



Effect of particle size on the speed and resolution of chiral separations using supercritical fluid chromatography



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ABSTRACT

Fast chiral supercritical fluid chromatography (SFC) separations have become important due to the increasing use of high-throughput experimentation (HTE) in organic synthesis. These HTE experiments can generate hundreds of samples for chiral analysis that need to be assayed in a short time. In general, chiral SFC can provide much faster analysis times compared to liquid chromatography (LC). Additionally, columns packed with smaller particles can provide faster and more efficient separations. In this study, the effect of the particle size on the speed and resolution of chiral separations by SFC was evaluated. The performance of Chiralcel OD columns packed with either 5 or 3 μm particles were compared using van Deemter or other kinetic plots. The benefits of using smaller particle columns for chiral SFC analysis are illustrated.

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1. Introduction

In recent years, fast chiral separations have become increasingly important as the use of high-throughput experimentation (HTE) in organic synthesis has grown. These HTE studies can generate a large number of samples that need to be evaluated in a short time. Therefore, there have been many significant efforts in recent years to speed the task of chiral analysis [1–5]. Studies have shown that chiral supercritical fluid chromatography (SFC) can provide much faster analysis times compared to high-performance liquid chromatography (HPLC) techniques, due to the advantageous physical properties of the carbon dioxide mobile phase used in SFC [6–10]. The low viscosity of the carbon dioxide mobile phase allows higher flow rates with low-pressure drop across the column leading to faster run times. In addition, the higher diffusivity of dissolved analytes in supercritical carbon dioxide allows for high-efficiency separations due to improved mass-transfer kinetics.

The recent rise of ultrahigh-pressure liquid chromatography (UHPLC) technology has shown that the use of columns packed with smaller, sub-2 μm particle stationary phases can provide faster and more efficient chromatographic separations [11,12]. The use of columns packed with smaller particles in SFC, however, has been

much less studied. As pointed out by Lesellier, systematic studies of the effect of particle size on SFC separations is inherently difficult, owing to the compressibility of the mobile phase [13], which makes interpreting the performance by constructing kinetic plots or van Deemter curves challenging [14,15]. One of the main problems for constructing kinetic plots in SFC is the accurate calculation of void time (t_0) values for different length columns, where the increase in column length induces an increase in the inlet pressure, which results in an additional change in the apparent void volume.

Some early studies by Gere et al. demonstrated the performance benefits of using columns packed with smaller particles in SFC, where van Deemter curves showed a decrease in the minimum height equivalent to theoretical plate (HETP) parameter with smaller particle sizes. In addition, columns packed with smaller particles exhibited a very flat van Deemter curve, allowing the use of higher linear velocities without sacrificing column efficiency [16]. Although these preliminary results showed the practical value of using smaller particles for SFC, research interest in exploiting this approach arose only in the last few years. More recent work in this research area has shown the use kinetic plots in SFC using smaller particle achiral columns [17–19].

The physical properties of the mobile phase (viscosity, diffusion, density, etc.) in any packed-column chromatography affect both the chromatographic efficiency and the mobile-phase linear velocity, both of which directly impact analysis time. When the outlet pressure for SFC separation is maintained at a constant value (e.g. 200 bar), the compressibility of the mobile phase leads to a change in density as a function of flow rate. Shen et al. [20] have

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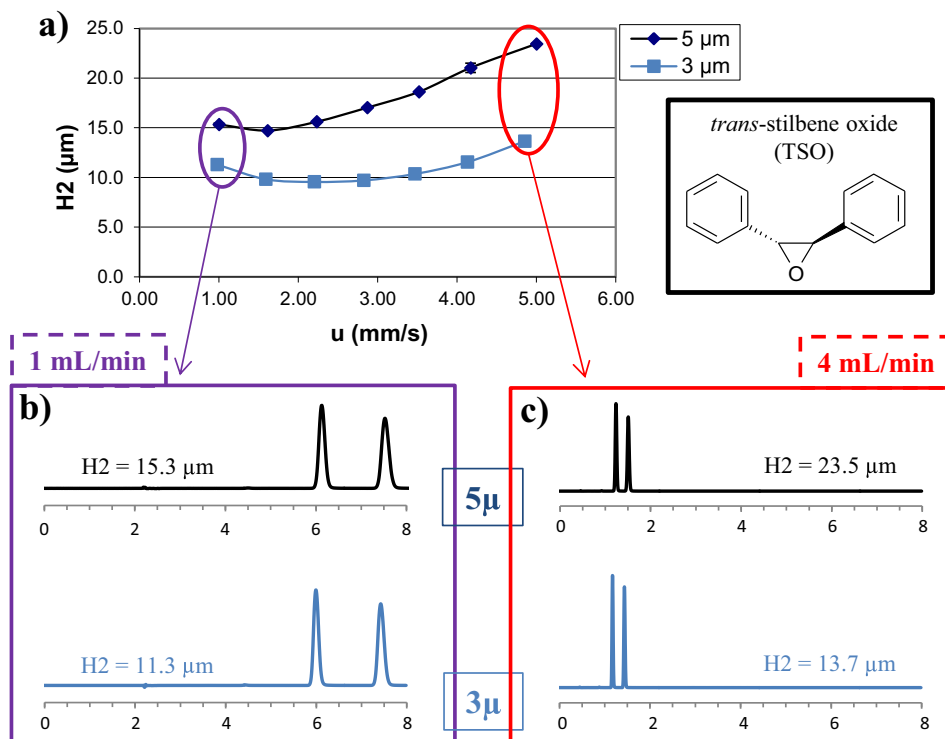


Fig. 1. (a) van Deemter plot showing the dependence of the theoretical plate height (H_2 ; peak 2) on the mobile-phase linear velocity (sample: 1 mg/mL TSO) using two different OD stationary phases (3 and 5 μm) ($n = 3$). Detailed chromatograms showing chromatographic performance for the two columns under (b) low and (c) high flow rate conditions.

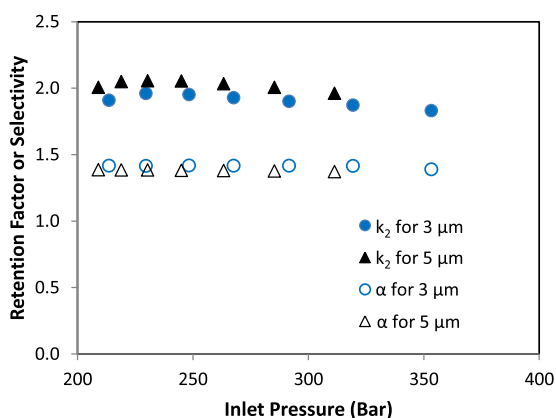


Fig. 2. Correlation between inlet column pressure values, retention and selectivity factors for the two OD stationary phases with a carbon dioxide mobile phase containing 10% MeOH. Conditions as described in Fig. 1.

proposed modifications for the van Deemter equation in SFC that take into account the compressibility of the mobile phase and suggest that the reduced plate height is a function of the mobile-phase linear velocity to the 1.5 order (i.e. $h = A + B/u^{1.5} + Cu^{1.5}$). Furthermore, in order to overcome the density variation issue in kinetic studies for chromatographic separations in SFC, Poe and Schroden [21] have described an isopycnic (i.e. constant density) method for the measurement of plate heights as a function of changing flow rate, while keeping the average density of the mobile phase constant. This approach has recently been used to evaluate some typical SFC columns and mobile-phase conditions, including C18 columns packed with 5.0, 3.5 and 1.8 μm fully-porous particles, as well as columns packed with 2.6 μm superficially porous particles [22]. Although the isopycnic way is often suggested for pure CO_2 , it has not been practical for most of typical SFC separations [23].

Another way to overcome the density variation is to operate in the subcritical area with fluid density higher than 0.9 g/mL.

In this study, we investigate the use of SFC for rapid chiral separations, comparing the performance of columns with either 5 or 3 μm diameter particles. These experiments were run using a constant back pressure (200 bar) and the change of inlet pressure was only due to the increasing flow rate. Furthermore, these experiments were run at subcritical conditions, using 10% methanol modifier and 40 °C temperature. Experimental van Deemter and other kinetic plots were compared for Chiralcel OD columns containing different particle sizes.

2. Experimental

2.1. Instrumentation

An Agilent 1100 LC with an Aurora A5 Fusion SFC module instrument was used for the results shown in Figs. 1–5. The Agilent stack was comprised of a G1322A vacuum degasser, G1312A binary pump, G1367A WPALS autosampler, G1316A column compartment, G1315B diode array detector. The system was controlled by Agilent ChemStation.

A Waters Acquity UPC² (Waters Corp., Milford, MA, USA) system was used for the results shown in Fig. 6, which was equipped with a fluid delivery module (a liquid CO_2 pump and a modifier pump), a sample manager–FL autosampler, a photodiode array detector, and MassLynx as software.

2.2. Chemicals and reagents

HPLC-grade methanol was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Isobutyl amine (IBA), *trans*-stilbene oxide (TSO), mianserin, lansoprazole, benzoin, warfarin, 4-hydroxywarfarin, 6-hydroxywarfarin, 7-hydroxywarfarin, 8-hydroxywarfarin,

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