary phase and a mobile liquid phase. The aim of the present review is to explore different On-Chip liquid chromatographic methods for the analysis of drugs. Liquid chromatography miniaturization has advanced significantly in order to provide reduced sample and minimal eluent consumption as well as simple electrospray-mass accessibility. Because microfluidics requires small sample volumes, quick analysis, and simple integration, it has garnered a lot of attention lately in the shrinking of HPLC systems. For polymer materials, grafting procedures, sol-gel processes, and traditional silane chemistry are frequently employed. The ease of preparation and the comparatively low pressures needed for driving are the main benefits of the open channel configuration. The on-chip microvalve, packed-particle analytical column, enrichment column, and nanospray emitter are the different types of columns used for on-chip liquid chromatography. The utilization of pillar array columns, packed-particle, open-tubular, and monolithic structures have been increased recently in the analysis of drugs with lowest concentration. These techniques have been used in proteomics and drug and small molecule analysis in a variety of biological samples. This technique has been successfully applied for proteome studies and analysis of small molecules like 7-aminoflunitrazepam in human urine, 8-isoprostaglandin F2α in human urine, hepcidin (a peptide marker of clinical disorders related to iron metabolism) in human plasma, pharmaceutical drugs with a wide range of hydrophilicities, fluoxetine and norfluoxetine in rat serum, illicit drugs and metabolites in human hair, and 7-ethyl-10-hydroxycamptothecin (SN38) in mouse plasma.*

***Keywords*— *Forensic, Monoliths, Microvalve, Nanospray.*

I. INTRODUCTION

Liquid chromatography is a technique used to separate and analyze components of a liquid mixture based on their interactions with a stationary phase and a mobile liquid phase. It is commonly employed in various scientific disciplines for its ability to separate and quantify compounds in complex mixtures with high precision. Since Mikhail Tsvet's invention of liquid chromatography in 1906, there has been continuous advancement in this field. Liquid chromatography (LC) and gas chromatography (GC) were developed as a result of pertinent research carried out in the first half of the 20th century.

Liquid chromatography is applied in biology, medicine, the environment, forensics, and other fields, making it an essential separation method. For LC, a variety of stationary phases can be employed, and the method yields very consistent outcomes. LC coupled to mass spectrometry (LC-MS) is an efficient analytical technique for extremely sensitive and accurate mass determination in omics research, including proteomics and metabolomics. Satisfactory separation is still challenging, nevertheless, especially when dealing with complex combinations like biological samples (tissue, plasma, serum, and urine). Thousands of metabolites need to be analyzed at once in metabolomics. In addition, substantial concentrations of other endogenous molecules that could cause interference coexist with certain significant biological compounds that are only present in trace amounts. Minimizing LC can help overcome these obstacles. [1]

Liquid chromatography miniaturization has advanced significantly in order to provide reduced sample and minimal eluent consumption as well as simple electrospray-mass accessibility. Because the resultant nanospray performed better in spectrometry. The use of nano-LC is sluggish despite these advantages. [2]

It is commonly referred to by terms that range from traditional modes to smaller versions. These names are typically derived from column I.D., the characteristics of the stationary phase, or the average mobile flow rate used in the investigation. Any kind of miniaturized LC in which the analytical column has an I.D. less than 1 mm (capillary dimensions) is commonly referred to as capillary liquid chromatography (CLC); from these values upwards, the technique is known as conventional LC or HPLC. Other LC denominations have been implemented by various writers, despite the fact that these big denominations have occasionally been applied effectively.

Four distinct on-chip LC techniques have been developed: the utilization of pillar array columns, packed-particle, open-tubular, and monolithic structures. These techniques have been used in proteomics and drug and small molecule analysis in a variety of biological samples.

The first and most turbulent stage of the development of miniaturized separation systems is essentially over. During this time, it was crucial to demonstrate that (a) conventional separation techniques could be successfully transferred to the miniaturized (planar) format and (b) completely new separation approaches that took advantage of physics at the micro- and nanoscale could be realized. We are now in the process of reducing, enhancing, perfecting, practicalizing, commercializing, and integrating smaller separation systems into bigger operations.

Because microfluidics requires small sample volumes, quick analysis, and simple integration, it has garnered a lot of attention lately in the shrinking of HPLC systems. But creating a small, high-pressure pumping system is one of the

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most difficult tasks involved in setting up a microfluidic HPLC system. Liquid is delivered into the microchannel by a number of pumps, such as the electrolysis pump, thermos pneumatic pump, electroosmotic pump, and thermostatic pump.[3]

Multi-dimensional separations are needed in emerging fields like proteomics to completely resolve complicated protein and/or peptide combinations. The use of porous silicon as a stationary phase in liquid chromatography chips has been covered by us before. Based on dye or nanoparticle distribution and separation studies, the porosity, pore size, and specific surface area were calculated in these papers. A phenomenological model that explains the chromatographic retention on porous-shell pillar columns was also created. An impartial assessment of the pore size, porosity, and specific surface area of porous silicon layers would be highly beneficial for the validation of the model. HPLC miniaturization happened slowly, despite early promises, mostly as a result of insufficient equipment. To ensure solvent conservation in capillary and nanoLC modes, the pumps must deliver gradient mobile phase in spitless mode while producing low flow rates (L to NL min−1) with minimal pulsation. In order to avoid band widening, tubing, unions, columns, sample introduction systems, and detectors must all have suitable volumes.[4]

II. Methods

There are four main ways to introduce a stationary phase into a micromachined channel: (a) applying the chromatographically active moieties only to the wall, which is the same as open tubular chromatography, or in this case, open channel chromatography; (b) using particles modified with retentive materials to create packed beds of these particles (a variation of this involves allowing the particles to self-assemble inside the channel); (c) building monoliths, or porous scaffolds, which can either be grafted with the retentive species later on or already include it; and (d) micromachined pillar arrays, also referred to in the literature as collocated monolithic stationary phase supports, or COMOSS, whose surfaces can be modified later on to provide retention capability.

Open tubular chromatography:

This is the simplest way to perform chromatography on chips.

For polymer materials, grafting procedures, sol-gel processes, and traditional silane chemistry are frequently employed. The ease of preparation and the comparatively low pressures needed for driving are the main benefits of the open channel configuration. The required pressures are tolerable even at channel depths of 5 m or less, which is crucial to obtain short diffusion lengths and a quick mass transfer to the stationary phase. Recently, Kato et al. demonstrated open-channel chromatography in submicron channels, demonstrating the ability to perform separations at pressures between 1 and 3 bars (14 and 43 psi). Shear forces between a fixed and a moving plate can be used as an alternative to pressure in order to drive the mobile phase. [5,6]

The earliest documented instance of on-chip LC is open-tubular chromatography, which is also the most straightforward method to implement chromatography on a chip. Manz et al. originally described this technology. An open-tubular column of 15 cm in length, 6 µm in breadth, and 2 µm in depth was constructed for their investigation; however, no attempts were made to separate the tubes. To achieve the required retention capacity, the channel wall of the chip was physically altered and employed as the stationary phase.

This method's poor surface-to-volume ratio results in low column capacity, which is its primary drawback. The specific surface area of the stationary phase needs to be raised in order to boost the capacity of open-tubular columns.[7]

Based on the kind of layer on the column wall, open-tubular columns fall into two primary categories: nonporous layers and porous layers (PLOT columns). The usage of non-porous layer OT columns in LC was restricted because of their subpar

low mass loadability due to its capacity. Additionally, because polar chemicals tend to elute close to the dead time, it is challenging to separate them in these columns due to their low capacity, which calls for low-volume injection and a particular detector.

There are two primary classifications of open-tubular columns: nonporous layers and porous layers (PLOT columns), based on the kind of layer on the column wall. Because of their low mass loadability and low capacity, non-porous-layer OT columns were not widely used in LC applications. Furthermore, because polar chemicals tend to elute close to the dead time in these columns, it is challenging to separate them due to their low capacity, which calls for low-volume injection and a particular detector.

Non-porous layer OT columns have a number of drawbacks; hence, coating techniques have been researched to prepare OT columns with a thick coating layer, which increases column capacity by enabling a larger injection volume. These techniques consist of applying a polyethoxysilane solution that has already gelled, layer-by-layer techniques, chemical bonding, and multilayer-by-multilayer techniques. The majority of the columns produced using these techniques are successfully used in CEC [8,9].

Packed Particle:

Most columns in conventional LC are particle-packed. Conventional-type stationary phases are transferred to the microfluidic chip when LC is performed on-chip. Thus, particle-packed on-chip LC systems are one of the easiest ways to achieve separation. A packed bed can be formed when pre-prepared particles flow into the microchannel on a chip. There are many particle types with various modifications available, such as ion exchange, C8, C18, and hydrophilic interaction chromatography (HILIC); particle-packed chips can be made with these particles. Particle-packed columns have a significantly larger specific surface area compared to open-tubular columns.[10]

The first chips that were available for commercial use were packed with particles. But they have only been used in

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combination with a mass spectrometer, for example. When the column and the mass spectrometer are connected, the packed particles are needed to remove the dead volumes. [11]

The on-chip microvalve, packed-particle analytical column, enrichment column, and nanospray emitter are all present in the Agilent LC particle-packed chip. It is widely used for proteome studies and analysis of small molecules like 7-aminoflunitrazepam in human urine, 8-isoprostaglandin F2α in human urine, hepcidin (a peptide marker of clinical disorders related to iron metabolism) in human plasma, pharmaceutical drugs with a wide range of hydrophilicities, fluoxetine and norfluoxetine in rat serum, illicit drugs and metabolites in human hair, and 7-ethyl-10-hydroxycamptothecin (SN38) in mouse plasma [12].

The immediate big advantage is that basically all the many particulate materials that have been developed over the years for LC can be adopted to the planar format. This wealth of available packing materials means that stationary phase chemistries for basically all possible scenarios are at one’s disposal. The size, size dispersity, and porosity of the particles are the next factors to take into account. It is extremely important to have a good understanding of all experimental parameters before choosing, e.g., the most appropriate particle size. [13]

These columns were operated at 3 bar (the maximum of the used pump), but it is not clear whether this constituted the optimum run conditions. In-depth studies over the past few years have begun to unravel the intricacies of packed beds and how their performance is influenced -. One of the key findings seems to be that the largest obstacle to correctly packed beds is the level of organization in the packing process. While modern particulate material is ever more monodisperse, the limitation of achieving high-order packings lies now in the used packing technique. [14-24]

Monoliths:

Monolithic columns are a type of stationary phase used in chromatography. Unlike traditional chromatographic columns packed with small particles, monolithic columns consist of a single continuous piece of material with a network of interconnected channels.

Many advantages of packed chromatographic beds, such as increased surface area and readily regulated surface chemistry, are also present in polymer monolithic stationary phases.

One clear benefit of monolithic stationary phases, though, is that they may be quickly and simply prepared by free radical polymerization inside the microdevice's channels—no frits or other holding structures are required. By modifying the initial monomer solution's composition and the polymerization conditions, the porosity, surface area, and pore size of the monolith can be adjusted. With careful monomer selection, a broad range of chemicals can be incorporated into the microfluidic device.

A sheet of silicon dioxide, 4 m thick, measured 30.6 cm in length, 40 m in width, and 20 m in depth. Before the polymer monolith forming, [3 - (methacryl - oyloxy) propyl] tri-methoxy-silane was applied to the channels. [25,26]. The pretreated surface's pendant methacryloyl groups were integrated into the polymer monolith during polymerization, covalently binding it to the channel walls. Using the redox initiating system ammonium per oxosulfate-tetramethylethyenediamine (TEMED), mixtures of vinyl sulfonic acid, methacrylamide, isopropyl acrylamide, piperazine di acrylamide (crosslinker), and ammonium peroxosulfate were polymerized to create a monolithic stationary phase for reversed-phase electrochromatography (fig 1).

The same team created a so-called hybrid microdevice more recently as an affordable and easier-to-manufacture substitute for quartz microchips. A short fused silica capillary column mounted in a 0.4 mm groove on a poly (vinyl chloride) (PVC) substrate made up the hybrid device.

Sample reservoirs, electrodes, and a slit for "on-tube" UV detection were all integrated in the PVC support plate.[27].

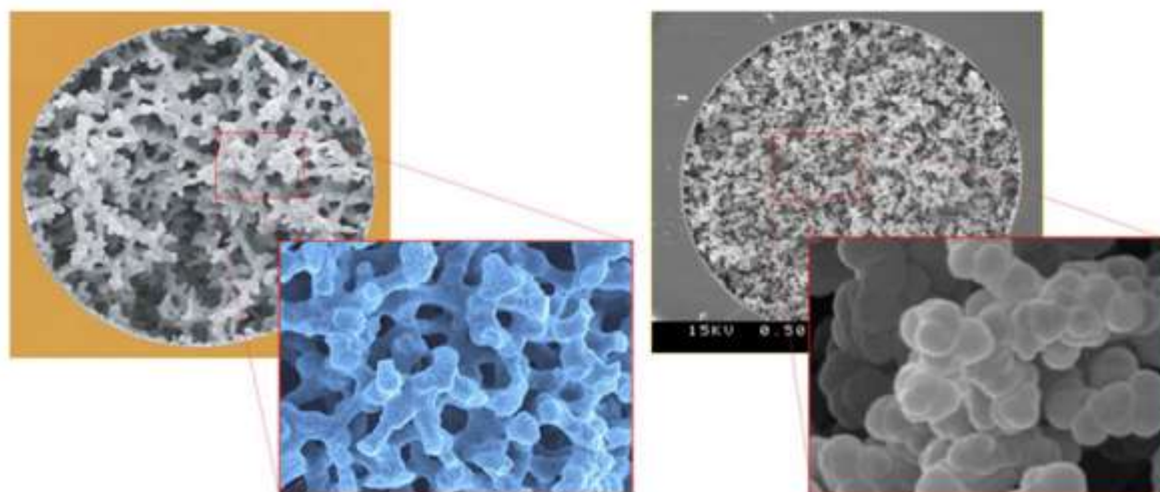

Figure 1: SEM micrographs showing difference in morphology of monolithic capillary columns prepared from silica (left) and poly(butyl methacrylate-co-ethylene dimethacrylate) (right). [34]

Silica based monoliths:

The newest kind of stationary phase for electrochromatography on microchips is monolithic silica. Monolithic silica devices benefit from their extremely high surface area, tunable pore size, and regulated surface chemistry. Usually, functional moieties are added to the silica monolith by silanizing the surface after the monolith is produced or by adding the proper silicon alkoxide to the precursor mixture. Large surface areas resulting from the existence of mesopores in monolithic silicon-based columns are typically recognized to offer higher performance in the HPLC separation of tiny compounds.[28].

Pillar Array Columns:

Pillar array columns refer to a microfabricated structure with an array of pillars designed to enhance separation efficiency. These columns provide a high surface area for interactions between the sample and the stationary phase, improving chromatographic performance in miniaturized systems.

Multidimensional LC (nD-LC) separations, in which peptides are separated by two or more distinct processes, have been extensively researched as a means of achieving high separation efficiency. The easiest ways to boost separation efficiency in one-dimensional liquid chromatography (1D-LC) are to either shorten the column's length or decrease the size of the particles (fig 2). Because of improved mass transfer kinetics and decreased eddy diffusion, modern columns packed with sub-2 mm superficially porous particles (SPP) and operated at ultra-high pressure (UHPLC) enable the conduct of separations with a very high peak capacity. [29,30].

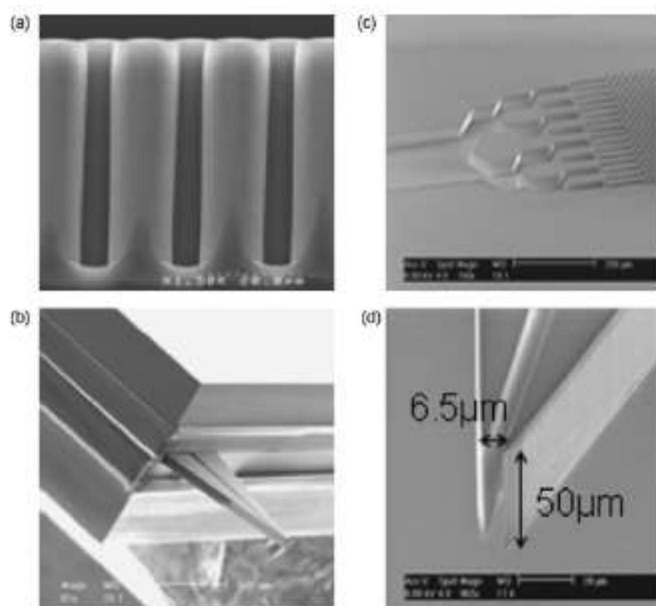

Figure 2: SEM images of a chip with a high-aspect ratio pillar array (panels a and c) and an integrated spray tip for introduction of eluate into an MS (panels b and d). [35]

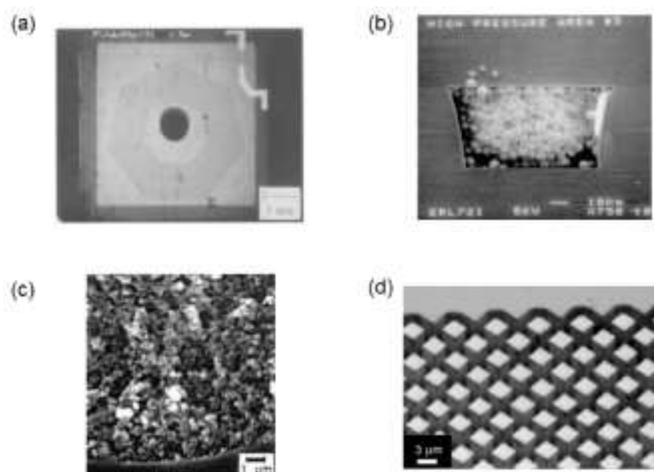

Figure 3: Four Approaches For On-Chip Lc: Use Of (A) Open-Tubular [1], (B) Packed-Particle [2], (C) Monolithic [3], And (D) Pillar Array Columns [1]

The design of the pillar arrays must be carefully considered in order to reduce the effects of the selected shape as well as contributions from eddy dispersion (based on variations in flow paths through the array) and dispersion resulting from impeded mass transfer.

Ordered pillar arrays are not the separative stationary phase itself; rather, they are only support structures that get closer to the ideal packed bed. Therefore, in order to deposit the stationary phase material onto the pillars, surface chemical processes must be carried out This can be achieved, for instance, in the case of oxidized silicon pillars by making use of silane processes that are comparable to those that derivatize the channel walls in open channel chromatography. [liquid]Studies on proteomics and lipidomics make use of these chips. Pharma Fluidics is presently offering pillar array columns for sale.

III. Discussion

A microcolumn that measured 1 mm in width and 20 mm in length produced the best enrichment results throughout the viral enrichment procedure, yielding an enrichment efficiency of 7.26. Microcolumns because of its lengthier structure, which makes more hydroxyapatite possible to pack into it. Using 5-HIAA and serotonin (5-HT), two neurochemicals, separation performance was tested between the non-grafted and grafted monolithic TPE columns.This outcome confirmed that improved performance in the monolithic column requires the grafting layer. The amount of the injected analyte was ascertained by integrating the fluorescence intensity. Fluorescence tests were performed utilizing chips with a column of 10 m depth and 11 cm length in order to have sufficient analyte intensity. The microchips even show somewhat improved performance at increasing mobile phase velocities (uav > 2 mm/s), despite the fact that the plate height data in nano-HPLC and microchip-HPLC are often close, notably around the plate height minimum (uav) of 1.0–1.5 mm/s. In the case of the packed capillaries, we relate this discovery to the preparation (sintering) of the entrance and outflow frits. After the detection window was constructed

directly behind the outlet frit, packed capillaries were cut at the inlet frit and directly attached to the injection valve.

IV. CONCLUSION

On-chip liquid chromatography offers significant advantages in terms of miniaturization, speed, and efficiency. Its conclusion typically emphasizes enhanced portability, reduced sample volumes, faster analysis times, and potential integration with other on-chip analytical techniques. However, challenges such as limited resolution and compatibility with complex samples may need further research for broader implementation. On-chip LC is expected to find extensive applications due to its portability,where application to clinical diagnosis is on of the importance examples. In clinical diagnosis,on-chip LC can be performed in hospitals and even at the bedside.

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