Journal of Chromatography A, 1228 (2012) 99–109

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

# Sub-2 μm porous silica materials for enhanced separation performance in liquid chromatography

Yong Wang, Feng Ai, Siu-Choon Ng*, Timothy Thatt Yang Tan*

*School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459, Singapore*

ARTICLE INFO

*Article history:*
Available online 3 September 2011

*Keywords:*
Sub-2 μm porous silica material
High efficiency
Ultra-high performance liquid chromatography
Capillary electrochromatography
Achiral separation
Enantioselective separation

ABSTRACT

Fully or partially sub-2 μm porous silica materials have garnered strong interests as column packing materials in separation and analytical technologies due to the promise of rapid separation, enhanced efficiency and separation resolution. Silica support materials of different morphology and sub-2 μm size have been developed to improve separation performances in liquid chromatography (LC) and capillary electrochromatography (CEC). The current review highlights the recent development of sub-2 μm fully/partially porous silica materials and the demonstrations of their enhanced performance in achiral and chiral chromatography.



**Contents**



## 1. Introduction

Chromatographic technique development has always strived towards higher efficiency and more rapid resolution in diverse areas such as clinical, pharmaceutical and toxicology analysis, as well as enantioselective separation, in order to reduce costs and enhance throughput [1–8]. Conventional liquid chromatography (LC), such as high performance liquid chromatography (HPLC), could not fully satisfy these requirements due to the relatively low efficiency and long analysis time. Several approaches have been undertaken to achieve these goals, such as increasing flow rates and shortening the column length by using monolithic columns [9–12]. However, these approaches may result in low phase ratio and low capacity factor. In addition, HPLC can only operate in relatively low pressure (up to 400 bar). Another way to enhance analysis speed is to operate separations at high temperatures. However, high temperature could destroy temperature-sensitive samples and column packing materials [13,14].

One promising approach is to use smaller size silica particle (less than 2 μm, as compared to conventional 3 and 5 μm size column

* Corresponding authors. Tel.: +65 6316 8829.
*E-mail addresses:* tytan@ntu.edu.sg (T.T.Y. Tan), ngsc@ntu.edu.sg (S.-C. Ng).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.08.085

packing materials). This is motivated by van Deemter's equation that shows an inversely proportional relationship between the separation efficiency and particle size. Therefore, nano- or sub-micron size supporting materials may be promising to improve separation performance. However, reduced particle size can induce high backpressure as demonstrated by Darcy's Law. The fundamentals of van Deemter's equation and Darcy's Law have already been reviewed by Wu and Clausen and hence will not be repeated here [15].

Different materials of less than 2 μm in size have been developed and demonstrated in various separation techniques. Zhang et al. reviewed the applications of nanomaterials in liquid chromatography [16]. This review summarized the applications of $C_{60}$, single-walled carbon nanotube (SWCNT), silica nanoparticle and metal oxide nanoparticle in capillary LC [17–19]. The review by Nilsson et al. [20] introduces the use of novel nanoparticles in capillary and microchip electrochromatography, in which the applications of different nanomaterials such as silica, gold, fullerenes, SWCNT, polymer, titanium oxide nanoparticles have been elaborated [21–25]. Future development of enhanced performance stationary phases will depend on innovative support materials such as novel nanocomposites of silica or polymer and precious metal with enhanced surface functionalities.

Pioneering works on the use of nonporous sub-2-μm silica particles in ultra-high pressure capillary liquid chromatography (UHPLC) with columns I.D. < 200 μm were performed between 1997 and 2003 [26–29]. The particles used were 1.0 and 1.5 μm. These particles were monodisperse and make very efficient chromatographic columns. Jorgenson et al. [28,29] prepared fused-silica capillaries (I.D. ~ 30 μm) packed with 1.0 and 1.5 μm nonporous $C_{18}$ modified silica particles at high pressure. These columns could generate more than 200,000 theoretical plates for the separation of small organic compounds using pressure as high as 72,000 psi (5000 bar). Spherical organosilica particles of 670 nm (stable in 1 < pH < 11) containing $C_{18}$ moieties were prepared by Cintrón and Colón using a simple one-step synthesis process [30]. When applied in UHPLC for the separation of a mixture of ascorbic acid, hydroquinone, resorcinol, catechol and 4-methylcatechol, a fast analysis time within 4 min and high theoretical plates (500,000 plates/m) were achieved. Elevated-temperature UHPLC was performed using polybutadiene-coated 1 μm nonporous zirconia particles in a study by Carr and co-workers [31]. Five herbicides were separated in 1 min at 26,000 psi and 90 °C with a column efficiency of 420,000 plates/m. It was shown that nonporous particles with uniform size (below 1.5 μm) could form robust packed column and bring about low plate height at elevated column pressure [28,31].

However, nonporous materials have comparatively lower surface area which results in low loading capacity. Another challenge is that the high pressure generated by using small (sub-micron) nonporous particles renders the instrument dangerous and impractical to operate [30]. Xiang et al. [32] studied the safety concerns of operating UHPLC with fused-silica capillary columns. Liquid jets and high speed projectiles of silica particles due to rupture of the capillary or failure of the ferrule in the capillary connection might lead to injuries. To avoid the high pressure and potential danger, some studies using sub-2 μm silica were carried out on capillary electrochromatography (CEC). CEC utilizes electroosmotic driving force instead of pressure drop to achieve high separation efficiency [33–36].

Recently, robust porous sub-2 μm silica materials are brought into the limelight for fast and efficient chromatographic separation [37]. Mesoporous sub-2 μm silica materials, being smaller in size compared to conventional 5 and 3 μm packing materials, could bring about enhanced separation efficiency. Their large surface area also significantly improves loading capacity. These factors allow for the use of column of shorter length and smaller inner diameter to achieve the same or even higher resolution in very short analysis time compared to conventional HPLC [37,38]. Many recent research efforts are devoted to the development of chromatographic application using sub-2 μm materials and the corresponding instrumentation. In 2007, a review article by Wu and Clausen elaborated the fundamental and practical aspects of UHPLC liquid chromatography for fast separations [15]. The article discussed the fundamental and practical aspects of UHPLC such as particle size, frictional heating, pressure drop, column diameter, pump and injection systems, detection as well as packing materials. It also summarized some practical applications using UHPLC with sub-2 μm porous and nonporous packing materials. Since 2007, the development of sub-2 μm porous silica material continues to contribute significantly to the field of liquid chromatography. These silica materials have demonstrated good performance in achiral chromatographic separation and have shown great promise in fast enantioselective separation of racemic compounds.

To better understand the impact of recent development and applications (especially in bioanalysis and chiral chromatography) of sub-2 μm porous silica column packing materials in liquid chromatography, herein, this review article highlights the emergence of sub-2 μm porous silica materials and their enhanced separation performance in achiral and chiral liquid chromatography (LC), and capillary electrochromatography (CEC). The various terminologies coined by researchers and vendors to describe enhanced performance LC systems can be confusing, such as ultrahigh pressure liquid chromatography (usually associated with fine capillary columns and ultrahigh pressure), ultra-high performance liquid chromatography (UHPLC) and rapid resolution liquid chromatography (RRLC). In this review, we use one expression, ultra-high performance liquid chromatography (UHPLC), to represent the fast LC techniques with smaller columns and higher pressures than conventional HPLC.

## 2. Sub-2 μm porous silica materials in chromatography

Porous sub-2 μm materials are promising "new" separation materials for fast and high efficiency separations. The development of chromatographic applications using stable mesoporous materials with narrow particle size distribution is attracting burgeoning interests. On one hand, reducing particle size leads to enhanced efficiency and allows the utilization of smaller columns to accelerate the analysis; on the other hand, the higher porosity brings about an increase surface area, hence significantly enhances loading capacity and reduces back pressure. The following sections discuss different types of sub-2-μm silica materials for chromatographic separation.

### 2.1. Sub-2 μm MCM materials

MCM-41 s is a widely used mesoporous silica material with highly ordered long-range mesopores developed by Mobil researchers in 1992 [39]. A typical synthesis of MCM-41 materials involved the use of silicate solution and long chain n-alkyl ammonium salts at elevated temperature. Its application in LC is deemed promising due to its large surface area [40]. However, problems were encountered when they were used for the separation of biomolecules. In addition, this material could not withstand high pressure due to its weak porous structure [40,41]. Recent efforts have seen the improvement of the material strength by modifying the synthetic approach. Büchel et al. prepared 0.5 μm porous silica particles using a modified Stöber process and employed them in capillary electrochromatography for fast separation of n-alkylbenzenes [42]. The synthesis was based on hydrolysis and subsequent condensation of alkoxysilanes in a short chain alcohol using ammonia as a catalyst. The obtained nonporous silica beads

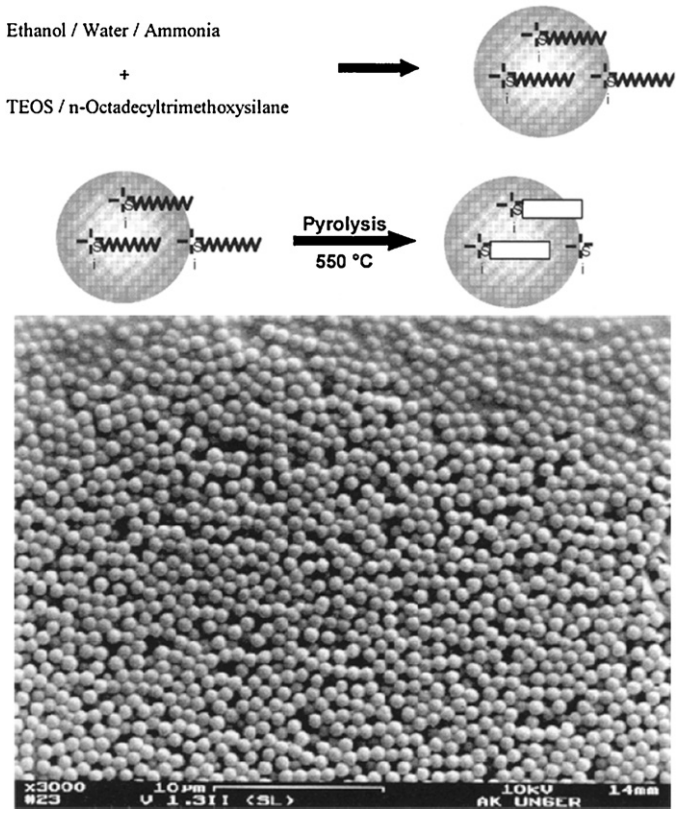


**Fig. 1.** Modified Stöber process and SEM image of 0.5 μm porous silica [42].

underwent calcination at high temperature over 550 °C to produce inner particle porosity (Fig. 1). Porous silica beads in a range of 0.2–3 μm were also prepared to investigate the influence of particle size on the electroosmotic flow (EOF).

Unger et al. reviewed the synthesis of micron and submicron spherical porous silica and discussed the opportunities as well as the challenges for high-resolution chromatographic and electrokinetic separations [43]. They indicated that high back pressure using the prepared materials strictly inhibited their applications in HPLC.

Recently, Liang et al. reported the preparation of large pores (up to 20 nm) monodisperse mesoporous silica spheres (MMSS) (1–1.7 μm) using a new surfactant poly(propylene oxide)amines and their application in fast HPLC analysis with low back pressure [44]. The particles were packed into a chromatographic column (50 mm × 2.1 mm I.D.) to separate a mixture of nucleic bases and nucleosides in hydrophilic interaction chromatography (HILIC) mode. Good separation was achieved in 3 min at a flow rate of 0.5 mL/min. The separation efficiency reached 174,280 plates/m while the back-pressure only increased to 23 MPa. The particles hence fulfilled the criteria of good column packing materials: high efficiency, high capacity and low back-pressure.

Our recent work [45] reported the synthesis and application of large pores spherical MCM-41S type material in chiral capillary electrochromatography. Porous silica beads of uniform particle sizes 660 nm and 810 nm were prepared using hexadecyltrimethylammonium bromide (CTAB) and tetramethoxysilane (TMOS). The pore size was expanded by a solvothermal treatment of the particles in an ammonia–ethanol solution. The obtained materials afforded better enantioselective separation efficiency and higher enantioselectivity compared to capillaries packed with normal 3 μm and 5 μm silica.

### 2.2. Sub-2 μm SBA materials

SBA material is another important sub-2 μm material applied in liquid chromatography because of its larger pore size, thicker pore wall and therefore more robust compared to MCM-41 materials [46]. A typical synthesis of these materials involves self-assembly of triblock copolymer template. The first application of SBA-15 in LC was demonstrated by Zhao et al. [47], in which large-pore mesoporous SBA-15 was prepared and functionalized with $C_{18}$ for the separation of glutathione, cysteine, dopamine and 6-thiopurine in UHPLC using capillary columns. However, as the morphology of this material is not spherical, the separation results were not quite satisfactory. Later on, high-speed CEC separation with submicron mesoporous SBA-15 was reported by Zou et al. [48]. The particle size of the spherical SBA-15 could reach 400 nm and the mesopores (12 nm) were highly ordered (Fig. 2). High-speed separation of some neutral compounds, anilines and some basic pharmaceuticals was achieved in CEC. High efficiency of 210,000 plates/m for thiourea probe was achieved and the lowest plate height of 2.0 μm was obtained at a linear velocity of 1.1 mm/s.

Yang and coworkers reported a modified synthetic approach for spherical large porous SBA-15 silica material (SLP-SBA-15) with particle size of 0.5–1 μm [46]. The application of co-template hexadecyltrimethylammonium bromide (CTAB) reduced the self-assembly time from 24 h to less than 1 h. The surface area of this

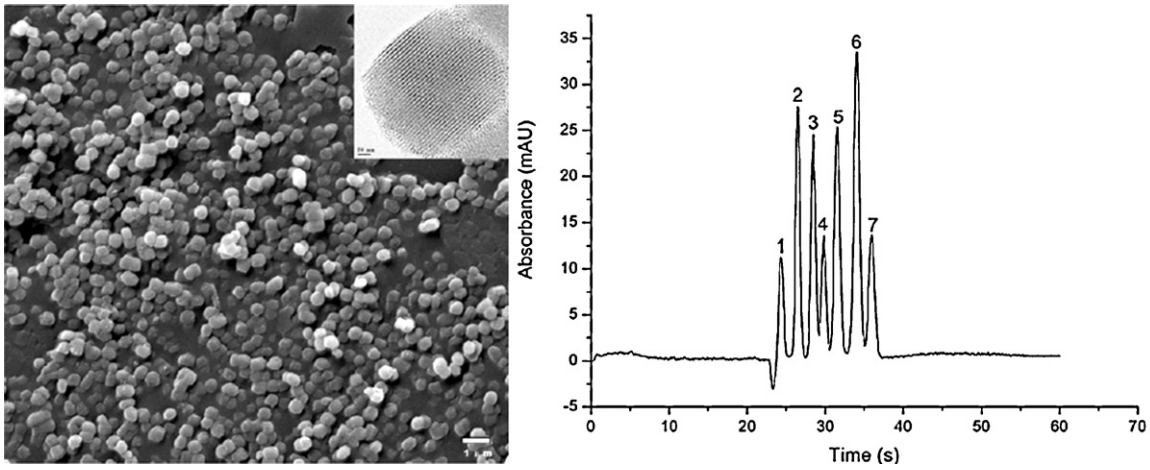


**Fig. 2.** (a) SEM image of 400 nm porous SBA-15 and (b) CEC separation of aromatic compounds. Experimental conditions: capillary column, 8.5/33 cm 650 μm I.D.; mobile phase, 10 mM Tris–HCl (pH 8.1) containing 80% ACN; separation voltage, 30 kV; injection, 2 kV for 5 s; detection wavelength, 214 nm. Solutes: (1) thiourea; (2) aniline; (3) benzene; (4) toluene; (5) dimethylbenzene; (6) 1,3,5-trimethylbenzene; and (7) 1,2,4,5-tetramethylbenzene [48].

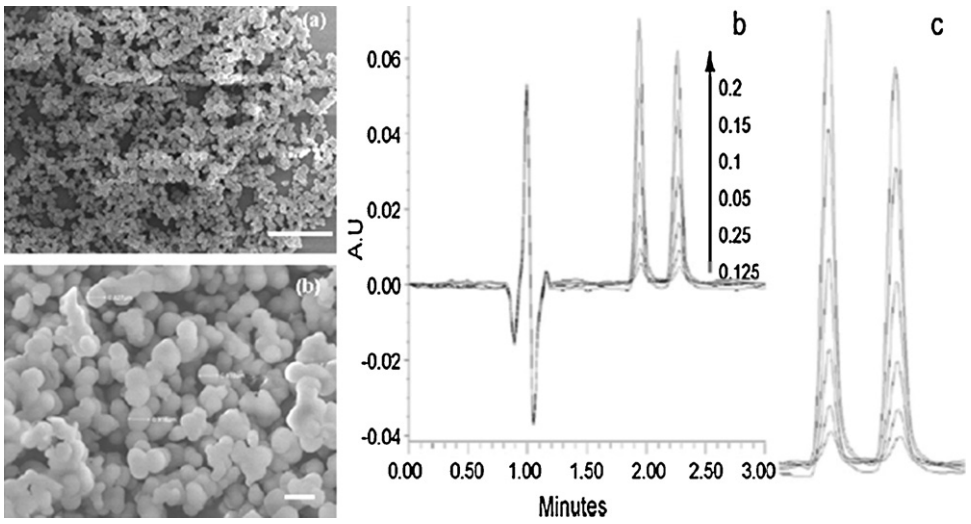


**Fig. 3.** (a) SEM images of submicron silica, and (b) chromatograms with different sample concentrations (0.125, 0.25, 0.5, 1.0, 1.5, and 2.0 mg/mL). Mobile phases: ACN/0.1% TEAA 60/40; flow rate: 0.7 mL/min; detection wavelength: 254 nm. (c) The magnified peaks from chromatograms [49].

material was determined to be over 600 $m^2/g$ with an average pore size of greater than 8 nm. SLP-SBA-15 was modified with $C_{18}$ and applied in UHPLC for separation of nonpolar alkyl aromatics. At a flow rate of 0.4 mL/min, the column pressure could be reduced to 2800 psi (column size: 4.6 mm × 50 mm). Reproducible separation of eight polycyclic aromatic hydrocarbons has been achieved with a relative standard deviation (RSD) of retention less than 1.6%. Liang et al. also reported a facile approach for the synthesis of mesoporous SBA-15 silica spheres for liquid chromatography application. 2–4.5 μm SBA-15 particles were synthesized and functionalized with dimethyloctadecylchlorosilane. The surface area of 2.0 μm particle was found to be 480 $m^2/g$. Separation efficiency could reach as high as 79,000 plates/m for toluene, and efficient separation of small molecules as well as larger biomolecules such as peptide and proteins has been achieved using this material.

The first enantioselective separation using submicron SBA materials was reported by Ai et al. [49]. Submicron mesoporous silica (0.6–0.9 μm) were successfully prepared with high surface area (480 $m^2/g$) and functionalized with cyclodextrins (CDs) followed by application in a Waters Acquity UPLC system for fast enantioselective separation of some racemic pairs using column with 2.1 μm I.D. and 5 cm length (Fig. 3). This will be discussed in a proceeding section.

### 2.3. Sub-2 μm hybrid materials

Ethyl-bridged hybrid (EBH) materials developed by Waters Corporation have contributed considerably to enhanced separation technology and have been widely used in the field of fast analysis. This material is prepared via Hybrid Particle Technology (HPT) by the co-condensation of 1,2-bis(triethoxysily)ethane with TEOS (Fig. 4) [50]. The particle size could range between the conventional 5 μm down to 1.5 μm with large pore size [51]. This material has several advantages such as decreased tailing factors for basic compounds, excellent mechanical strength and chemical stability in high pH environment over other silica-based packing materials [51,52]. Jorgenson et al. applied 1.0 μm EBH particles in capillary ultrahigh-pressure LC and achieved good separation of hydroquinone, benzene, catechol and 4-MeCat [51]. The separation was accomplished within 250 s with an efficiency as high as 730,000 plates/m.

An interesting work by Liang et al. reported the preparation of large porous phenylene-bridged organosilica spheres (PHS) and their potential in chromatographic applications [53]. PHS with particle size range of 1.5–3.5 μm and pore size range of 50–85 Å were synthesized and further modified by $C_{18}$ for reversed-phase LC application. Their results demonstrated higher separation efficiency to anilines compounds and significant enhancement in column stability at high pH.

### 2.4. Sub-2 μm core–shell silica material

An important sub-2 μm porous silica packing material in liquid chromatography is the core–shell materials (partially porous). The concept of core–shell silica materials was introduced about 40 years ago by Horváth et al. [54] and some initial works were

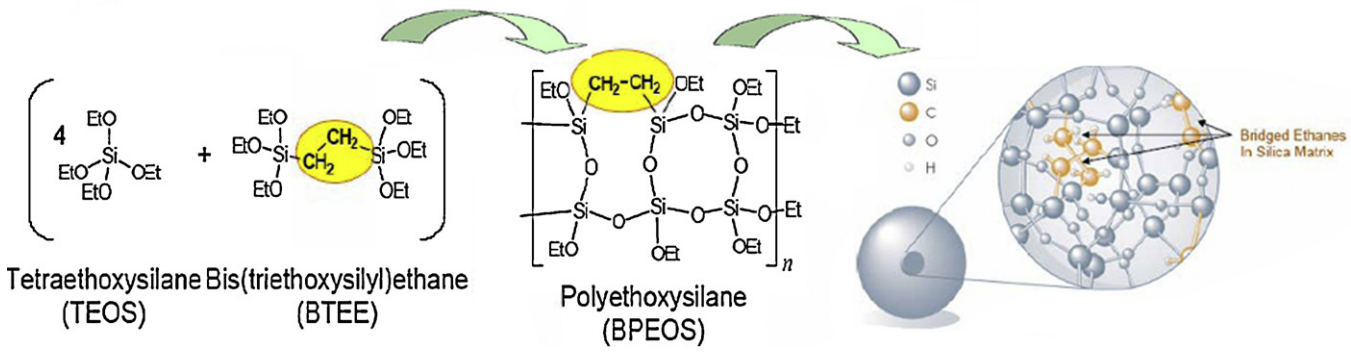


**Fig. 4.** Preparation of EBH materials [50].

performed successfully in preparing good packing materials with large particle size (over 20 μm) [55,56]. However, this type of core–shell material did not attain much commercial success, probably due to the rapid development of smaller fully porous particles. Recently, the emergence of commercial Poroshell, Halo and Kinetex column materials has brought the attention back to core–shell silica materials [57–60]. The particle size of Poroshell is 5 μm with a shell of 0.25 μm. Although this material afforded greater peak capacity over conventional fully porous 5 μm particle, its minimum reduced plate height of 2.5 (for heptanophenone) is greater than that of current commercial standards. Halo particles, marketed in 2007, have an average size of 2.7 μm (1.7 μm core and 0.5 μm shell) and afford a minimum reduced plate height of 1.4 for small compounds, compared to that of over 2 for sub-2 μm fully porous silica packing materials [57]. In 2009, a new core–shell silica material with an average size of 2.5 μm was commercialized by Kinetex, which exhibits a minimum reduced plate height around 1.1 [59]. Following this, a "genuine" sub-2 μm core–shell silica (1.25 μm core diameter, 0.23 μm shell thickness) was further developed by Kinetex using the same technology [61]. This technology involves a sol–gel process incorporating nanostructuring techniques to generate durable and homogeneous porous shell on solid silica beads. The advantages of applying this material in chromatography include low column back-pressure and therefore allow high flow rate. Most importantly, they can afford UHPLC separation results (high resolution, high selectivity and fast analysis) on most LC instruments using columns packed with 2.7 μm core–shell particle. Kinetex has commercialized the columns using this core–shell silica materials bonded with $C_{18}$ and $C_8$. Gritti and Guiochon compared peptide separation performance using 2.6 μm Kinetex-$C_{18}$ to 1.7 μm BEH-$C_{18}$ porous particles and the 2.7 μm Halo-$C_{18}$ shell particles [62]. They found that, by increasing linear velocity, the peak capacity of the Kinetex column was constant while those of the other two columns decreased almost by twenty percent. Kinetex column also afforded similar or even better resolution of biomolecules with molecular size around 4 nm. The comparison of 1.7 μm Kinetex-$C_{18}$ with 1.7 μm Waters-BEH in a peptide separation study by Fekete et al. indicated that the former showed about 50% improvement in column efficiency compared to the latter [61].

## 3. Sub-2 μm porous silica materials in UHPLC

### 3.1. UHPLC using capillary columns

With the development of nanotechnology, high efficient and fast separation LC techniques employing small particles are garnering more attention. The study on the application of small particles in liquid chromatography started from the use of nonporous silica particle in capillary ultrahigh pressure liquid chromatography (UHPLC). This technique provides high column efficiency but its loading capacity is compromised. Furthermore, the low permeability of the long capillary columns (over 30 cm) with small non-porous silica particle produces high back-pressure, which makes operation of the equipment challenging and is also dangerous for the operators [29,30,32]. Recent development using capillary columns for UHPLC system focuses on the reduction of column length and diameter to achieve fast and efficient analysis, and the utilization of porous silica particles to improve the capacity and reduce backpressure. Utilization of porous silica beads in UHPLC was first performed by Jorgenson et al. [51]. $C_{18}$-EBH 1.5 μm particles supplied by Waters Corporation were packed into 30-μm-I.D. fused-silica capillary columns. However, these columns have a length of up to 50 cm and dead time was about 5.6 min with an inlet pressure of 23,000 psi. Anspach et al. packed the same particles into 1 mm diameter, 150 mm in length columns and evaluated their performance in UHPLC [52]. The required pressure to achieve optimum plate height was 16,000 psi. Thomas et al. used a Waters nanoAcquity coupled in tandem with MS to determine synacthen in urine for sports drug testing at a pressure of 10,000 psi [63]. This method enables a fast (<6 h/sample), sensitive (1 fmol/mL), less laborious (>25 samples/day) and highly specific analysis using commonly available chemicals, reagents and instrumentation. In the authors' opinion, the development of UHPLC in the coming future will drive towards the utilization of smaller capillary columns (small diameter or shorter column length) coupled with porous sub-2 μm silica particles (fully porous or core–shell materials) in order to speed up analysis with enhanced separation efficiency.

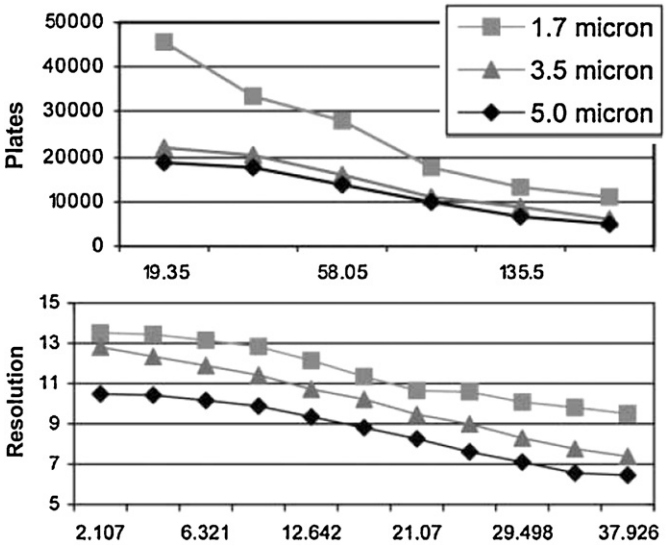


**Fig. 5.** Comparison of 1.7, 3.5 and 5.0 μm particles for preparative LC [64].

### 3.2. UHPLC using 2.1 mm diameter columns

Conventional HPLC with small porous particles may provide efficient separation using high flow rate. However, the high back-pressure generated can go beyond hardware capability of HPLC and the large consumption of organic solvent due to large geometry of HPLC columns increases the cost. UHPLC using capillary columns suffers from safety issues associated with extremely high pressure and the fragile nature of the fused silica capillary. In recent years, much work has focused on the miniaturization of conventional HPLC columns by using sub-2 μm porous spherical materials to achieve fast and efficient separations with acceptable pressure (up to 1000 bar). Comparison of 1.7, 3.5 and 5.0 μm particles in LC was done by Durham and Hurley using an Agilent 1200 RRLC system [64]. Both the column efficiency and resolution on the 1.7 μm packed column were superior compared to the other two columns (Fig. 5). Comparison of UHPLC and HPLC on the efficiency, analysis speed and resolution has been investigated extensively. For the separation and quantification of lincomycin traces, UHPLC afforded fast sample throughput and about 10 times lower consumption of solvents [65]. The superiority of UHPLC on the analysis of phenolic compounds over HPLC was shown in a work by Solich et al. [66]. UHPLC offered 5 times faster analysis speed over that of HPLC and about 2 times higher sensitivity for the separation of phenolic acids and flavonoids (Figs. 6 and 7). Comparison of UHPLC–MS/MS and HPLC–MS/MS for the determination of pesticides in baby foods was carried out by Fussell et al. [67]. The study revealed that UHPLC/MS allowed improved detection of disulfoton in baby foods and afforded 2.5 times faster analysis speed than by HPLC/MS.

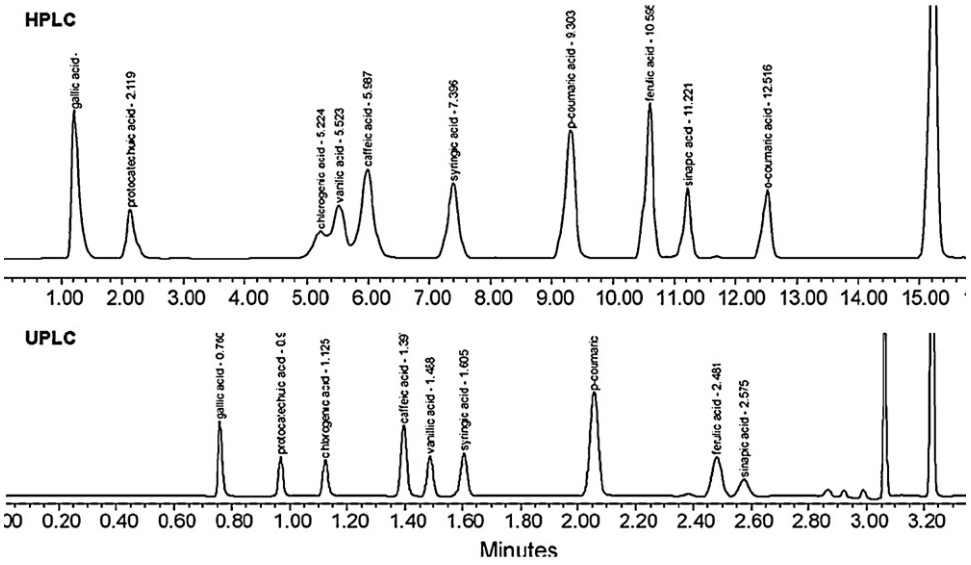


**Fig. 6.** Separation of phenolic acids on HPLC and UHPLC. HPLC: 0.1% formic acid–methanol, from 85:15 to 50:50 (v/v), 1.0 mL/min; UHPLC: 0.1% formic acid–methanol, from 88.5:11.5 to 30:70 (v/v), 0.45 mL/min [66].

### 3.3. Achiral application of UHPLC

A survey of published literatures in UHPLC applications has found most works on achiral analysis using $C_{18}$ or $C_8$ bonded materials. There are over four hundred papers published in the past five years on achiral analysis and separation. In general, UHPLC systems have shown at least five times faster separation/analysis compared to conventional HPLC with the same or improved sensitivity and resolution. The advantages of using sub-2 μm chromatographic silica materials for UHPLC over conventional HPLC have been verified by many researchers, as highlighted in the previous sections. Since Waters Corporation first commercialized its UPLC system in 2004, most of the earlier published works were done using their UPLC system with $C_{18}$-EBH columns. Analytical work in genomic and metabolomic research is amongst the first application of UHPLC systems, especially when coupled to MS. Xu et al. summarized the application of UHPLC using sub-2 μm particles in high throughput quantitative bioanalysis [6]. Wu and Clausen summarized the fundamentals and applications of UHPLC in pharmaceutical analysis, proteomics assay and environmental analysis in 2007 [15]. Table 1 summarizes some representative applications with such materials in UHPLC after 2007.

A greater number of publications emerge on the application of UHPLC (especially UHPLC/MS) in food chemistry, metabolic identification, pesticide residue analysis, environmental water analysis and pharmaceutical industry [74–82]. Benešová et al. used UHPLC coupled with fluorescence detection to rapidly determine ochratoxin A in brewing materials and beer within 3 min [83]. Fast determination of pesticides in tea by UHPLC/MS was carried out by Chen et al. [84]. UHPLC/MS for tamoxifen metabolites profiling in plasma was performed by Dahmane et al. [85]. Gikas et al. implemented simultaneous quantification of daptomycin (DPT) and rifampicin (RFM) in plasma in UHPLC and also carried out a pharmacokinetic study [86]. The total analysis time was 4.5 min

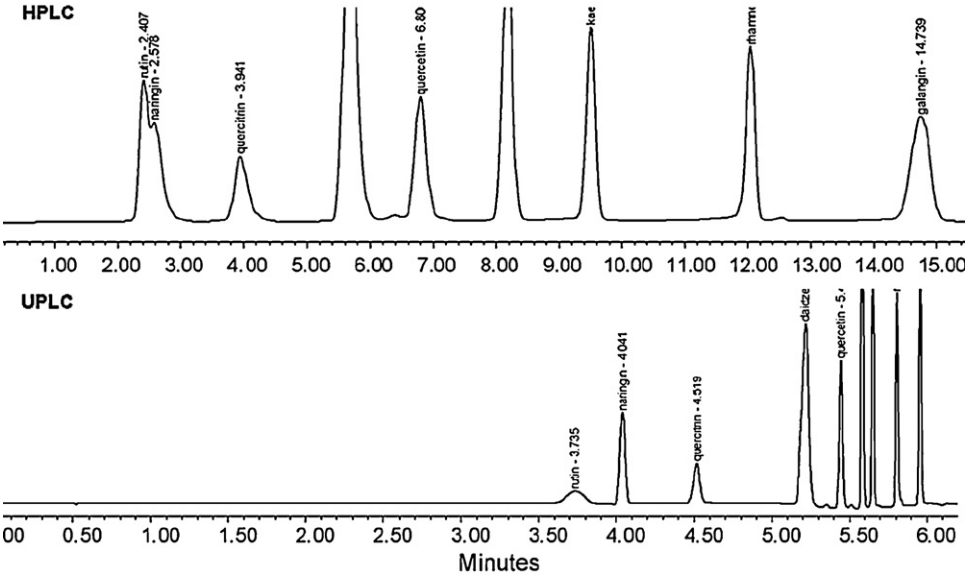


**Fig. 7.** Separation of flavonoids on HPLC and UHPLC. 0.1% formic acid–methanol, from 60:40 to 40:60 (v/v), 1.0 mL/min; UHPLC: 0.1% formic acid–methanol, from 69:31 to 10:90 (v/v), 0.45 mL/min [66].

**Table 1**
Achiral separations using different sub-2 μm particles in UHPLC.

| Materials | Particle size (μm) | Method | Applications | Performances | Column | Refs |
|---|---|---|---|---|---|---|
| Mesoporous $C_{18}$-MCM41S | 1–2 | UHPLC | Separation of the mixture of uracil, dimethyl phthalate, toluene, biphenyl and phenanthrene | The achieved pore size was ~20 nm. Column efficiency was 105,000 plates/m for phenanthrene | I.D. 2.1 mm, length 50 mm | [68] |
| Mesoporous $C_{18}$-SBA-15 | 0.5–1 | UHPLC | Separation of non-polar alkyl aromatics | Well ordered 2*d* hexagonal structure with poresize enlarged up to 8.2 nm. The back pressure could be reduced to below 190 bar at a flow rate of 0.4 mL/min. Good reproducibility | I.D. 4.6 mm, length 50 mm | [46] |
| Fully porous BEH-130$C_{18}$ | 1.7 | Nano UHPLC/MS | Analysis of synacthen in urine | A fast (<6 h/sample), less laborious (>25 samples/day), sensitive (1 fmol/mL) and high specific analysis was achieved | I.D. 75 μm, length 100 mm | [63] |
| Core–shell Kinetex-$C_{18}$<br>Fully porous BEH-$C_{18}$ | 1.7<br>1.7 | UHPLC | Separation of the mixture of uracil, acetophenone, toluene and naphthalene | Efficiency for acetophenone was over 130,000 plates/m<br>Separation was well performed within 3 min | I.D. 2.1 mm, length 150 mm | [69] |
| Fully porous BEH-$C_{18}$<br>Hypersil Gold $C_{18}$ | 1.7<br>1.9 | UHPLC | Separation of the polar neutral mixture of estradiols and steroids | Minimum reduced plate heights reached 2.7 and 2.8, respectively for estradiol. Fast separation of steroids (neutral polar API) swabbed from stainless steel model surface was achieved within 1.5 min | I.D. 2.1 mm, length 50 mm | [70] |
| Fully porous BEH-$C_{18}$ | 1.7 | UHPLC/MS | Separation of the biological mixture of brguanosine, labetalol, reserpine and SB243213A | Sub-minute separation of the four molecules was achieved at 65 °C with high reproducibility | I.D. 2.1 mm, length 50 mm | [71] |
| Fully porous BEH-$C_{18}$ | 1.7 | UHPLC | Separation of antibiotics (tetracyclines and macrolides) | Baseline gradient separation was achieved within 8 min. The maximum experimental peak capacities for tetracyclines and macrolides were 51.8 and 46.7, respectively | I.D. 2.1 mm, length 50 mm | [72] |
| Core–shell Kinetex-$C_{18}$ | 1.7 | UHPLC | Separation of the mixture of small polar neutral analytes with a polypeptide and different sized proteins | Minimum plate height for 4.1 kDa polypeptide reached 6.3 μm and 232 μm for 38.9 kDa proteins | I.D. 2.1 mm, length 50 mm | [73] |

with DPT and RFM, eluting at 1.9 and 2.1 min, respectively. The determination of aristolochic acid-derived DNA adducts in exfoliated urothelial cells was performed by Guo et al. using UHPLC/MS [87]. Fast classification of polysaccharides from traditional Chinese medicines (TCM) using UHPLC coupled with multivariate analysis was done by Kuang et al. [88]. Rapid analysis of TCM Niu Huang Jie Du Pill using UHPLC with UV detector was carried out by Liang et al. [89].

### *3.4. Enantioselective separation using UHPLC with sub-2 μm particles*

The chirality of drugs is important in pharmaceutical industry due to the different pharmacological activities and pharmacokinetic/metabolic behaviors of the enantiomers. Therefore, analytical scale enantioselective separation has been widely performed in HPLC, gas chromatography (GC) and supercritical fluid chromatography (SFC). Enantioselective separation can be achieved by using chiral stationary phases (CSPs) or chiral mobile phase additives in chromatography, the former deemed more practical such that the often expensive chiral additives are not wasted. Various strategies have been developed to afford cyclodextrin functionalization onto silica particles to form CSP for enantioselective separation in chromatography [45,49,90–95].

To improve chiral analysis speed and efficiency, a promising solution may be the combination of sub-2 μm CSP and UHPLC technique. However, the potential of UHPLC technique in enantioselective separation has not been fully realized. So far, there are only a few publications describing the enantioselective separation using UHPLC with sub-2 μm CSP (Table 2).

As early as 2003, Gong et al. used diaza-18-crown-6-capped β-cyclodextrin bonded 1.5 μm nonporous silica particles for enantioselective separation in UHPLC [96]. High efficiency was obtained while the columns demonstrated low sample capacity. Guillarme et al. summarized some previous enantioselective separation applications using capillary liquid chromatography with sub-2 μm nonporous silica CSP or mobile phase additives [97]. They indicated that all the separations afforded high column efficiency but very low sensitivity. Hence, this group applied commercial HP-β-CD as chiral mobile phase additives in a Waters Acquity UPLC

**Table 2**
Enantioselective separation using sub-2 μm materials in UHPLC.

| Materials | Particle size (μm) | Selectors | Column dimensions | Applications | Performance | Refs |
|---|---|---|---|---|---|---|
| Non-porous silica | 1.5 | 18-Crown-6-β-CD immobilized onto silica | I.D. 75 μm, length 23 cm | Separation of indapamide and 2-phenylpropionaldehyde enantiomers | Seven enantiomer pairs were resolved with resolutions ($R_s$) over 2. The resolution of 2-phenylpropionaldehyde reached 24.5 | [96] |
| Fully porous Acquity Phenyl BEH silica | 1.7 | HP-β-CD added in the mobile phase | I.D. 2.1 mm, length 100 mm | Separation of methamphetamine, methylenedioxyamphetamine and 3,4-methylenedioxyethylamphetamine enantiomers | Separations were completed within 5 min by using the HP-β-CD directly. Fast and high efficient separations were achieved after precolumn derivatization | [97] |
| Hypurity Thermo fully porous silica | 1.9 | Brush-type selectors immobilized onto silica | I.D. 4.1 mm; length 50, 75, and 100 mm | Separation of sulfoxide enantiomers | Very high speed and resolution were achieved. Separations are routinely obtained with analysis time in 15–40 s | [98] |
| Mesoporous SBA-15 | 0.6–0.9 | Phenylcarbamoylated-β-CD immobilized onto silica | I.D. 4.6 mm, length 50 mm | Separation of aminoglutechimide, diltiazem, propranolol, tolerisone, 1-(4-iodophenyl)ethanol and 1-(4-phenylphenyl)but-3-ol enantiomers | All the separations were performed within 7 min and good day-to-day reproducibility | [49] |
| Fullyporous Agilent ZORBAX Eclipse Plus $C_{18}$ | 1.8 | Cationic β-CD added in the mobile phase | I.D. 2.1 mm, length 50 mm | Separation of a series of dansyl amino acids enantiomers | Most of the dansyl amino acids were resolved within 10 min | [99] |

system to carry out enantioselective separation of some amphetamine enantiomer pairs. Separation was achieved in 2–5 min with high efficiency and selectivity. They also performed fast indirect enantioselective determination of β-blockers after precolumn derivatization with an optically pure reagent (Fig. 8). Remarkably, the 10 enantiomers were well separated in less than 3 min. Moreover, the individual separation of β-blocker enantiomers was performed in 1 min or less.

Gasparrini et al. [98] and Tan et al. [49] prepared sub-2 μm CSP based on brush-type (Fig. 9) and β-cyclodextrin (Fig. 10) for fast enantioselective separation in UHPLC systems. In the study by Gasparrini et al., the comparison of enantioselective separation results with 4.3, 2.6 and 1.9 μm brush-type indicated that 1.9 μm CSP could considerably reduce the analysis time and solvents consumption to achieve the same resolution as that of 4.3 μm CSP. Improved throughput and enantioselective separation in sub-minute were demonstrated with shorter columns with sub-2 μm CSP. In our recent work, β-cyclodextrin modified sub-2 μm CSPs were shown to display rapid and good enantioselective separation (within 10 min) of six different neutral and basic drug enantiomers [49].

A very recent work on fast enantioselective separation of dansyl amino acids using Agilent 1200 series UHPLC technique was performed by Xiao et al. [99]. Three novel cationic β-cyclodextrins were applied as mobile phase additives for fast enantioselective separation of a series of dansyl amino acids on an Agilent $C_{18}$ column (2.1 mm I.D. and 5 cm length). Most of the analytes could be baseline resolved within 10 min (Fig. 11), which was a great improvement compared to conventional HPLC columns (4.6 mm I.D. and 15 cm length).

## 4. Sub-2 μm materials in capillary electrochromatography (CEC)

CEC, as a hybrid of HPLC and CE, offers the combined positive attributes of both separation techniques, such as high efficiency, rapid analysis and micro molar quantities of analytes [100,101].

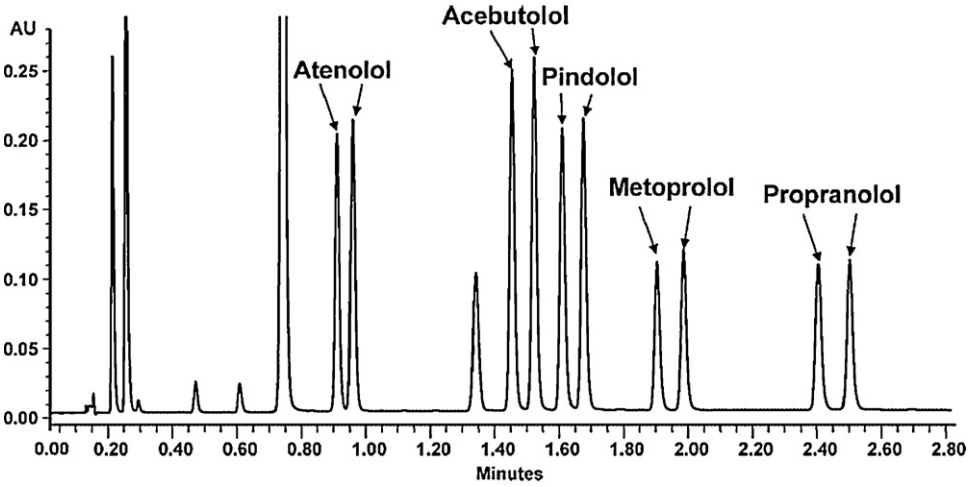


**Fig. 8.** Enantioselective separation of β-blockers after derivatization with AITC reagent in UHPLC. Mobile phase: acetate buffer 20 mM pH 5-MeCN, from 23.5 to 56.5% MeCN in 2.34 min. Temperature: 25 °C [97].

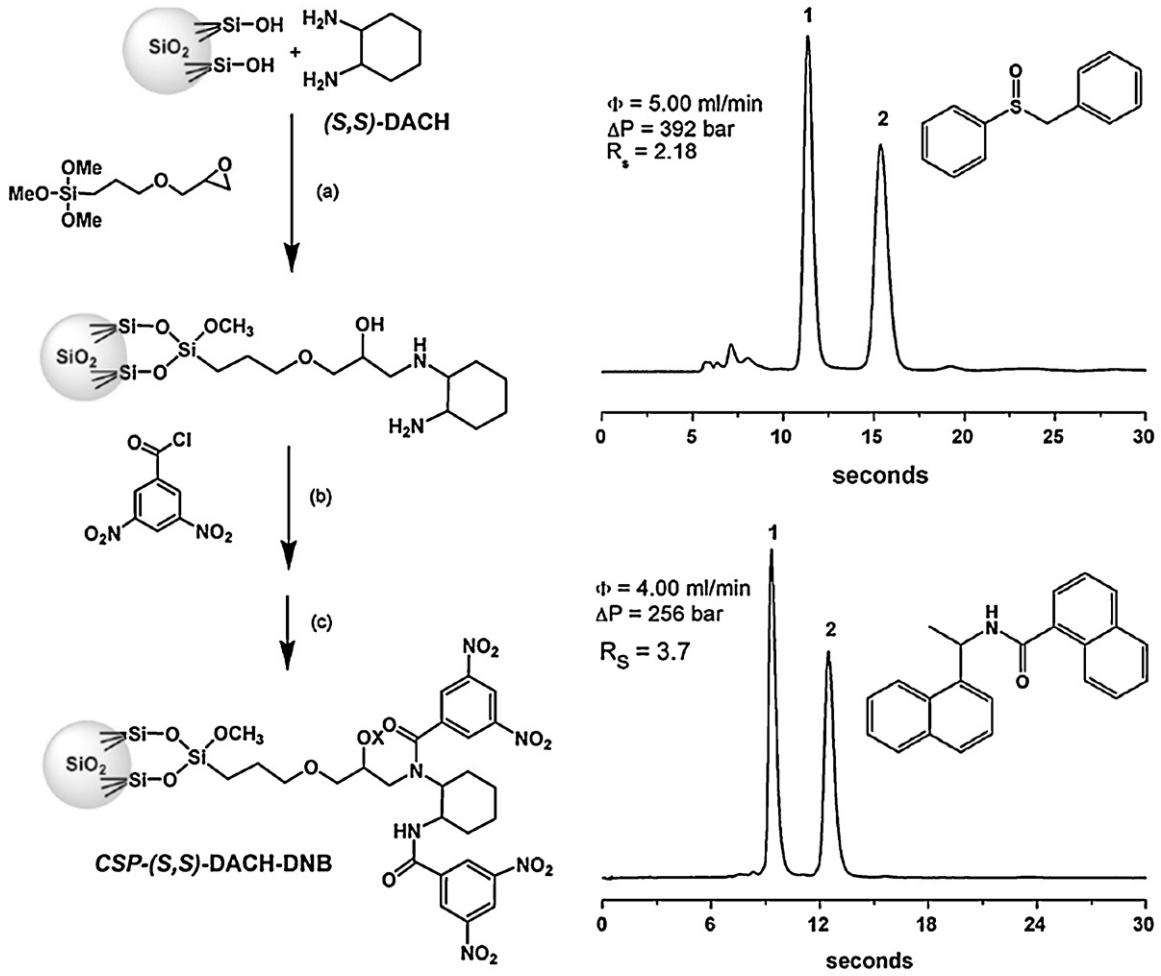


**Fig. 9.** Preparation of sub-2 μm brush-type CSPs and sub-minute enantioselective separation [98].

In CEC, the electroosmotic flow (EOF) generated from the double charged layer of the silica surface is used to propel the analytes and mobile phase through the stationary phase packed bed inside the capillary columns. Mayer and Schurig first reported the enantioseparation of 1,1′-binaphthyl-2,2′-diyl hydrogenphosphate and 1-phenylethanol enantiomers using an open-tubular (OT) CEC columns. The theoretical plates per meter were calculated to about 325,000 while the resolution was modest due to the low selector loading on the capillary inner wall. So far, there have appeared three types of CEC: packed-bed CEC, open-tubular CEC and monolith CEC. The use of packed columns is still dominant in the field of chiral CEC. Packed bed CEC columns are fabricated from 50 to 100 μm I.D. fused-silica capillaries, typically packed with normal size silica particles (3–5 μm).

Compared with HPLC, the generation of electroosmotic flow (EOF) (similar to a plug flow profile) in packed CEC capillary allows for the use of very small particles without back-pressure consideration, and hence resulting in enhanced efficiency. Although nonporous silica particle affords good efficiency, the low surface area means low surface loadings of functional molecules and loading capacity of analytes. Early work on the application of porous small particles in CEC was performed by Unger et al. in which they prepared 0.2–3 μm porous silica particles with uniform size and systematically evaluated their performances in CEC [33]. The advantages of small particle in achiral CEC have also been demonstrated in their study. The performance of porous SBA-15 in CEC was investigated by Zou et al. as discussed in Section 2.2 [48]. Enhanced enantioselective separation of submicron particles in CEC

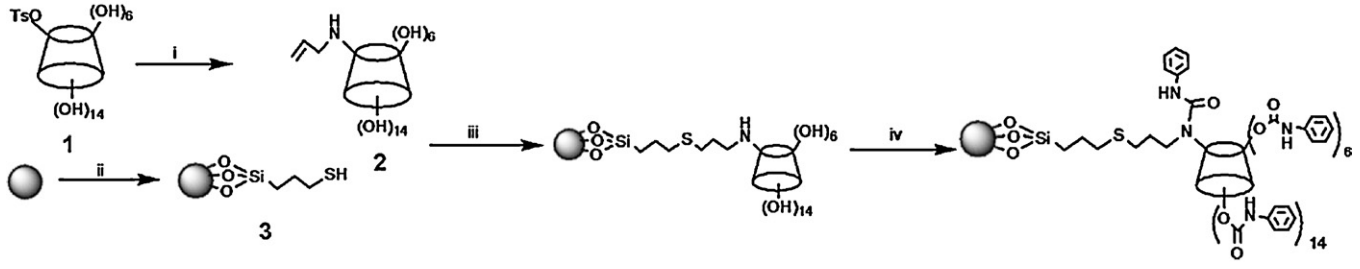


**Fig. 10.** The synthetic scheme of sub-2 μm β-cyclodextrin CSP [49].

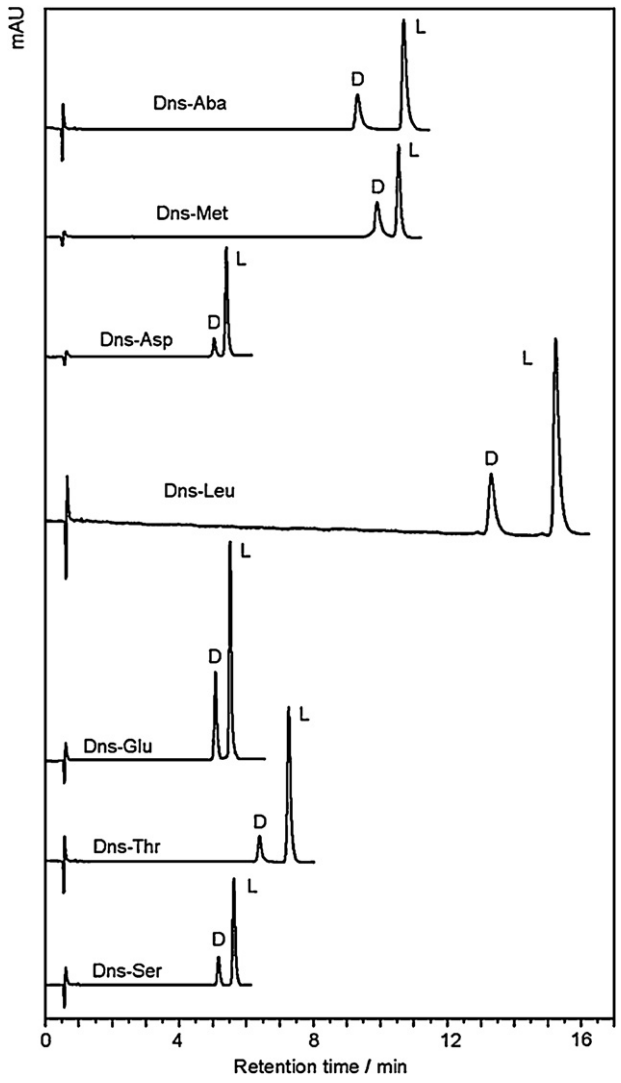


**Fig. 11.** Elution of dansyl amino acids in UHPLC using β-CD based chiral mobile phase additives. Conditions: flow rate 0.4 mL/min; mobile phase: acetonitrile–triethylammonium acetate buffer (1% TEAA, pH = 4.65) (12.5:87.5, v/v) + 45 mM selector; temperature 25 °C [99].

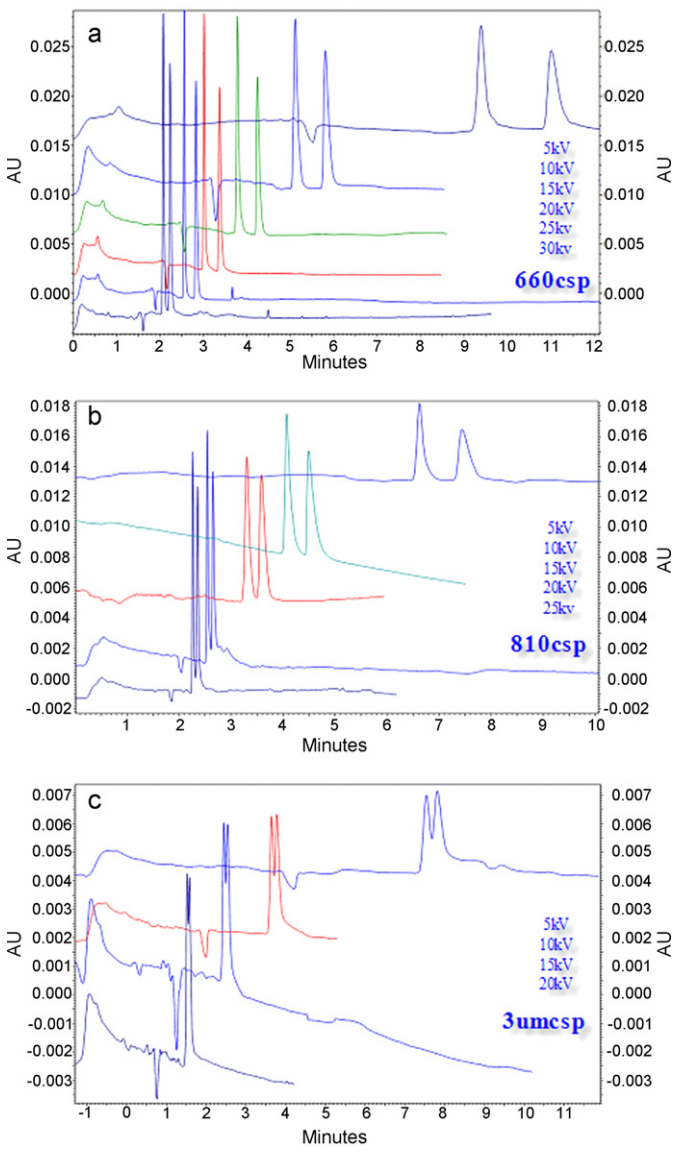


**Fig. 12.** CEC enantioselective separation of aryl alcohols using 660 nm, 810 nm and 3 μm CSP [45].

has been demonstrated by our group [45]. The prepared submicron CSPs were packed into 9 cm long and 50 mm I.D. capillaries using monolithic frits and evaluated on CEC by altering the separation parameters. Fast and efficient separation of a series of racemic aryl alcohols was performed, and a remarkable column efficiency of 476,000 plates/m was achieved under high voltages. The resolution and efficiency were far better than those of 3 μm CSP under the same separation conditions (Fig. 12).

Although CEC with small particles affords fast and highly efficient analysis, a few issues remained to be resolved. The first issue is the difficulty in packed-bed column fabrication. The packing of reduced size materials into a column requires high packing pressure, which requires a robust packing instrument and consistent packing technique. Besides, as the inlet and outlet frits of a capillary are commonly fabricated by sintering the packing materials inside the capillary, there are at least three parts of the fabricated capillary unprotected by the polyimide (inlet frit, outlet frit and the detection window) which makes the fabricated capillary very fragile. Besides, bubble formation and Joule heating effect during the operation may be significantly increased with inconsistent packing materials [45,100].

## 5. Conclusion

In this article, we have reviewed the recent development of sub-2 μm porous silica materials in fast chromatographic separation. Sub-2 μm porous silica materials have the potential to become the next generation column packing materials for chromatographic techniques due to its reduced size and porous structure, therefore promising enhanced efficiency. Several kinds of porous sub-2 μm materials have been successfully developed such as EBH, SBA and core–shell spherical beads. UHPLC using fused-silica capillary combined with small particles could provide improved separation results, however, the associated difficulty and danger in handling the capillary column and instrument need to be resolved. Recent works have chosen the faster and more efficient UHPLC technique over conventional HPLC. The advantages of sub-2 μm porous silica materials in UHPLC have been extensively investigated and this technique will continue to be developed. With the development of novel chiral stationary phases based on sub-2 μm porous silica materials and simpler chiral derivatization chemistries, the potential of UHPLC in enantioselective separation could be fully realized

in the coming future by simply transferring the broad enantioselective separations from conventional HPLC to this technique.

## Acknowledgement

Funding from the Agency for Science, Technology and Research SERC grant (grant no: 092 101 0056) in support of this project is acknowledged.

## References

[1] D.T.-T. Nguyen, D. Guillarme, S. Rudaz, J.-L. Veuthey, J. Sep. Sci. 29 (2006) 1836.
[2] I.M. Mutton, Chromatographia 47 (1998) 291.
[3] J.J. Van Deemter, F.J. Zuiderweg, A. Kinkenberg, Chem. Eng. Sci. 5 (1956) 271.
[4] J.H. Knox, M. Saleem, J. Chromatogr. Sci. 7 (1969) 614.
[5] J.H. Knox, J. Chromatogr. Sci. 15 (1977) 352.
[6] R.N. Xu, L. Fan, M.J. Rieser, T.A. El-Shourbagy, J. Pharm. Biomed. Anal. 44 (2007) 342.
[7] T. Yamashita, Y. Dohta, T. Nakamura, T. Fukami, J. Chromatogr. A 1182 (2008) 72.
[8] J. Tang, R. Zhu, R. Zhao, G. Cheng, W. Peng, J. Pharm. Biomed. Anal. 47 (2008) 923.
[9] S. Hjertén, J.L. Liao, R. Zhang, J. Chromatogr. 473 (1989) 273.
[10] I. Gusev, X. Huang, C. Horvath, J. Chromatogr. A 885 (1999) 273.
[11] T. Ikegami, E. Dicks, H. Kobayashi, H. Morisaka, D. Tokuda, K. Cabrera, N. Tanaka, J. Sep. Sci. 27 (2004) 1292.
[12] H. Oberacher, A. Premstaller, C.G. Huber, J. Chromatogr. A 1030 (2004) 201.
[13] J. Nawrocki, C. Dunlap, J. Li, J. Zhao, C.V. McNeff, A. McCormick, P.W. Carr, J. Chromatogr. A 1028 (2004) 31.
[14] D. Guillarme, S. Heinisch, Sep. Purif. Rev. 34 (2005) 181.
[15] N. Wu, A.M. Clausen, J. Sep. Sci. 30 (2007) 1167.
[16] Z. Zhang, Z. Wang, Y. Liao, H. Liu, J. Sep. Sci. 29 (2006) 1872.
[17] K. Jinno, K. Yamamoto, J.C. Fetzer, W.R. Biggs, J. Microcolumn Sep. 5 (1993) 517.
[18] J. Nawrocki, C. Dunlap, A. McCormick, P.W. Carr, J. Chromatogr. A 1028 (2004) 1.
[19] L.X. Chen, Y.F. Guan, J.P. Ma, G.A. Luo, K.H. Liu, J. Chromatogr. A 1064 (2005) 19.
[20] C. Nilsson, S. Birnbaum, S. Nilsson, J. Chromatogr. A 1168 (2007) 212.
[21] T. de Boer, R. Mol, R.A. de Zeeuw, G.J. de Jong, D.C. Sherrington, P.A.G. Cormack, K. Ensing, Electrophoresis 23 (2002) 1296.
[22] B. Neiman, E. Grushka, J. Gun, O. Lev, Anal. Chem. 74 (2002) 3484.
[23] Y. Xu, S.F.Y. Li, Electrophoresis 27 (2006) 4025.
[24] Y.L. Hsieh, T.H. Chen, C.Y. Liu, Electrophoresis 26 (2005) 4089.
[25] Y.W. Lin, M.J. Huang, H.T. Chang, J. Chromatogr. A 1014 (2003) 47.
[26] Y. Xiang, N. Wu, A.J. Lippert, M.L. Lee, Chromatographia 55 (2002) 399.
[27] Y. Xiang, B. Yan, C.V. McNeff, P.W. Carr, M.L. Lee, J. Chromatogr. A 1002 (2003) 71.
[28] J.E. MacNair, K.D. Patel, J.W. Jorgenson, Anal. Chem. 71 (1999) 700.
[29] J.E. MacNair, K.C. Lewis, J.W. Jorgenson, Anal. Chem. 69 (1997) 983.
[30] J.M. Cintrón, L.A. Colón, Analyst 127 (2002) 701.
[31] Y. Xiang, B. Yan, B. Yue, C.V. McNeff, P.W. Carr, M.L. Lee, J. Chromatogr. A 983 (2003) 83.
[32] Y. Xiang, D.R. Maynes, M.L. Lee, J. Chromatogr. A 991 (2003) 189.
[33] T. Adam, K.K. Unger, M.M. Dittmann, G.P. Rozing, J. Chromatogr. A 887 (2000) 327.
[34] Y. Okamoto, Y. Ikawa, F. Kitagawa, K. Otsuka, J. Chromatogr. A 1143 (2007) 264.
[35] M.F. Huang, Y.C. Kuo, C.C. Huang, H.T. Chang, Anal. Chem. 76 (2004) 192.
[36] P.S. Doyle, J. Bibette, A. Bancaud, J.L. Viovy, Science 295 (2002) 2237.
[37] Y. Shen, R.D. Smith, K.K. Unger, D. Kumar, D. Lubda, Anal. Chem. 77 (2005) 6692.
[38] M. Swartz, L. Krull, LC–GC 24 (2006) 770.
[39] I.Y. Ying, C.P. Mehnert, M.S. Wong, Angew. Chem. Int. Ed. 38 (1999) 56.
[40] M. Raimondo, G. Perez, M. Sinibaldi, A. De Stefanis, A.A.G. Tomlinson, Chem. Commun. (1997) 1343.
[41] K.W. Gallis, J.T. Araujo, K.J. Duff, J.G. Moore, C.C. Landry, Adv. Mater. 11 (1999) 1452.
[42] T. Adam, S. Lüdtke, K.K. Unger, Chromatographia 49 (1999) S49; G. Büchel, M. Grün, K.K. Unger, A. Matsumoto, K. Tsutsumi, J. Supramol. Sci. 5 (1998) 253.
[43] K.K. Unger, D. Kumar, M. Grun, G. Buchel, S. Ludtke, T. Adam, K. Schumacher, S. Renker, J. Chromatogr. A 892 (2000) 47.
[44] Y. Li, S. Cheng, P. Dai, X. Liang, Y. Ke, Chem. Commun. (2009) 1085.
[45] L.S. Li, Y. Wang, D.J. Young, S.C. Ng, T.T.Y. Tan, Electrophoresis 31 (2010) 378.
[46] X. Liu, L. Li, Y. Du, Z. Guo, T.T. Ong, Y. Chen, S.C. Ng, Y. Yang, J. Chromatogr. A 1216 (2009) 7767.
[47] J. Zhao, F. Gao, Y. Fu, W. Jin, P. Yang, D. Zhao, Chem. Commun. (2002) 752.
[48] R. Tian, J. Sun, H. Zhang, M. Ye, C. Xie, J. Dong, J. Hu, D. Ma, X. Bao, H. Zou, Electrophoresis 27 (2006) 742.
[49] F. Ai, L. Li, S.C. Ng, T.T.Y. Tan, J. Chromatogr. A 1217 (2010) 7502.
[50] L. Nováková, D. Solichová, P. Solich, J. Sep. Sci. 29 (2006) 2433.
[51] K.D. Patel, A.D. Jerkovich, J.C. Link, J.W. Jorgenson, Anal. Chem. 76 (2004) 5777.
[52] J.A. Anspach, T.D. Maloney, L.A. Colón, J. Sep. Sci. 30 (2007) 1207.
[53] Y. Zhang, Y. Jin, H. Yu, P. Dai, Y. Ke, X. Liang, Talanta 81 (2010) 824.
[54] C. Horváth, S.R. Lipsky, J. Chromatogr. Sci. 7 (1969) 109.
[55] C. Horváth, S.R. Lipsky, Anal. Chem. 41 (1969) 1227.
[56] J.J. Kirkland, Anal. Chem. 41 (1969) 218.
[57] J.J. DeStefano, T.J. Langlois, J.J. Kirkland, J. Chromatogr. Sci. 46 (2008) 254.
[58] F. Gritti, G. Guiochon, J. Chromatogr. A 1157 (2007) 289.
[59] F. Gritti, I. Leonardis, D. Shock, P. Stevenson, A. Shalliker, G. Guiochon, J. Chromatogr. A 1217 (2010) 1589.
[60] X. Wang, W.E. Barber, P.W. Carr, J. Chromatogr. A 1107 (2006) 139.
[61] S. Fekete, K. Ganzler, J. Fekete, J. Chromatogr. A 1217 (2010) 3819.
[62] F. Gritti, G. Guiochon, J. Chromatogr. A 1217 (2010) 1604.
[63] A. Thomas, M. Kohler, W. Schänzer, M. Kamber, P. Delahaut, M. Thevis, Rapid Commun. Mass Spectrom. 23 (2009) 2669.
[64] D.K. Durham, T.R. Hurley, J. Liq. Chromatogr. Relat. Technol. 30 (2007) 1895.
[65] J. Olšovskš, M. Jelínková, P. Man, M. Koběrská, J. Janata, M. Flieger, J. Chromatogr. A 1139 (2007) 214.
[66] Z. Spáčil, L. Nováková, P. Solich, Talanta 76 (2008) 189.
[67] C.C. Leandro, P. Hancock, R.J. Fussell, B.J. Keely, J. Chromatogr. A 1103 (2006) 94.
[68] D.A. Keane, J.P. Hanrahan, M.P. Copley, J.D. Holmes, M.A. Morris, J. Porous Mater. 17 (2010) 145.
[69] F. Gritti, G. Guiochon, J. Chromatogr. A 1217 (2010) 5069.
[70] E. Oláh, S. Fekete, J. Fekete, K. Ganzler, J. Chromatogr. A 1217 (2010) 3642.
[71] D.N. Mallett, C. Ramírez-Molina, J. Pharm. Biomed. Anal. 49 (2009) 100.
[72] T. Tylová, Z. Kameník, M. Flieger, J. Olšovaká, Chromatographia 74 (2011) 19.
[73] S. Fekete, K. Ganzler, J. Fekete, J. Pharm. Biomed. Anal. 54 (2011) 482.
[74] S. Babaee, A. Beiraghi, Anal. Chim. Acta 662 (2010) 9.
[75] M. Clariana, M. Gratacós-Cubarsí, M. Hortós, J.A. Barcía-Regueiro, M. Castellari, J. Chromatogr. A 1217 (2010) 4294.
[76] F. Gosetti, U. Chiuminatto, D. Zampieri, E. Mazzucco, E. Robotti, G. Calabrese, J. Chromatogr. A 1217 (2010) 7864.
[77] H.J. Kang, H.J. Yang, M.J. Kim, E.S. Han, H.J. Kim, D.Y. Kwon, Food Chem. 127 (2011) 1056.
[78] X. Lu, Z. Xiong, J. Li, S. Zheng, T. Huo, F. Li, Talanta 83 (2011) 700.
[79] K. Sakai-Kato, E. Saito, K. Ishikura, T. kawanishi, J. Chromatogr. B 878 (2010) 1466.
[80] S.A.C. Wren, P. Tchelitcheff, J. Chromatogr. A 1119 (2006) 140.
[81] Q. Zhou, L. Pang, J. Xiao, J. Chromatogr. A 1216 (2009) 6680.
[82] A. Zuniga, L. Li, Anal. Chim. Acta 689 (2011) 77.
[83] S. Běláková, K. Benešová, R. Mikulíková, Z. Svoboda, Food Chem. 126 (2011) 321.
[84] G. Chen, P. Cao, R. Liu, Food Chem. 125 (2011) 1406.
[85] E. Dahmane, T. Mercier, B. Zanolari, S. Crucon, N. Guignard, T. Buclin, J. Chromatogr. B 878 (2010) 3402.
[86] E. Gikas, F.N. Bazoti, P. Fanourgiakis, E. Perivolioti, A. Roussidis, A. Skoutelis, J. Pharm. Biomed. Anal. 51 (2010) 901.
[87] L. Guo, H. Wu, H. Yue, S. Lin, Y. Lai, Z. Cai, J. Chromatogr. B 879 (2011) 153.
[88] H. Kuang, Y. Xia, J. Liang, B. Yang, Q. Wang, Y. Sun, Carbohydr. Polym. 84 (2011) 1258.
[89] X. Liang, L. Zhang, X. Zhang, W. Dai, H. Li, L. Hu, et al., J. Pharm. Biomed. Anal. 51 (2010) 565.
[90] Y. Wang, D.J. Young, T.T.Y. Tan, S.C. Ng, J. Chromatogr. A 1217 (2010) 7878.
[91] Y. Wang, D.J. Young, T.T.Y. Tan, S.C. Ng, J. Chromatogr. A 1217 (2010) 5103.
[92] Y. Xiao, Y. Wang, T.T. Ong, L.Y. Ge, S.N. Tan, D.J. Young, T.T.Y. Tan, S.C. Ng, J. Sep. Sci. 33 (2010) 1797.
[93] P. Sun, C. Wang, Z.S. Breitbach, Y. Zhang, D.W. Armstrong, Anal. Chem. 81 (2009) 10215.
[94] X. Han, T. Yao, Y. Liu, R.C. Larock, D.W. Armstrong, J. Chromatogr. A 1063 (2005) 111.
[95] Y. Wang, Y. Xiao, T.T.Y. Tan, S.C. Ng, Tetrahedron Lett. 49 (2008) 5190.
[96] Y. Gong, Y. Xiang, B. Yue, G. Xue, J.S. Bradshaw, H.K. Lee, J. Chromatogr. A 1002 (2003) 63.
[97] D. Guillarme, G. Bonvin, F. Badoud, J. Schappler, S. Rudaz, J.L. Veuthey, Chirality 22 (2010) 320.
[98] G. Cancelliere, A. Ciogli, I. D'Acquarica, F. Gasparrini, J. Kocergin, D. Misiti, J. Chromatogr. A 1217 (2010) 990.
[99] Y. Xiao, T.T.Y. Tan, S.C. Ng, Analyst 136 (2011) 1433.
[100] Y. Wang, Y. Xiao, T.T.Y. Tan, S.C. Ng, Electrophoresis 30 (2009) 705.
[101] B. Preinerstorfer, M. Lammerhofer, Electrophoresis 28 (2007) 2527.