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Sub-Micron Plate Heights for Capillaries Packed with Silica Colloidal Crystals

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Abstract

Extremely uniform packing of colloidal silica in capillaries is shown. Reversed-phase electrochromatograms of DiI-C₁₂ exhibit plate heights as low as 0.13 μm and a reduced plate height as low as 0.4, using 75 μm i.d. capillaries packed with 330 nm silica particles. The contribution from the A term is 0±20 nm in electrochromatography. The particles are shown to form colloidal crystals inside the capillaries. Optical images show Bragg diffraction, indicative of crystallinity; SEM images show face-centered cubic crystallinity; and the porosity is 0.25±0.01, which is in agreement with that for face-centered cubic crystals. The capillaries are fritless, and 100 μm i.d. capillaries packed with silica colloidal crystals withstand pressures of at least 12,400 psi.

Introduction

The introduction of sub-2 μm particles to RPLC has delivered improved speed and resolution to separations.^{1, 2} The improvement is explained by the contribution of the particle diameter, d_p , to the plate height, H .³

$$H = A + \frac{B}{v} + Cv \quad (1)$$

where the A and C parameters are dependent upon particle diameter, d_p .

$$A = 2\lambda d_p; B = 2\gamma D; C = \frac{\omega d_p^2}{D} \quad (2)$$

This model neglects contributions from injection, detection, and slow desorption from the surface. The parameters λ , γ and ω are due to details of the particles and the packing. Using smaller particle diameters increases the optimal velocity and gives lower plate heights; consequently, sub-2 μm particles have had a significant impact on the practice of HPLC.⁴

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Supporting Information Available: Supporting Information for Malkin.pdf.

Detailed studies have shown that λ increases as the particle size becomes smaller,³ indicating that efficiency is limited by nonuniform packing. This arises from a radial distribution of packing densities in the cylindrical tube, giving a radial distribution of velocities that broadens peaks.^{5–8} Recent experiments bear this out: nonporous 1.0 μm particles in capillaries of varying diameter show that the A term is primarily due to the radial heterogeneity of the packing.⁹

The purpose of this paper is to investigate whether better packing is possible with silica colloidal crystals because these form face centered cubic lattices.¹⁰ Slabs of silica colloidal crystals have been used for separations of small molecules,¹¹ and for DNA and protein sieving.¹² The plate heights are well above 1 μm ,^{11, 12} yet these should achieve sub-micron plate heights by virtue of the crystalline packing. In this work, silica colloidal crystals packed inside capillaries¹³ are used to determine whether sub-micron plate heights are attainable.

Experimental Section

Fused silica capillaries of 75 and 100 μm i.d. were cleaned in 0.1 M NaOH and rinsed with ultrapure water, then ethanol, and dried in a vacuum oven. The capillaries were dipped in colloidal suspensions of silica particles (330-nm diameter), allowing the slurry to wick into the capillaries. Drying over a few days allowed silica colloidal crystals to form in the capillaries, without the need for frits. The colloidal crystals were chemically modified by horizontal polymerization of trichlorosilanes bearing the functional groups C_{18} and C_1 , to give a C_{18} density of 3 $\mu\text{m}/\text{m}^2$.¹⁴ The capillaries were then rinsed with dry toluene, dried in an oven at 120 $^{\circ}\text{C}$ for 1 hr, and stored in glass vials. Just before use, the capillaries were rinsed with mobile phase: 90:10 MeOH:water, to which 0.1% formic acid was added.

SEM images were obtained with a JEOL Neoscope. Pressure vs. flow rate was measured using a CVC Micro Tech XtremeSimple nanoLC. The porosity was measured from the volume of water that wicks into a known length of colloidal crystal. A water plug was wicked into the open region of a capillary, and its length was measured. It was then pushed by syringe to touch the colloidal crystal, where it then wicked into the crystal. After filling the full length of the colloidal crystal, which is evident from the increase in transparency, the length of the remaining water plug was measured. For a change in the length of the plug of water, Δl_w , and a length of colloidal crystal l_c , the porosity, ε , is the ratio of the two values.

$$\varepsilon = \frac{\Delta l_w}{l_c} \quad (3)$$

For electrochromatography, the capillary spanned a pair of reservoirs made from PDMS. Injection was done by quickly dipping the capillary, pre-filled with mobile phase, into a solution of 13 μM solution of DiI (1,1-didodecyl-3, 3, 3,3-tetramethyl-indocarbocyanine perchlorate) in methanol, which allowed the analyte to adsorb at the entry of the capillary. The electromigration was driven using Pt electrodes in the reservoirs, with electric fields ranging from 500 to 2000 V/cm. The peaks were imaged using an inverted fluorescence microscope equipped with a fluorescence cube for Cy3 and a 2 $\times$ objective. Movies of the migrating dye were acquired using a sensitive CCD camera (Princeton Instruments Photonmax) to view the migration from injection to final separation. The same equipment was used for measuring diffusion coefficients.

Results and Discussion

Figures 1a and 1b show photographs of a typical packed capillary through a zoom optical microscope with color camera. The photographs illustrate the blue color, which is indicative of crystalline packing of the particles. The color is due to Bragg diffraction from the even spacing of particles on the order of the wavelength of blue light.¹⁵ Figure 1c shows the SEM image of a small region of the capillary. The packing is polycrystalline, with two different crystal faces evident: the (111) face, which has hexagonal symmetry, and the (100) face, which has square symmetry. The packing exhibits mostly domains of hexagonal symmetry, with occasional domains of square symmetry. The full SEM image (Supporting Information) shows that this polycrystalline packing extends all the way across the capillary. The region near the wall is dominated by colloidal crystal of square symmetry, while hexagonal symmetry is more prevalent for the bulk, but both are face-centered cubic colloidal crystal. In short, what is different about this capillary is that crystalline structure extends all the way across the capillary up to the walls, whereas conventional columns exhibit order only near the wall, while the bulk is amorphous. If this polycrystalline packing that is visible at the end of the capillary persists throughout the length of the capillary, it would avoid the radial heterogeneity of packing density, yielding smaller plate heights.

One cannot image the interior of the colloidal crystal, but resistance to flow and porosity can reveal whether the particles are close-packed. The Supporting Information provides a plot of volume flow rate vs. pressure for a pair of capillaries having inner diameters of 100 μm i.d. and packing lengths of 2.31 and 2.40 cm. The highest volume flow rates are 200 nL/min at 12,400 psi, which are more than sufficient to achieve stable nanospray ionization. The dimensionless resistance to flow, ϕ , is calculated from the linear velocity, v , through the area πr_c^2 , for capillary radius r_c , pressure, P , length, L , viscosity, η , and particle diameter, d_p .¹⁶

$$\phi = \frac{P \cdot d_p^2}{v \cdot \eta \cdot L} \quad (4)$$

For the two capillaries, the values of ϕ are 747 and 733. To compare with pressure-packed capillaries, for the nonporous 1.0 μm particles,¹⁷ $\phi=363(\pm 10)$, which is two-fold lower. The flow resistance thus indicates that the colloidal crystal is packed more densely. A quantitative indicator of close-packed structure is the porosity, which is 0.39 for pressured-packed particles¹⁸ and 0.26 for face-centered cubic crystals.¹⁹ A histogram of porosity values (Supporting Information) shows that $\varepsilon=0.25\pm 0.01$ (95% CI). This is in agreement with the porosity of face-centered cubic crystals, revealing that the entire volume of the packing is a colloidal crystal.

To determine plate height, DiI-C₁₂ was electromigrated over distances on the order of 1.5 mm at varying electric fields. The plate height was determined by fitting each peak to a Gaussian after traveling to the end of the field of view of the fluorescence microscope. Raw data for representative electrochromatograms are shown in Figure 2, covering the range of electric fields. These raw data show plate heights that are well under 1 μm . The Supporting Information provides 1) these electrochromatograms, plotted on expanded scales, and Gaussian fits, 2) raw data for injected widths at varying electric fields, which typically contribute ~ 100 nm to the plate height, and 3) a calculation showing that the detection window contributes negligibly.

The plate-height, with the contribution from injected width subtracted out, is plotted vs. migration rate in Figure 3. The velocity axis is analyte migration rate, rather than flow rate, to facilitate an independent determination of the B term. The B term was measured by

migrating the peak well into the packed capillary, and monitoring peak variance vs. time with no field. This plot (Supporting Information) shows $B=2\gamma D=18.4(\pm 0.02) \mu\text{m}^2/\text{s}$. Knowing the B term allows A as the only fitting parameter. The best-fit curve from nonlinear regression, shows that $A=0\pm 20$ nm. There is evidence of heating (Supporting Information), but this does not affect plate height, in agreement with our previous report.¹¹ This is perhaps a consequence of the high thermal conductivity of a densely packed material. At the highest electric field, a plate height of $0.13 \mu\text{m}$ is observed after correction for the contribution from injection.

One might wonder if the sub-micron plate heights are simply from using sub-micron particles. Table 1 compares the plate height, as well as the reduced plate height ($h=H/d_p$), with earlier reports for electrochromatography with colloidal silica. The plate height for the material in this paper is significantly lower in every case. A Knox plot is given in the Supporting Information. The lower values of the plate height and reduced plate height reveal that the ultrahigh efficiency is not simply due to using smaller particles, instead, more uniform packing is achieved.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Images of a silica colloidal crystal packed in a capillary. Photographs through an optical microscope for a) end-on view and b) side-on view. c) SEM image of the end-on view of a region of the capillary.

Figure 2.

Raw electrochromatograms for DiI-C₁₂ at four different electric fields, superimposed on the same time axis, without removing the contribution from injected width. The mobile phase was 90:10 MeOH:water and 0.1 % formic acid, and the stationary phase was horizontally polymerized C₁₈/C₁.

Figure 3.

Plot of plate height vs. analyte migration rate. The circles are experimental data and the solid curve is $H=2\gamma D/v$. Regression showed that $A=0\pm 20$ nm.

Table 1

Electrochromatographic plate heights for nonporous colloidal silica spheres.

Column type	d_p	H	h	Analyte	Reference
Capillary	0.50 μm	1.5 μm	3.0	Thiourea	20
Capillary	0.33 μm	0.13 μm	0.4	DiI	This paper
Slab	0.20 μm	2.4 μm	12	DiI	11
Microchannel	0.16 μm	2.2 μm	14	Proteins in SDS	12