



Open tubular liquid chromatographic system for using columns with inner diameter of 2 μm . A tutorial

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ABSTRACT

We have recently demonstrated the remarkable performances of liquid chromatography (LC) using 2- μm -i.d. open tubular (OT) columns; peak capacities of 2000+ within less than three hours have been routinely obtained at an elution pressure of around 100 bar or less. However, only a small number of researchers have been involved in the research in this area; part of the reason is due to the issues associated with setting up open tubular liquid chromatography (OTLC) systems. While cautions should be taken here and there in carrying out separations, but none of the issues can inhibit us from performing OTLC separations. Therefore, we feel it desirable to write a tutorial on how to build an OTLC system. In this tutorial, we introduce the key components for the apparatus, how to construct/prepare them or where to purchase them, and how to assemble them together into a complete system. We further discuss the advantages and disadvantages of the system; we mention particularly the practical issues from using the narrow (2- μm -i.d.) columns and how to mitigate these issues.

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1. Introduction

System miniaturization has attracted increasing attention in liquid chromatography (LC) [1], because it brings a lot of benefits such as short analysis time, low sample/reagent consumption and low waste generation, improved portability, and reduced instrument and consumable costs [2,3]. An effective way toward LC miniaturization is to use narrow open tubular (OT) columns [4]. Compared to packed columns, narrow OT columns not only yield improved resolving power but also brings about other benefits. For example, narrow OT columns reduce the pump's pressure demand, ~50–100 bar for OTLC vs ~300–400 bars for high-performance liquid chromatography (HPLC) or ~1000 bar for ultrahigh performance liquid chromatography (UPLC). These columns hold potential to lower the sample consumptions and waste generations dramatically. Column temperature programming and on-column detection are readily implemented for these columns [4].

A significant advantage of a narrow OT column is its high separation efficiency. Based on Golay's theory [5,6], one can use OT columns having inner diameters (i.d.) of a few μm [7] to achieve high-efficiency LC separations. In 2018, Chen et al. [8] and Yang

et al. [9], for the first time, reported the ultrahigh efficiency of open tubular liquid chromatographic (OTLC) separations using 2- μm -i.d. columns. The inner wall of the column was coated with octadecyltrimethoxysilane (OTMS), and the effective length of the column was 44 cm. One can always increase the peak capacity number by increasing the column effective length or shorten the separation time by decreasing the column length. For instances, Yang et al. [10] obtained the peak capacities of >2000 in <3 h routinely by utilizing a 155-cm-long, while Xiang et al. [11] resolved 6 amino acids in <700 ms by utilizing a 2.7-cm-long OT column.

Narrow OT columns can be an excellent solution to the challenges in Omics research. For example, proteomics aims to identify and quantitate expressed proteins and their isoforms, and sample quantity is frequently a limiting factor, such as the case in biomedical and discovery proteomics that deal with low sample quantities. Using a 2- μm -i.d. OT column coupled with an Orbitrap MS, Xiang et al. [12] demonstrated that ~1000 proteins/isoforms could be reliably identified using only 75 pg tryptic peptides, representing over 10–100-fold improvement in sensitivity compared with previously developed 15 or 30- μm -i.d. packed-column LC [13,14] and capillary electrophoresis (CE) MS systems [15]. Metabolomics biomarkers are often present in extremely low amounts in biological matrices containing high concentrations of other compounds and contaminants, making these matrices extremely complex [16].

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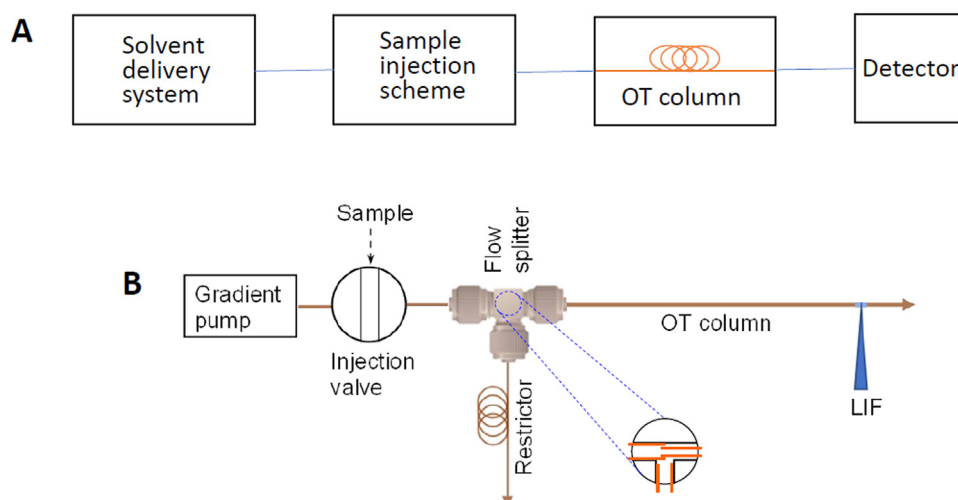


Fig. 1. (A) Block diagram of OTLC system, (B) Schematic diagram of OTLC apparatus (Reprinted with permission from ref. 4).

Sample dilution is a straightforward solution to alleviate this issue, leading to small quantities of biomarkers for a constrained sample volume. These challenges can potentially be solved using narrow OT columns (or miniaturized LC columns in general [17]), because OT columns with various coatings can be derivatized and 2-dimension separation can be more readily setup with an OT column for the high-speed 2nd-dimension separation.

In this tutorial, we focus on how to construct the system, more precisely the experimental apparatus, for using 2- μm -i.d. OT columns. Because of the use of a 2- μm -i.d. column, the flow rate through the column is low (<100 pL/min), the effect of the dead volume is magnified, and the sample volume injected into the column is low (low pL to fL), etc. All of these can impose challenges for constructing the OTLC system. We will first introduce the key components for the apparatus, how to construct/prepare them or where to purchase them, and how to assemble them together into a complete system.

2. System configuration in general

Fig. 1A presents a schematic block diagram of the key system components; it consists of a pump for solvent delivery, a scheme for sample injection, a column for analyte separation, and a detector for resolved analyte monitoring. Fig. 1B presents an actual apparatus that was used in ref. [5]. For utilizing a 2- μm -i.d. OT column, special attention should be paid to the design or selection of each of the above components so that they can work together properly, as shown in the following sections.

2.1. Solvent delivery system

To perform a separation using a 2- μm -i.d. OT column, the flow rate through the column is normally a few hundred pL per min or lower, and the elution pressure is often around 50–100 bar. Constant-flow pumps at this rate/pressure range are not commercially available. While electroosmotic pumps (EOPs) [18] have been used to execute OTLC, constant-pressure sources such as a pressure chamber [19] or a commercial HPLC pump in conjunction with a flow splitter [9,20] are more commonly employed.

The development and application of EOPs have been reviewed recently by Li et al. [21], and Gu et al. [22] reported an EOP configuration that was particularly suited for OTLC. A fundamental unit of this pump is presented in Fig. 2A; it consists of a +EOP (a capillary tube filled with a positively-charged monolith) and

a -EOP (a capillary filled with a negatively-charged monolith) connected together via a piece of capillary tubing. Because electroosmotic flow (EOF) goes from ground electrode to positive electrode in the positive monolith and from positive electrode to ground electrode in the negative monolith, as an external high voltage (HV) is applied across the monoliths via a conducting gel immobilized inside a capillary, the pump solution moves from the inlet to the outlet smoothly. An excellent feature of this pump is that if the positive monolith generates a pressure of P1 (P1 will be present in the solution in the connecting capillary tubing) and the negative monolith generates a pressure of P2, the pressure at the outlet will have a value of $P1 + P2$. Since both the inlet and outlet of the pump unit are electrically grounded, several such units can be connected in series (see Fig. 2B). The overall pressure output of this pump assembly will be $n \times (P1 + P2)$, where n is the number of pump units connected. The overall flow rate will remain the same as that of each pump unit. With three units connected in series, pressure of up to 1200 bar and flow rate of up to 280 nL/min have been achieved. Following the same approach, Wang et al. [23] developed a microchip EOP with a pressure of up to 170 bar and a flow rate of up to 500 nL/min. Other advantage of EOP include its compactness (all the necessary parts can be assembled in a limited space and hence an EOP is for system miniaturization) and its capability of generating high pumping pressures (increased pump pressures are required if long OT columns are desired).

Constant-pressure sources have been frequently employed to drive separations using 2- μm -i.d. OT columns [8–12]. A pressure chamber [19,24] was used to perform OTLC in our lab, and its fabrication was straightforward and described previously [19,24]. Fig. 3 presents a schematic diagram of the device [19]; it consists of a chamber base and a chamber cap made from a stainless or acrylic rod. A hole is drilled into the base forming an open space. The base and the cap are tightened together via threads, and a septum is put in between to ensure an air-tight seal. Two small (~1-mm-diameter) holes are drilled on the cap so that a pressurized gas line and an OT column can be inserted into the chamber. The sampling end of the column is inserted through the septum and dipped into the solution (either a sample or a solvent solution) in the vial inside the chamber. By switching the three-way valve to the gas-line position, a pressurized gas such as helium is introduced to the pressure chamber to drive the solution in the vial into the column. The pressure inside the chamber is controlled by a pressure regulator attached to the gas cylinder. The chamber pressure can also be released by switching the three-way valve to

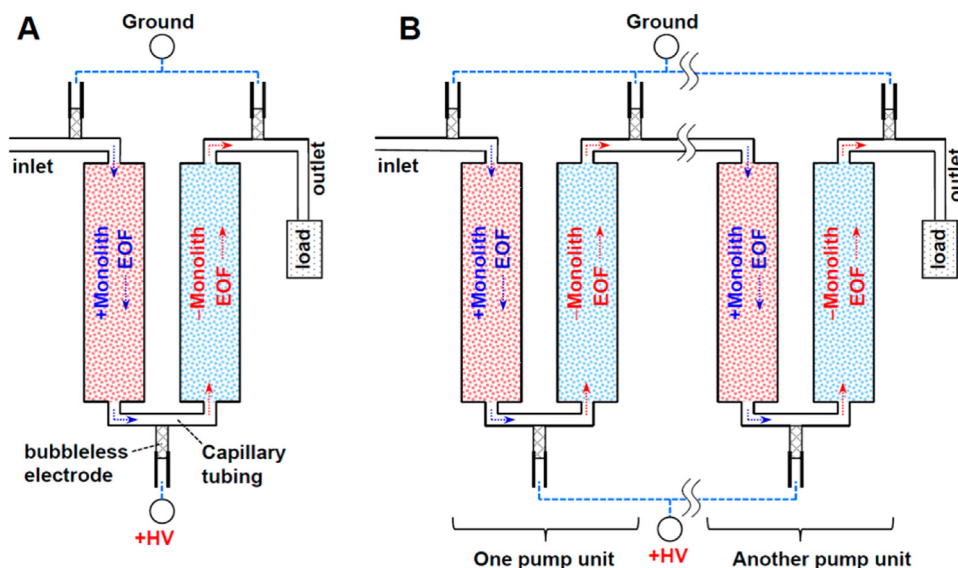


Fig. 2. Schematic configuration of EOP assembly. (A) Fundamental pump unit. (B) Pump assembly (Reprinted from ref. 22 with permission).

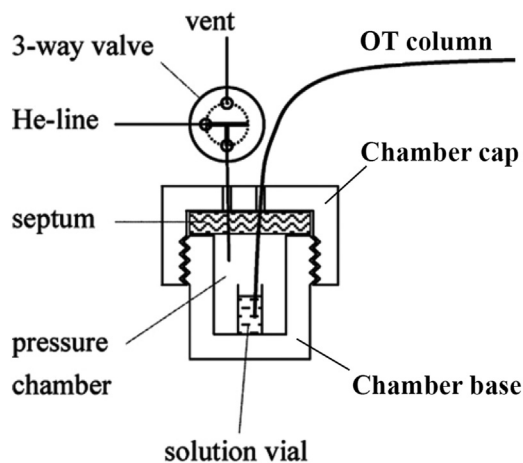


Fig. 3. Schematic diagram of pressure chamber.

the vent position.[Note: Similar parts have been manufactured (e.g., in the capillary electrophoresis instrument by Agilent), but they are usually not available commercially]

Another way to develop a constant pressure source is to use a commercial pump in conjunction with a flow splitter. Because usually the majority of the flow goes to the restriction capillary, the flow splitter regulates the pressure on rather than the flow in to the separation column. Therefore, we consider this type of pump a constant pressure source, although some researchers have considered it a constant flow pump [25,26]. The pressure upper limit is constrained by that of the HPLC pump and the HPLC accessories, including tubing and connection parts, while the output pressure is determined by the pressure drop across the splitting capillary. Building such a pump is common, and examples can be seen in the literature [9,20,27].

2.2. Sample injection scheme

Because of the low flow rate through a 2- μm -i.d. column, a small volume of sample is injected into the column. Under a flow rate of 100 pL/min and an injection time of 5 min, the injected volume is only 0.5 nL. Injection valves for such low volumes are not available commercially. In our lab, a common solution to this issue

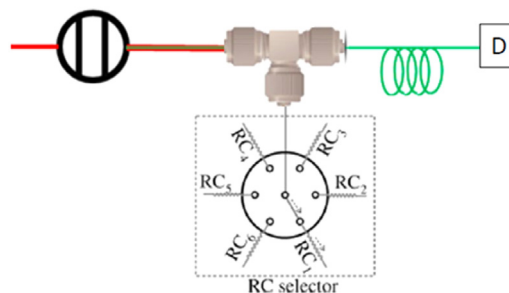


Fig. 4. Schematic diagram of injection scheme.

is to use a regular injection valve combined with a flow splitter to accomplish a low volume sample injection. The setup in Fig. 1B also presents a schematic injection scheme. An injection valve is arranged between the HPLC pump and the T. The i.d. of the connection tube should be larger than but close to the outer diameter (o.d.) of the OT column. Inside the T (see inset), the entrance end of the OT column should be inserted into the connection tube to avoid the adverse effect of the dead volume in the T. If we have a valve with an injection volume of 2 μL and need to inject 0.4 nL sample into the OT column, we can achieve this simply by adjusting the split ratio of the flow splitter to 1:5000 (1 to OT column and 5000 to restriction capillary). As the pump drives the 2 μL sample from the injection valve to the flow splitter, only 1/5000 of the solution, that is, 0.4 nL goes to the OT column. The injected volume is independent of the pump rate as long as the split ratio remains the same. The injected volume can also be changed by replacing the capillary restrictor with a selection valve connected to multiple restriction capillaries [28], as presented in Fig. 4.

When the 2- μm -i.d. OT column was initially used, the sample was directly injected into the column via a pressure chamber [19] as shown in Fig. 3. Briefly, after the column was properly mounted on the LIF detector and focused, it was flushed with ~ 3 –4 column-volumes of eluent. Then, a vial containing a sample solution was put inside the chamber, the chamber was capped, and the three-way valve was set at the vent position. The inlet end of the column was inserted, through the septum, into the sample solution. Sample injection was started when the three-way valve was switched to the pressurized gas line to drive the sample solution

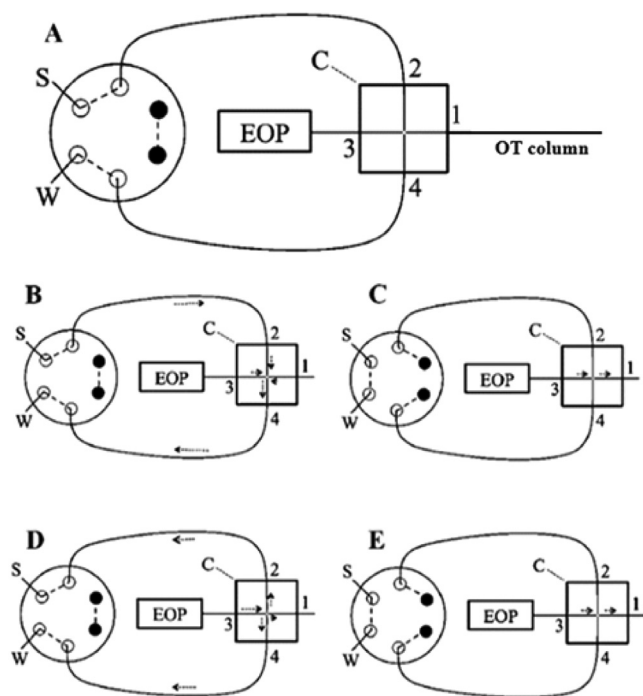


Fig. 5. Schematic diagram of injection scheme. A) Experimental setup: S=sample, W=waste, and C=chip injector. The six-port injection valve is showing on the left. The solid dots indicate ports that are blocked. Capillaries connected to positions 1, 2, 3, and 4 on the chip injector are separation capillary, sample capillary, pump capillary, and waste capillary, respectively. The separation capillary has a length of from 40 cm to 70 cm, an o.d. of 150 μm , and an i.d. of 2 mm, the other three capillaries had a length of 15 cm, an o.d. of 150 μm , and an i.d. of 15 μm . B)–E) Schematic diagram depicting sample injection. The arrows indicate the flow directions (see text for details). (Reprinted from Ref. 50 with permission).

into the column. After a preset period, the three-way valve was switched to the vent position to stop the injection. This process is similar to the pressure injection in capillary electrophoresis. The volume of the sample injected into the column depends on the injection time and chamber pressure. Theoretically, an electrokinetic injection can be implemented using the above device as well after electrodes are put at both ends of the OT column and an electric field is applied.

Zhu et al. [29] have reported a sample injection scheme between the split-flow and direction injection approaches. Fig. 5A presents a schematic arrangement of the injection scheme to illustrate the working principle. The microfabricated chip cross and the six-port valve constituted the injector. The EOP, used as a solvent delivery source, was connected to position 3, the OT column was connected to position 1, and two auxiliary capillaries from the six-port valve were connected to positions 2 and 4 on the chip cross. When the valve was switched to an “open” position (see Fig. 5B), the two auxiliary capillaries from positions 2 and 4 on the chip were connected to a sample solution (S) and a waste reservoir (W). By applying a vacuum to W, S was aspirated through the chip cross into W. The cross region inside the chip cross usually had a volume of several nL. When the valve was switched to a “closed” position (see Fig. 5C), the two auxiliary capillaries were connected to the blocked ports on the valve. By turning on the EOP, the sample residing in the cross-region was injected into the column. The volume injected (often no more than a few hundred pL) depended on the injection time and pump rate. After sample injection, the valve was switched to “open” again (see Fig. 5D). While the EOP was on, the residue sample inside the chip cross was flushed to W. Finally, the valve was switched to “closed” (see Fig. 5E) to carry out the separation.

2.3. 2- μm -i.d. OT column

Both coated and uncoated columns have been used for LC separations. The coated columns are commonly termed Wall Coated Open Tubular (WCOT) columns, while the uncoated columns are termed Bare Open Tubular (BOT) columns in recent literature [4].

BOT columns. When the wall of a BOT column is in contact with an alkaline solution, the surface silanol groups are dissociated, and a negatively charged surface is formed. The negative surface repulses anions and attracts cations, leading to anions being accumulated in the center region and cations being enriched near the wall of the column. The higher charged (absolute value) anions are concentrated more in the center region, while higher charged cations are enriched more near the wall of the column than the lower charged anions/cations. As a solution (eluent) is forced through the column, a Poiseuille flow is induced. The compounds residing in the center region move faster while the compounds near the wall move slower than those in the wall/center regions. As a result, differently charged species are eluted according to the following order (from the first to the last): the higher charged (absolute value) anions, the lower charged anions, the neutral species, the lower charged cations, and the higher charged cations. Examples of separations via this principle can be seen in the literature [19,30].

BOT columns have also been utilized for large-molecule separations [19,29,31–33] based on a hydrodynamic chromatography (HDC) principle. HDC was first published in 1974 [34], and high-resolution HDC separations were obtained for DNA fragments [19,35] using 2- μm -i.d. BOT columns. A detailed separation mechanism was developed in the literature [36].

WCOT columns. A WCOT column is an open capillary tube whose inner wall is coated with a layer of stationary phase. Low loadability or loading capacity [37] was a concern of using WCOT columns, but in a recent study, Yang et al. [38] demonstrated that this issue could be mitigated with the use of 2- μm -i.d. OT columns. The reason is that a reduced column i.d. increases the surface-to-volume ratio, leading to a good match between stationary-phase loadability and mobile-phase loadability. To prepare a good coating Yang and Liu used a relatively high concentration of the coating reagent to derivatize the column wall.

The procedure for preparing an octadecyltrimethoxysilane (OTMS) coated 2- μm -i.d. OT column has been described previously [8,24,38]. Briefly, after a segment (e.g., 1 m in length) of a capillary is flushed with 1 M NaOH solution at 100 $^{\circ}\text{C}$ for 2 h and rinsed with distilled and de-ionized (DDI) water for 1 h, it is flushed with acetonitrile for about 30 min at ambient temperature and dried with nitrogen overnight. The dried capillary is then flushed with 70% OTMS in toluene at 50 $^{\circ}\text{C}$ for 16 h. The column is ready for use after the OTMS-coated capillary is rinsed with toluene for 1 h and dried with nitrogen. The resulted coating has a thickness of 10–20 nm [38].

Technically, most of the methods used to create coatings for larger i.d. OT columns can be applied to prepare 2- μm -i.d. columns. A straightforward and conveniently implemented method is via physical adsorption. For example, taking advantage of the property of a fused-silica surface which can be either negatively or positively charged, OT columns with a methylamine and 1,4-butanedioldiglycidyl ether copolymer coating [39] or a poly (butadiene-maleic acid) (PBMA) co-polymer coating have been prepared for anion or cation separations [40]. Hydroxypropylcellulose (HPC) and micro- and nano-particles can also be physically adsorbed onto silica surfaces, and OT columns with such material coatings have been prepared for protein [31] and ion exchange [41,42] separations. A potential issue with adsorbed coating is its limited stability and lifetime. Coating materials are more commonly chemically bonded to the column walls. The most popular

example of such material is a thin layer of porous polymer synthesized via *in situ* polymerization [20,43]. Synthesizing this polymer layer inside a narrow column requires additional attention to prevent clogging, but it has been demonstrated that it is possible to for such coating in 900-nm-i.d. columns [44]. OT columns are prepared with ionic material coating for hydrophilic interaction chromatography [45] and β -cyclodextrin for chiral compound separations [46]. Nanomaterials such as graphene oxide [47], C60-fullerene [48] and nanoparticles [49] and metal-organic frameworks [50,51] can also be attached to column walls as stationary phases. Lam et al. have recently published a comprehensive review article for preparing OT columns through both adsorption and chemical derivatization [52].

2.4. Detector

Up to date, only two kinds of detectors have been practically utilized for 2- μ m-i.d. OTLC separations: laser-induced fluorescence (LIF) detector and mass spectrometer (MS).

LIF detector. Because LIF detections for 2- μ m-i.d. columns require accurate alignment and focusing, such LIF detectors are not yet available commercially. To attack this problem, Weaver et al. [53] developed a confocal LIF detector, and the detector was successfully utilized for 2- μ m-i.d. OTLC detection [9,44,54]. The configuration of the detector is schematically presented in Fig. 6. Briefly, after a 488 nm laser beam from a solid-state laser module (Melles Griot, Rochester, NY) passed through a 488-nm laser line filter (Melles Griot), it was reflected by a dichroic mirror (491 nm cut-on wavelength, Semrock, Rochester, NY) and focused on to the solution inside the OT column by an objective lens (0.32 NA, 16 \times magnification, Melles Griot). Fluorescence from the inside of the column was collected and collimated by the same objective lens, passed through the same dichroic mirror, reflected by a mirror (Melles Griot), filtered by a 510 nm long-pass filter (Semrock, Rochester, NY), and focused by a lens (Melles Griot) through an 800- μ m pinhole onto a photomultiplier tube (H5784-01, Hamamatsu, Shizuoka, Japan). The output signal was acquired by a USB data acquisition card (Measurement Computing, Norton, MA) and displayed on a computer via an in-lab-developed LabView program. The system construction and characterization were described in detail by Weaver et al. [53].

Accurate alignment of the OT column with the objective lens was critical to achieving a sensitive detection. For the LIF detector presented in Fig. 6, an optical window was created at an appropriate position on the column by removing the polyimide coating with flame, and the optical window was then affixed on an x-y-z translation stage above the objective lens. After a fluorescent dye (e.g., 1 μ M fluorescein in 10 mM TE buffer at pH \sim 8.0) was set to flow through the column at a constant flow rate, the LIF detector was turned on, and the fluorescence signal was continuously monitored. The position of the optical window was then adjusted via the translation stage until the maximum signal output was obtained. At this time, the positions of the translation stage were locked; the optical window was aligned with the LIF detector. After the dye solution inside the OT column was washed out, sample injection and OTLC separation could be performed.

MS detector. MS instrument has been advanced rapidly recently with much improved detection sensitivity and mass resolution, which has made MS one of the most popular detectors and sometimes a mandatory detector for chemical and biochemical analyses. Due to the low flow rate through a 2- μ m-i.d. column, attention needs to be paid to the interface, although Marginean et al. [55] had reported a picospray emitter delivering a flow at 400 pL/min to MS for detection. Xiang et al. [12] recently described an emitter that had successfully interfaced 2- μ m-i.d. OTLC with MS. Fig. 7 presents the overall system configuration. In this system, a

cross was utilized to construct the flow splitter; the additional outlet was blocked by a metal stopper. The exit-end of the OT column was sharpened via HF etching to a tip o.d. of ≤ 5 μ m to achieve efficient electrospray ionization (ESI) at a flow rate of pL per min. A +HV was applied through the metal stopper on the cross to initiate and maintain ESI. A T junction was used to introduce a nitrogen sheath flow at the emitter to improve electrospray stability. An Orbitrap Fusion Lumos Tribrid Mass Spectrometer was used as the detector.

3. Discussion

An OTLC system has four major components: a solvent delivery system, a sample injection scheme, a 2- μ m-i.d. OT column, and a detector; all have issues and need improvements. Accurate and precise solvent delivery is key to obtaining reproducible separations. Delivering a solvent at sub-nL/min has been achieved, but it is challenging to do so accurately and precisely, especially for delivering a gradient solvent [56,57]. While a pressure chamber is suitable for delivering an isocratic solvent, it cannot deliver a gradient solvent. The flow from an LC pump in conjunction with a flow splitter can fluctuate when the composition and viscosity of a gradient change. Since constant-flow pumps at a rate of a few hundred pL per min and a pressure of about 50–100 bar are not commercially available, a robust and reliable solvent delivery system is highly desired for LC using 2- μ m-i.d. OT columns.

One potential solution to this problem is to take a similar approach as that used in the splitless pumps [26] but further scale the flow rates down; more sensitive and accurate pressure gauges and flowmeters may be required. Alternatively, EOPs [58] combined with accurate flowmeters for feedback signals to adjust the voltages on the EOPs; a low-rate flowmeter suitable for has recently been designed and tested [59].

Sample injection is another issue. In case one wish to concentrate analyte via column-head stacking, a relatively large volume (e.g., 20 nL) of sample need to be injected through the column, the injection time would be very long (e.g., ≥ 200 min). We had tested injecting a sample into a pre-column but failed, primarily due to the relatively large dead volume when a sample-loaded pre-column was connected to the 2- μ m-i.d. OT column. We remain cautiously optimistic about this approach since the dead volume may be reduced to an acceptable level with an appropriate pre-column-to-OT-column coupler. Fabricating both the pre-column and the OT column on a microchip could be an alternative solution.

Preparing 2- μ m-i.d. OT columns with high quality, long-lasting and consistent performances are critical toward its success and widespread in chemical and biochemical applications. To achieve this, we need to have capillaries with consistent i.d. near 2 μ m. From Molex [60], the i.d. of their 2- μ m-i.d. capillaries has a tolerance of ± 1 μ m. Efforts need to be invested in the manufacturing process to reduce the tolerance to ± 0.3 μ m and preferably ± 0.1 μ m. Owing to the low flow rate through the capillary, evaporation at the capillary end is relatively fast. Any residual solution at the end may lead to some solid deposits, causing capillary clogging. In case a chemical chain reaction is used to synthesize the coating, caution must be taken not to allow the chemicals overreacted, causing capillary clogging.

LIF is well suited for 2- μ m-i.d. OTLC because: a) it is very sensitive and can detect single molecules, b) it is an on-column detection scheme resulting in little sacrifices in separation performances, and c) it can be made portable, which holds tremendous potential for point-of-care applications. A major limitation of a LIF detector is that the analytes must be fluorescent or derivatized to be fluorescent, which constrains its applications.

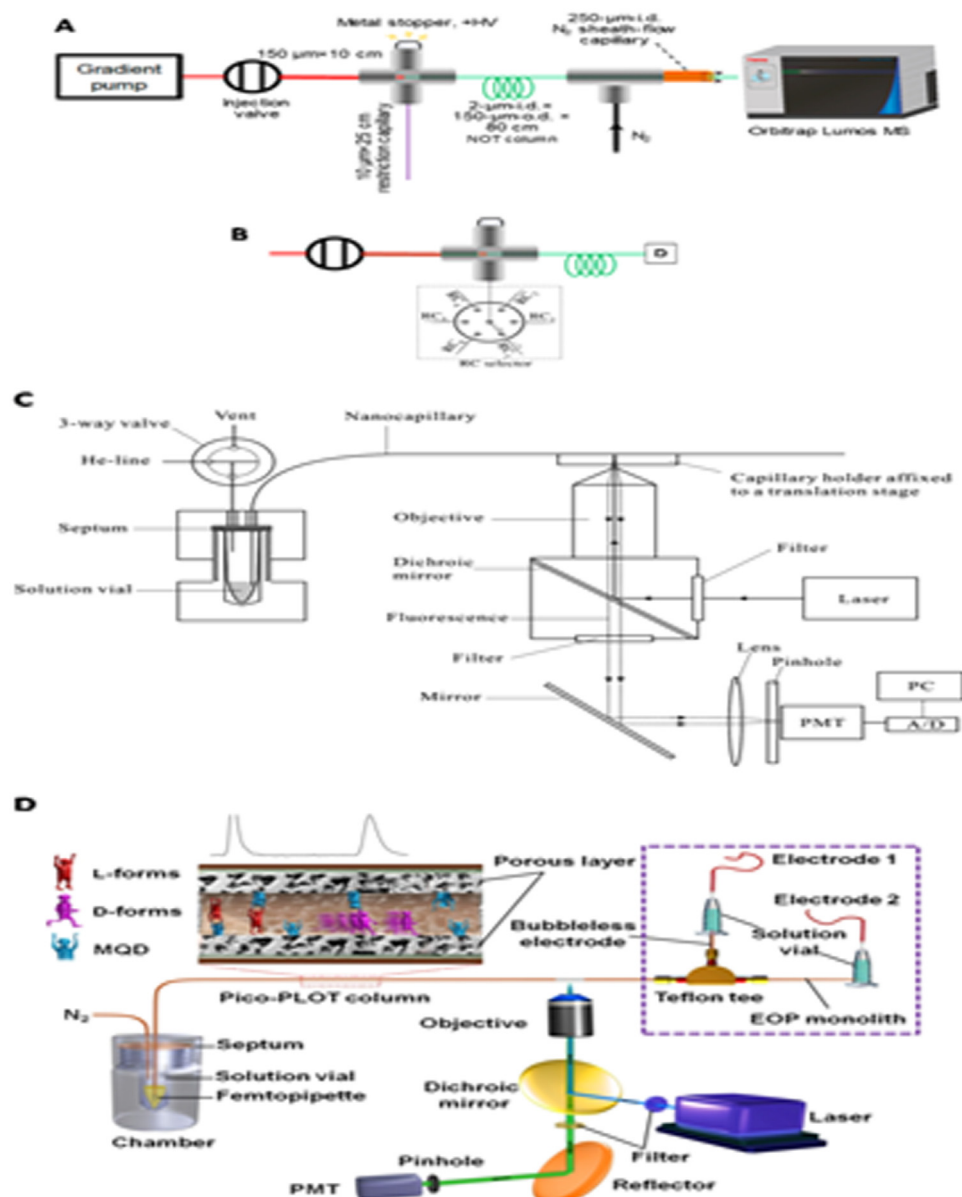


Fig. 6. Schematic diagram of OTLC system with LIF detection. (Reprinted with permission from ref.19).

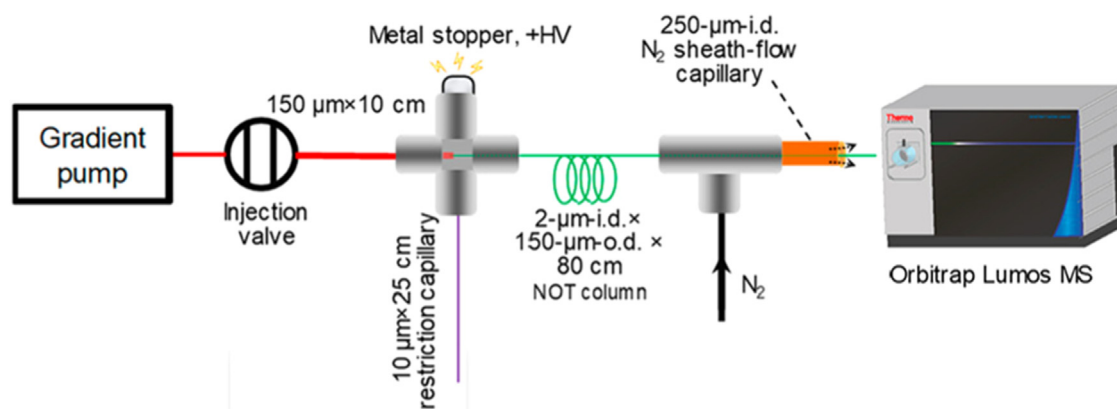


Fig. 7. Schematic illustration of the experimental setup of the picoLC-MS system. (Reprinted from ref. 12 with permission).

The advancement of MS has made it the most desirable detector for 2- μm -i.d. OT column separations. Low flow LC is readily incorporated with MS via a nanospray interface; ionization efficiencies will be high, and the majority (>50%) of the analytes initially in the solution phase can be transmitted to the MS detector [61]. It can be problematic to maintain a stable spray under the 100 pL/min flow rate. An appropriate sheath flow may be added to enhance the flow rate to stabilize the spray.

Ion-selective microelectrode [62] and potentiometric microelectrode [63] have been tested for narrow OT columns and may eventually be applied for 2- μm -i.d. OT column separations. However, the UV/VIS absorption detectors routinely used in today's LC are not suitable for 2- μm -i.d. OT columns because of the short optical pathlength.

4. Conclusion

Open tubular liquid chromatography using 2- μm -i.d. OT columns is a relatively new area of research and applications. Advantages of this technique include ultrahigh resolving power, ultrafast separation, extremely low solvent/sample consumption, little waste generation, reduced instrument and consumable cost, etc. Because it uses a single straight pore for the separation, it provides an excellent testbed for LC theoretical studies. However, this technique also has some disadvantages. A stable constant-flow pump capable of operating at nL/min to low pL/min regime is not commercially available, a scheme capable of injecting nL to fL sample into a 2- μm -i.d. OT column is not developed, protocols for preparing columns with long-lasting, high quality, and reproducible performances are not established, and detectors with adequate data sampling rate and sensitivity for 2- μm -i.d. OT columns are not popular. We are optimistic that all these challenges can be mitigated soon, but more scientists need to be involved in the research and development. We anticipate that LC using 2- μm -i.d. OT columns will become an indispensable tool for chemical and biochemical analyses of samples especially from proteomic research or single cell analysis in a few years.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Apeng Chen: Conceptualization, Writing – original draft, Writing – review & editing. **Shaorong Liu:** Conceptualization, Writing – original draft, Writing – review & editing.

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