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Obstructed Diffusion in Silica Colloidal Crystals

Benjamin J. Rogers and Mary J. Wirth*

Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, IN 47907

Abstract

The hindered diffusion in silica colloidal crystals was studied experimentally, both by fluorescence recovery after photobleaching and by measurement of ionic conductivity. Particle size was varied to include 120, 220, 470 and 1,300 nm, and the porosities were determined by flow measurements. For fluorescein, the results showed that the obstruction factor, which is the ratio of the diffusion coefficients inside the media and in open solution, is equal to the porosity within experimental error. For proteins, the same conclusion is made after correction for size exclusion of the pores. The obstruction factors for these media are two-fold lower than those measured for chromatographic media, 60% higher than theoretical predictions, and equal to what is assumed for electrophoretic sieving in random fibers.

Keywords

chromatography; pore exclusion; size exclusion; zone broadening; ionic conductivity

The understanding of diffusion through beds of packed spheres is important for designing new materials for chromatography. Diffusion in chromatography has long been known to be both a blessing and a curse: diffusion is the essential process that enables equilibrium to be established between the mobile and stationary phases, yet diffusion also causes bands to broaden. These two roles of the diffusion coefficient, D_0 , are summarized by the van Deemter equation, which describes the normalized zone variance, H , commonly called the plate height, as a function of mobile phase velocity, v .¹

$$H = A + \frac{2\gamma D_0}{v} + \omega \frac{d_p^2}{2D_0} v \quad (1)$$

The A term accounts for heterogeneity of path lengths through the medium, d_p is the particle diameter, and γ and ω are parameters that are typically determined by fitting data to Eq. 1. The equation is written for nonporous particles, for which ω accounts for the velocity distribution from Poiseuille flow. The term γ is the obstruction factor, which accounts for diffusion being slowed along the column axis by the particles. The obstruction factor is defined as the ratio of diffusion coefficients inside the medium, D , to that in open solution, D_0 .

$$\gamma = \frac{D}{D_0} \quad (2)$$

*Corresponding author: Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, IN 47907, 765-494-5328, mwirth@purdue.edu.

Knowing what controls obstruction of diffusion is useful for projecting the minimum plate height, H_{min} .

$$H_{min}=A+2d_p \sqrt{\gamma\omega} \quad (3)$$

As $A \rightarrow 0$ from improved homogeneity, the prediction of plate height requires knowing γ .

There is no clear guidance from the literature to predict γ . Fundamentally, obstruction of diffusion is related to the porosity, ε , which is the fractional free volume in the medium, and to tortuosity, τ , which accounts for extra distance that needs to be traversed in the medium.²

$$\gamma = \frac{\varepsilon}{\tau} \quad (4)$$

The value of ε can be readily measured, but τ needs to be determined experimentally.² In early work, Knox modeled tortuosity for a gas chromatography column, predicting and also determining experimentally that $\gamma=0.6$ for a porosity of 0.38.³ Recently, Richard and Striegel showed that reported values of γ range from 0.1 to 0.8, and they performed measurements that indicated $\gamma=0.7$, also for a porosity of 0.38.⁴ A review of experimental results in the chemical engineering literature shows general agreement, where γ is typically somewhat less than unity.⁵ In our previous work, we had assumed that the obstruction factor was equal to the porosity.⁶ This assumption was based on the model used to describe electrophoresis in media of random fibers.⁷ Theory of obstructed diffusion in packed beds predicts even lower values for γ . A numerical solution by Volgin *et al.* for spheres arranged as face-centered cubic crystals, where the porosity is 0.26, gives a value of $\gamma=0.16$,⁸ and the same result was obtained with a Monte Carlo simulation by Kim and Chen.⁹ Both results give good agreement with an analytic approximation by Weissberg.¹⁰ Further, the Weissberg approximation applies to higher porosities, predicting for the chromatographic conditions that $\gamma=0.3$ for $\varepsilon=0.38$. Other analytic solutions agree with Weissberg's result.⁸ In all cases, theory predicts much more obstruction than has been observed experimentally.

Chromatographic columns are known to be radially heterogeneous in packing, giving significant band broadening from the A term in Eq. 1.¹¹ The nonzero A term means that the tortuosity is non-uniform in the column, perhaps contributing to the disagreement with theory. Further, the theory used monodisperse particles, whereas chromatographic particles have a distribution of $\pm 50\%$ in particle diameter.¹² Our group recently showed that sonication gives homogeneous packing of nonporous silica spheres, resulting in an A term of Eq. 1 that is negligible.¹³ Further, these spheres are virtually monodisperse, with a size distribution of less than $\pm 3\%$.¹⁴ The purpose of this work is to measure γ and its experimental error for these homogeneous packed beds of varying, monodisperse particle diameter and varying size of the diffusing species.

Experimental section

Monodisperse silica spheres were obtained from two sources (Nanogiant, Temple, AZ; and Fibre Optics, New Bedford, MA) and were calcined by heating to 600 °C for 8 hours, followed by annealing by heating to 1050 °C for 3 hours. The surfaces were rehydroxylated under reflux in 50:50 (v/v) HNO₃:H₂O for 3 hours. 75 μ m fused silica capillaries (Polymicro, Phoenix, AZ) were conditioned by pumping 0.1 M NaOH, 18 M Ω -cm water and ethanol with a syringe pump each for 30 min at 25 μ L/min. Colloidal crystals were formed in the capillaries and then chemically modified by self assembly of methyl and n-butyl trichlorosilanes (Gelest, Morrisville, PA) using methods described previously.^{15–16}

A Thermo Accela UHPLC (Thermo-Fisher Scientific, Waltham, MA) was used to generate pressure-flow curves aided by flow splitting provided by a microfluidic Tee (Vici Valco, Houston, TX). Eluent was directly collected in a 400 μm id glass tube (Drummond Scientific Co; Broomall, PA). The volume was determined by measuring the length of liquid eluted into the tube with a Nikon SMZ 1500 Zoom Microscope (Nikon, Tokyo, Japan). HPLC grade toluene (Sigma Aldrich, St. Louis, MO) was used to measure resistance to flow in the no-slip condition.⁶ The pressure-flow curves were fit using Origin@software (Microcal, Northampton, MA).

Both sodium fluorescein and FITC-labeling kits were obtained from Sigma Aldrich (St. Louis, MO). Cytochrome C and bovine serum albumin were obtained from Sigma Aldrich (St. Louis, MO). Monoclonal antibody was donated by Eli Lilly (Indianapolis, IN). The proteins and fluorescence were all constituted in 25/75 $\text{H}_2\text{O}/\text{ACN}$ at 0.01 mg/mL.

Fluorescence recovery after photobleaching (FRAP) experiments were carried out using a Nikon Eclipse E2000 U inverted microscope (Nikon, Tokyo, Japan). After a capillary was filled with analyte, the capillary was placed between two glass cover slips, with the index matched by immersion oil. An argon ion laser (Melles Griot, Albuquerque, NM) and focused into the capillary using a 2X microscope objective (Nikon, Tokyo, Japan) was used for photobleaching. The recovery was monitored by imaging using the microscope with a halogen light source (Nikon, Tokyo, Japan) and a Cascade II CCD camera (Photometrics, Tucson, AZ). Winview@software (Photometrics, Tucson, AZ) was used to record the images as a function of time. Three replicates were performed for each measurement, wherein the capillary was rinsed and filled with fresh solution between each measurement.

To measure the conductivity across each column, packed beds were filled with HPLC grade water with 0.1% trifluoroacetic acid (Sigma Aldrich, St. Louis, MO). The capillary ends were each immersed in reservoirs made of PDMS and filled with the same HPLC grade liquid. Varying electric fields were applied using a high voltage power supply (Ultravolt, Ronkonkoma, NY), and current was recorded using National Instrument LabVIEW hardware and software (Austin, TX).

Results and discussion

Diffusion was measured by fluorescence recovery after photobleaching (FRAP). With this technique, the fluorescence intensity, F , recovers to its initial values, F_0 over time, t . The time scale for the recovery is determined by the standard deviation of the hole bleached, w , as well as the diffusion coefficient.¹⁷ The normalized fluorescence recovery follows a simple exponential function.

$$\frac{F_0 - F}{F_0} = \exp\left(-\frac{w^2 t}{D}\right) \quad (5)$$

The recovery is written for diffusion coefficient, D , and the same function, of course, describes recovery inside the medium, where D is operative. Inspection of Eq. 5 shows that the decay curves can be linearized in a more convenient form by plotting $\ln((F_0 - F)/F_0)$ vs. w^2/t .

$$\ln\left(\frac{F_0 - F}{F_0}\right) = -\frac{D}{w^2 t} \quad (6)$$

Plotted in this form, $D=w^2/t$ when $\ln((F_0-F)/F_0)=-1$, allowing easy reading of the diffusion coefficient from the graph. This new method of presenting FRAP data allows curves for all species and particle sizes to be readily compared and interpreted.

The FRAP recovery curves are presented in the linearized form in Figure 1. A line is drawn across the value of $\ln((F_0-F)/F_0)=-1.0$ to assist in reading the diffusion coefficient for each data set. The raw data show the expected trend, whereby diffusion coefficient for each species is by far the largest in open solution, and the diffusion coefficients decrease with increasing molecular weight. The diffusion coefficients are listed in Table 1, along with their standard deviations. In accord with Eq. 2, the ratios of diffusion coefficients, D/D_0 , are used to calculate the obstruction factors, γ , which are listed in Table 1. The obstruction factors are all considerably smaller than the 0.6 or 0.7 values previously reported for chromatographic media. Before interpreting these, it is important to consider hindrance to diffusion by size-exclusion due to the limited pore diameters. Size exclusion is sometimes an overwhelming effect. Monoclonal antibody was so excluded from the capillary packed with the smallest and the second smallest particles that it did not diffuse significantly enough into the capillary to allow a diffusion measurement. In each of these cases, "NA" is entered in Table 1. The strong exclusion demonstrates that not all of the fluid volume in the medium is accessible to the diffusing species. To interpret the obstruction factors, size exclusion must be considered for all of the species. The Stokes radius is calculated from the friction coefficient, f , which is related to the diffusion coefficient in the open capillary by $D=kT/f$, where D is the measured diffusion coefficient for the open capillary.

$$f=6\pi\eta R \quad (7)$$

The Stokes radius for each of the species is included in Table 1. These range from 1.0 to 5.2 nm. Next, the effective pore size of the media must be determined by flow rate measurements.

Figure 2 shows plots of flow rate vs. pressure for each particle size. To avoid slip flow, the particles had been chemically modified with hydrocarbon and the fluid was toluene. The viscosity of toluene depends on pressure,¹⁸ and the flow rate is normalized for the pressure-dependent viscosity to maintain linearity. The lines represent least-squares fits to the Kozeny-Carman relation of Eq. 8 to determine the porosity.

$$\frac{P}{L} = \frac{180 \cdot \nu \cdot \eta}{d_p^2} \frac{(1-\epsilon)^2}{\epsilon^3} \quad (8)$$

In this equation, P , L , d_p , and η are pressure, bed length, particle diameter, and viscosity, respectively. The values of the porosity recovered from the linear regressions are listed in Table 2. The data indicate that the smallest two particle diameters, 120 nm and 220 nm, each have a porosity of 0.26, which is indicative of face-centered cubic packing. The larger particle diameters, 470 and 1,300 nm have higher porosities, but these are still less than the value of 0.38 that one would have for dense random packing.¹⁹

We define the size exclusion factor, ϵ^* , to be the fraction of the liquid volume that is accessible to an analyte. The functional form of ϵ^* has been derived for size-exclusion chromatography by Giddings,²⁰ where the pore radius of the material is r and the diffusing species is a sphere of radius R .

$$\varepsilon^* = \left(\frac{r-R}{r} \right)^2 \quad (9)$$

To calculate the size-exclusion factor of Eq. 9, we use the Stokes radius for R . The Stokes radius treats the molecule as spherical, which is reasonable for the proteins, but might not be sufficiently accurate for fluorescein in anisotropic media, such as the regions where spherical particles meet. For r , we use the hydraulic radius, r_{hyd} , which is the radius of an open capillary having the same resistance to flow as the packed medium.²

$$r_{hyd} = \frac{d_p}{3} \frac{\varepsilon}{(1-\varepsilon)} \quad (10)$$

The hydraulic radius corresponding to each particle diameter is listed in Table 2. These range from 14 nm for the smallest particle size to over 200 nm for the largest particle size. Based on Eqs. 8–10, the calculated size exclusion factors are listed in Table 2.

The size exclusion factor is smallest for the largest species, the monoclonal antibody, and approaches unity for fluorescein, which is the expected trend. The exclusion factor is virtually unity for all species in the capillaries packed with the 1,300 nm particles. To assess how well ε^* corrects for size, the relation for γ is re-stated to include the size exclusion factor.

$$\gamma = \frac{\varepsilon \cdot \varepsilon^*}{\tau} \quad (11)$$

Figure 3 shows a plot of $\varepsilon \cdot \varepsilon^*$ vs. D/D_0 for the three proteins, which Eq. 11 predicts to be linear with a slope of $1/\tau$ if the ε^* is a valid correction. The error bars represent standard deviations. The data follow the dashed line, which is drawn from $\varepsilon^*=0$ to the points for which $\varepsilon^*=1$, the latter of which corresponds to the data points having the highest values of D/D_0 . The data closely overlay for the points that represent no obstruction, *i.e.*, for the 1,300 nm particles. For the smaller particle sizes, there is more than random scatter from the line, but the scatter is not more than two standard deviations. The graph also illustrates what would happen without the correction factor of ε^* : the data do not reasonably extrapolate to zero. The data for Figure 3 thus show that the correction factor is imperfect but has the correct functionality. Finally, we can now interpret the slope of the plot in Figure 3, which is $1/\tau$, and the slope is unity within experimental error. This indicates that $\gamma \sim \varepsilon \cdot \varepsilon^*$ for these packed media.

The obstruction factor, even with size-exclusion, is distinctly higher than predicted by theory for face-centered cubic crystals. A confirmatory experiment is performed, based on the idea that theory equates the obstruction factor for diffusion to that for conductivity, as discussed nicely by Volgin *et al.*⁸ The equation for obstructed conductivity by Maxwell²¹ gives results virtually identical to the equation for obstructed diffusion by Weissberg¹⁰. Delgado demonstrated that obstruction of diffusion could be measured simply by measuring the ionic conductivity of the packed medium compared to that for an open column.²² Following Delgado, we measured ionic current for the same capillaries that were used in the diffusion experiments, where trifluoroacetic acid was included in the liquid to provide protons as charge carriers. The ratios of current, i , in the packed capillaries relative to the current, i_0 , in the open capillaries for the various particle diameters are listed in Table 3. Also listed for convenient comparison are the obstruction factors for fluorescein and the porosities measured by flow. The data show that porosity, obstruction factor and current

ratio are all similar. To facilitate a comparison of these three parameters, a bar graph with standard deviations for each measurement is given in Figure 4. One can see from the bar graph that the measurement of γ by diffusion and by current ratio agree, and they both equal the porosity within experimental error. In short, in the absence of size exclusion the data show that $\gamma \approx \epsilon$.

This notion that $\gamma \approx \epsilon$ agrees with what has been assumed in electrophoresis for random fibers.²³ The obstruction factor is approximately two-fold lower than for chromatographic media. This is not a disagreement *per se*, it is likely a consequence of these media being more homogeneous. The disagreement with theory for hindered diffusion in colloidal crystals is puzzling. It is possibly a consequence of the structure of the colloidal crystals being imperfect. The crystalline domains of the packed capillary are tiny, and the domains are randomly arranged, as indicated by the microscopic color variations shown in an image in earlier work.¹⁵ Slight gaps at the domain boundaries could provide gaps that speed diffusion. If this were the case, it remains difficult to explain why these results disagree with the theory of Weissberg, which does not require structural order. It is possible that the theory does not account for the composite structure, where ordered domains are randomly arranged.

The results of this work indicate that approximately a two-fold smaller value for γ can be obtained experimentally, compared to what has previously been reported for chromatographic media. The lower obstruction factor would contribute to a lower plate height by a factor of approximate 2ly. Based on theory, an additional factor of 2 might be gained if the ideal colloidal crystalline structure could be approached.

Acknowledgments

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Figure 1.

FRAP data, with the $1/e$ point indicated for each experiment by the horizontal line. Data are for four fluorophores: A) fluorescein, B) cytochrome c, C) BSA and D) a monoclonal. For each species, a FRAP decay curve is shown for the each particle diameter, including open solution ($\square$), 1300 nm ($\blacklozenge$), 470 nm ($\times$), 220 nm ($\circ$) and 120 nm ($\triangle$).

Figure 2. Pressure vs. flow rate of toluene in nL/min, normalized by viscosity in cP for varying particle diameter: A) 120 nm B) 220 nm C) 470 nm D) 1300 nm.

Figure 3.
Plot of hindrance factor vs. diffusion based obstruction factor for porosity alone ($\circ$) and porosity and pore hindrance ($\bullet$).

Figure 4.
Bar graph to compare porosity (ϵ), obstruction factor (D/D_0) and current ratio (i/i_0).

Table 1

Measured transport properties for each particle diameter, d_p : diffusion coefficient, Stokes radii, and obstruction factor, γ .

d_p (nm)	Diffusion coefficient ($\mu\text{m}^2/\text{s}$)			
	Fluor	Cyt c	BSA	mAb
120	66.1 $\pm$ 1.2	24.1 $\pm$ 0.4	10.2 $\pm$ 0.05	N/A
220	68.3 $\pm$ 1.6	27.6 $\pm$ 0.9	11.8 $\pm$ 1.2	N/A
470	71.3 $\pm$ 1.3	30.7 $\pm$ 0.7	16.3 $\pm$ 0.5	9.8 $\pm$ 0.3
1300	84.6 $\pm$ 5.1	39.5 $\pm$ 1.1	24.8 $\pm$ 0.9	18.1 $\pm$ 0.5
open	260.4 $\pm$ 19.5	119.8 $\pm$ 3.5	77.5 $\pm$ 0.8	53.3 $\pm$ 1.1
Stokes radius (nm):				
	1.03 $\pm$ 0.07	2.23 $\pm$ 0.07	3.4 $\pm$ 0.04	5.20 $\pm$ 0.11
d_p (nm)	γ			
120	0.254 $\pm$ 0.019	0.201 $\pm$ 0.006	0.13 $\pm$ 0.0052	N/A
220	0.262 $\pm$ 0.008	0.230 $\pm$ 0.008	0.172 $\pm$ 0.013	N/A
470	0.273 $\pm$ 0.009	0.256 $\pm$ 0.010	0.211 $\pm$ 0.009	0.185 $\pm$ 0.016
1300	0.325 $\pm$ 0.020	0.330 $\pm$ 0.012	0.320 $\pm$ 0.05	0.322 $\pm$ 0.030
open	1	1	1	1

Table 2

Porosity (ϵ), hydraulic radius (r_{hyd}), and pore hindrance of diffusion (ϵ^*)

d_p (nm)	ϵ^*					
	ϵ	r_{hyd} (nm)	Fluor	Cyt c	BSA	mAb
120	0.260 $\pm$ 0.013	14.0 $\pm$ 0.7	0.86 $\pm$ 0.07	0.71 $\pm$ 0.04	0.57 $\pm$ 0.02	N/A
220	0.260 $\pm$ 0.010	26.0 $\pm$ 1.0	0.92 $\pm$ 0.07	0.84 $\pm$ 0.04	0.76 $\pm$ 0.03	N/A
470	0.285 $\pm$ 0.017	59.0 $\pm$ 3.5	0.97 $\pm$ 0.09	0.93 $\pm$ 0.06	0.89 $\pm$ 0.05	0.83 $\pm$ 0.05
1300	0.335 $\pm$ 0.014	213 $\pm$ 8.9	0.99 $\pm$ 0.08	0.98 $\pm$ 0.05	0.97 $\pm$ 0.04	0.95 $\pm$ 0.04

Table 3

Current ratio, i/i_0 , and its comparison to porosity, ϵ , and the fluorescein diffusion coefficient ratio, D/D_0

d_p	i/i_0	D/D_0 (Fluor)	ϵ
120	0.242 ± 0.006	0.254 ± 0.019	0.260 ± 0.013
220	0.243 ± 0.007	0.262 ± 0.008	0.260 ± 0.010
470	0.279 ± 0.001	0.273 ± 0.009	0.285 ± 0.017
1300	0.317 ± 0.013	0.325 ± 0.020	0.335 ± 0.014