

Characterization of Amorphous Silica Supports for Reversed Phase HPLC

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Abstract

We have measured physical characteristics and metals content for a variety of silicas and chromatographically compared their relative levels of base deactivation after bonding monomeric alkyl stationary phases to them. Some aggressive base deactivation procedures significantly altered the characteristics of the silica, particularly pore size and surface area. Some base deactivated silica can provide superior chromatography for peak shape over type B materials. This study attempts to correlate the physical and chemical properties of different silica varieties with their level of base deactivation.

Introduction

- Reversed Phase HPLC columns are manufactured from a wide range of silicas that vary in many chemical and physical properties. Differences in the properties of the underlying silica may have a major influence on the resulting chromatographic properties of the finished column. Important physical properties include particle size distribution, pore size distribution, pore volume, and the surface area. An important chemical property is the concentration of metals.

Introduction

- The first generation of HPLC silica was type A made from inorganic sols. The second generation includes base deactivated silica prepared by various chemical treatments of type A silica to remove surface metals. Metal impurities in silica are commonly known to have a detrimental effect on peak shape of basic analytes. It has been theorized that at least some of the improvement in base deactivation obtained following chemical removal of metals may be due to the resulting physical changes of the silica (i.e. pore shaping).

Introduction

- The latest and third generation of silica is high purity, Type B silica, synthesized from an organic sol. Type B silica contains extremely low concentrations of metals and has a major advantage over earlier generations of silica for some applications.

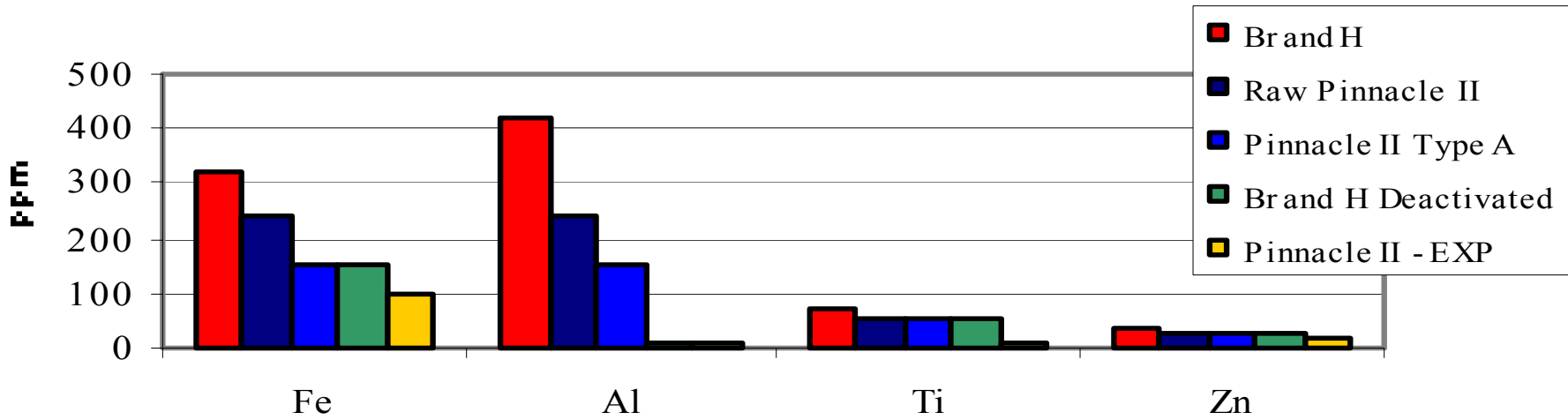
Measured Silica Properties

- Metals Content (ppm)
- Mean particle size and distribution (μm)
- Total pore volume (ml/g) with average pore size (A°) and distribution
- Mean surface area (m^2/g)

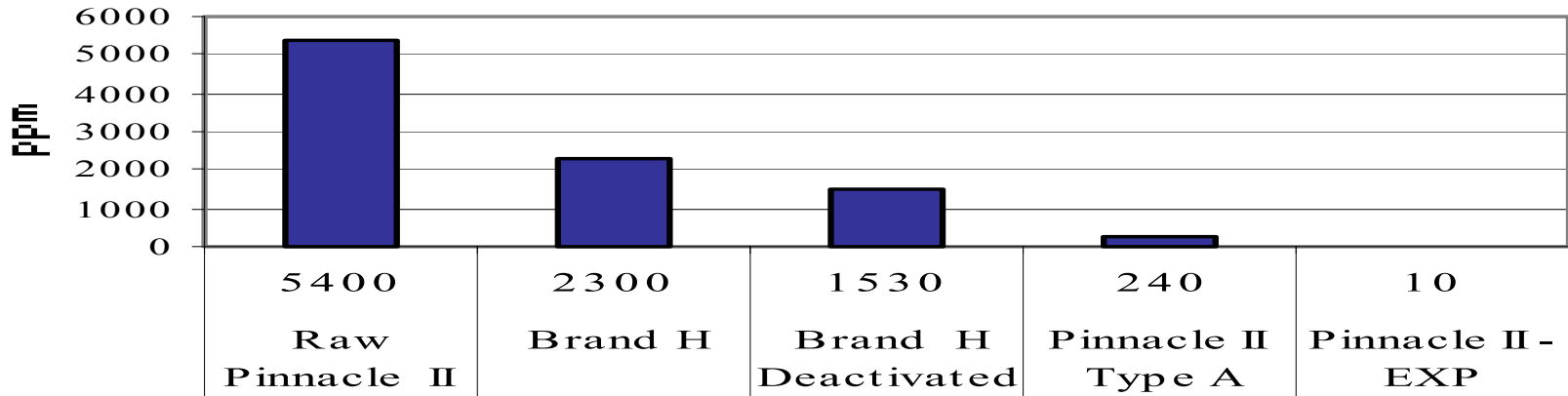
Silica Metals Content

- Silica derived from inorganic sources (Type A) must undergo metals removal procedures to create usable materials for HPLC chromatography.
- Refined metal removal processes using the type A silica can create a base deactivated silica.
- Type B silica is derived from “metal free” organic sols. Metals content is due to post-particle contamination.
- Certain processes can produce a base deactivated silica that rival type B for metals content.

Metals content of silica derived from Inorganic Sols

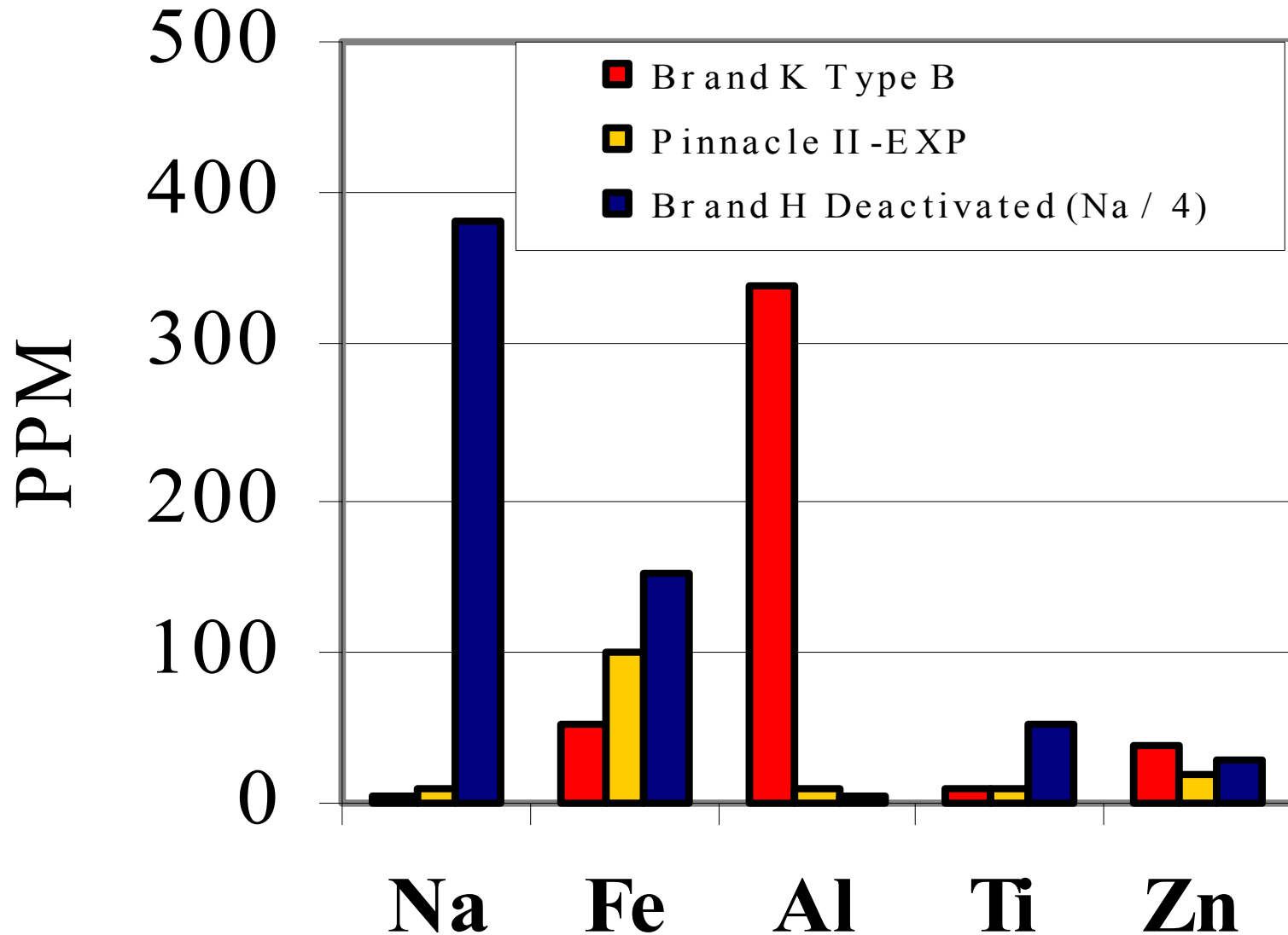


Sodium Content



Raw, Type A, and Base Deactivated Materials

Base Deactivated Silica vs Type B Silica



Pyridine/Phenol Test for Base Deactivation

Column Dimensions: 150x4.6mm, 5 μ m

Mobile Phase: 80/20 20mM K_xPO₄, pH 7.0/Acetonitrile

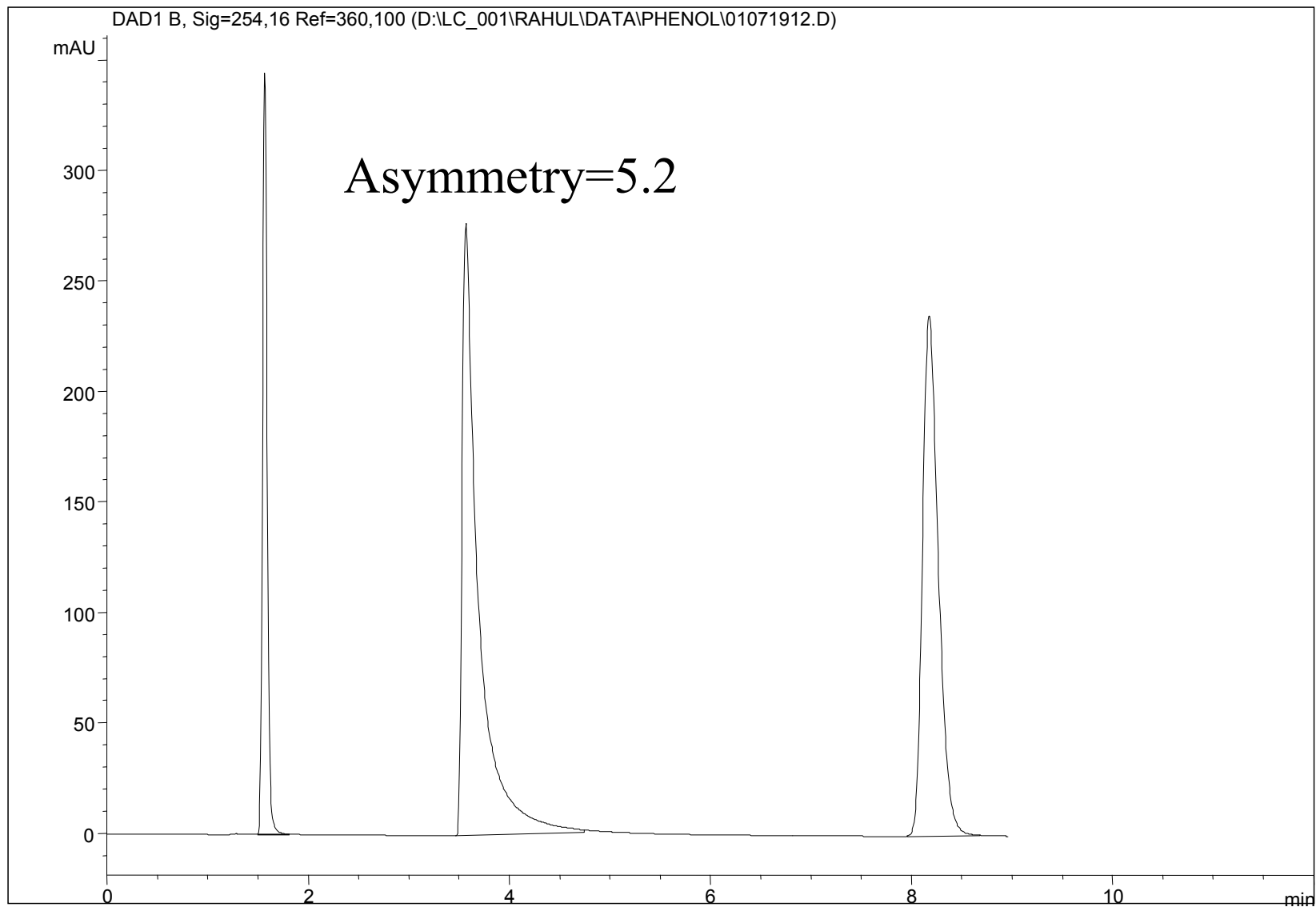
Flow rate: 1ml/min

Detector: UV @ 254 nm

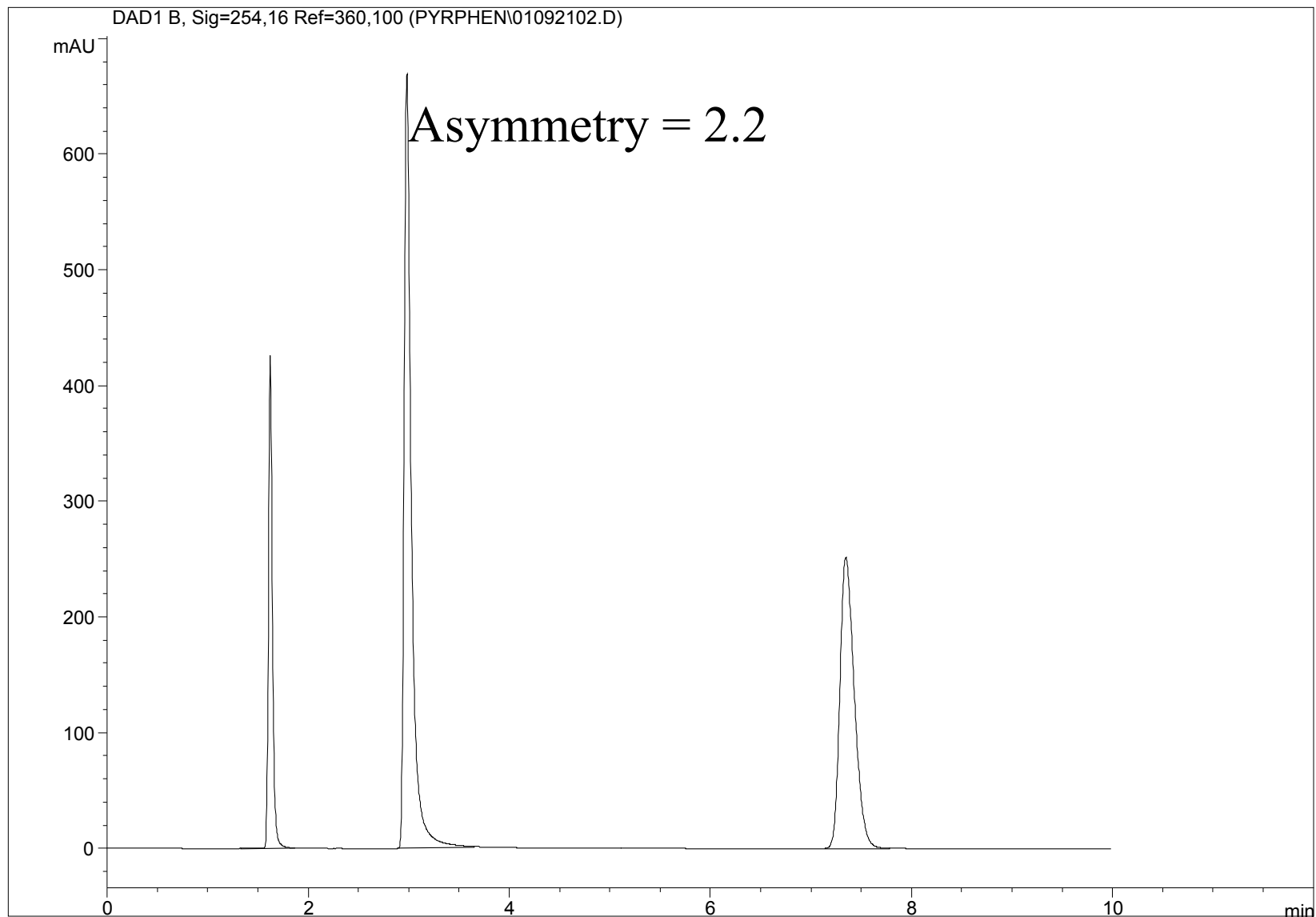
Injection volume: 5 μ l

Peaks: 1. Uracil
2. Pyridine
3. Phenol

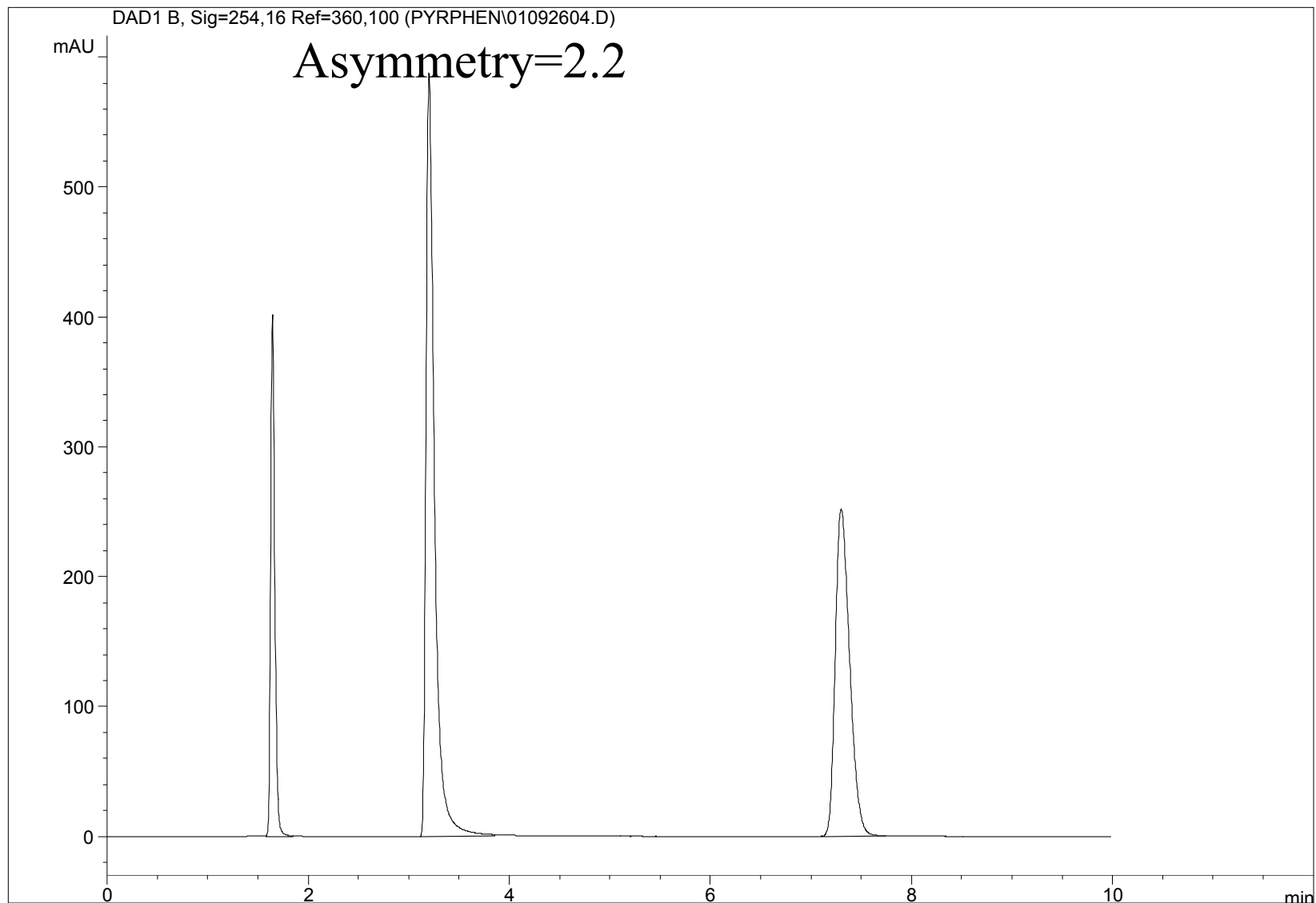
Type A with C18 Bonding



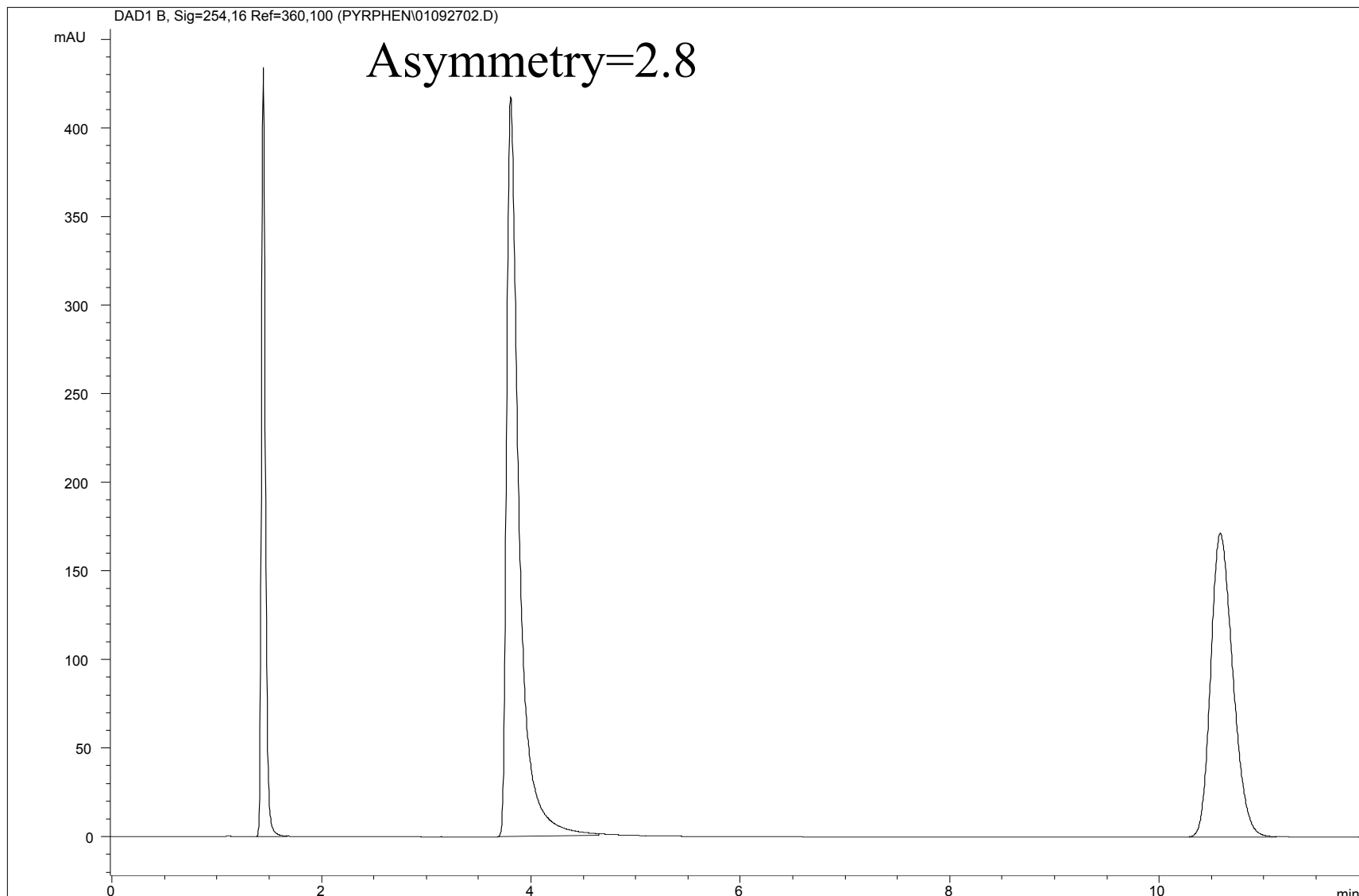
Brand H – Deactivated with C18 Bonding



Pinnacle II-Exp with C18 Bonding



Brand K – Type B with C18 Bonding

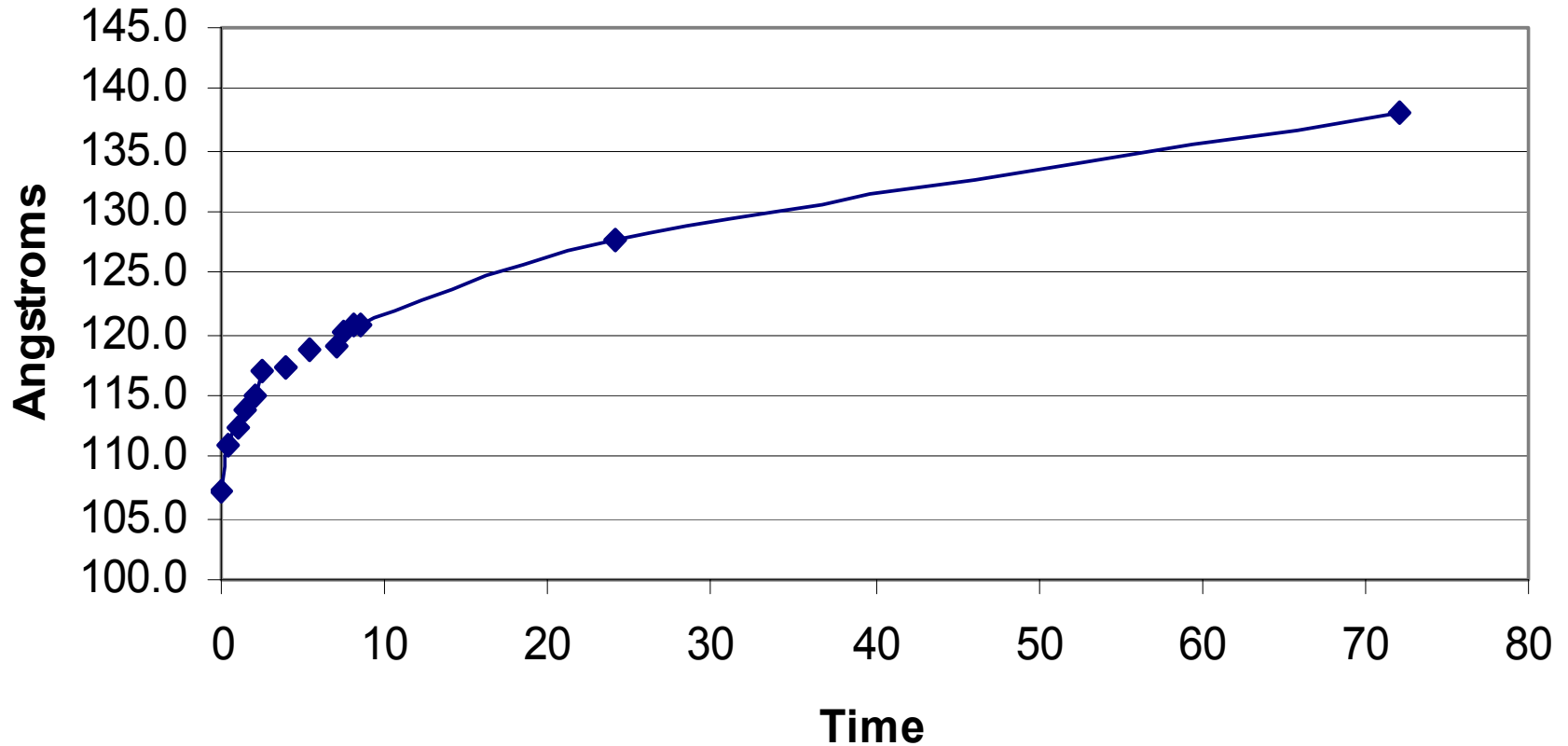


Base Deactivation Treatments

- Over time the metal extraction treatments enlarge the pore size of the silica.
- An increase in the pore size creates an increase in the pore volume. This increases the void volume.
- As pore size increases for the silica particle surface area decreases.
- The loss in surface area reduces the maximum amount of phase that can be placed onto the silica surface relative to the parent material.
- Treatment and synthesis routes also effect the surface of the particle.

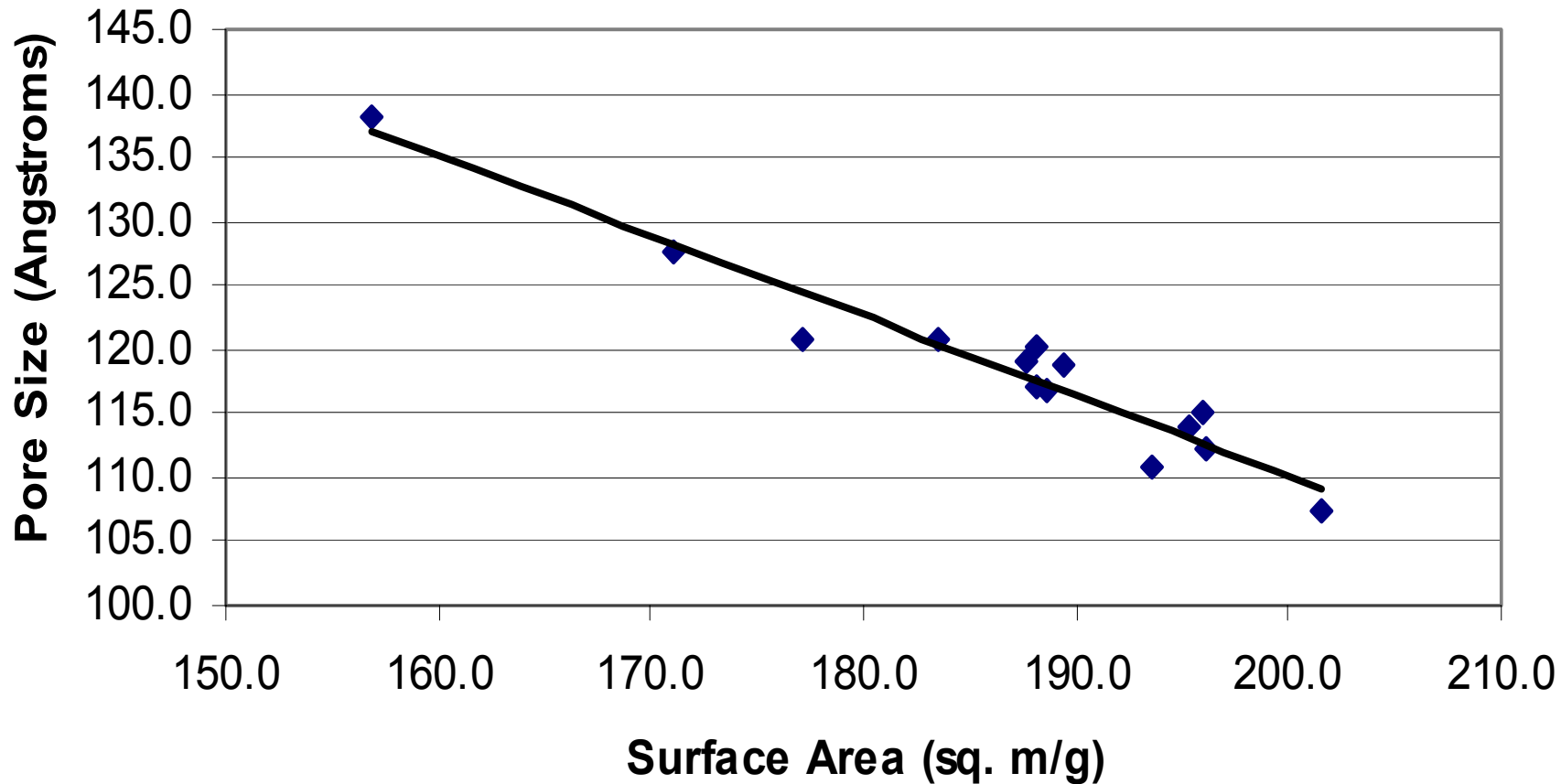
Base Deactivation Treatments

Mean Pore Size Increase vs Time



Base Deactivation Treatments

Mean Pore Size vs Surface Area



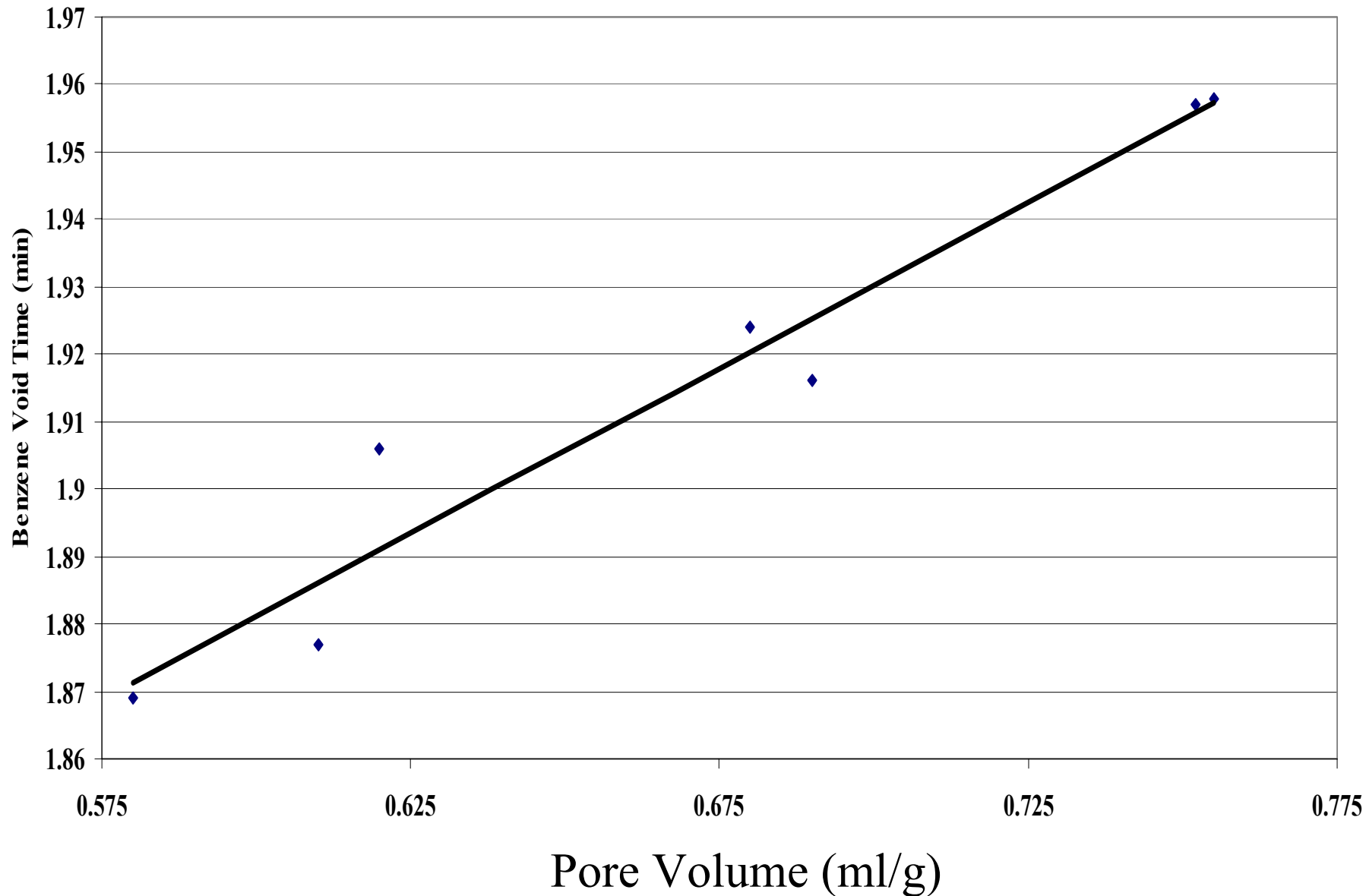
Silica Pore Volume vs Retention

- As the pore volume of a silica particle increases, the surface area decreases.
- Increased pore volume creates a linear increase in V_0 . Unretained components take longer to elute from the column.
- An increase in pore volume will decrease k' though the retention of retained components may appear to be the same.

Pore Volume vs Void Time

$$y = 0.4915x + 1.5862$$

$$R^2 = 0.9434$$



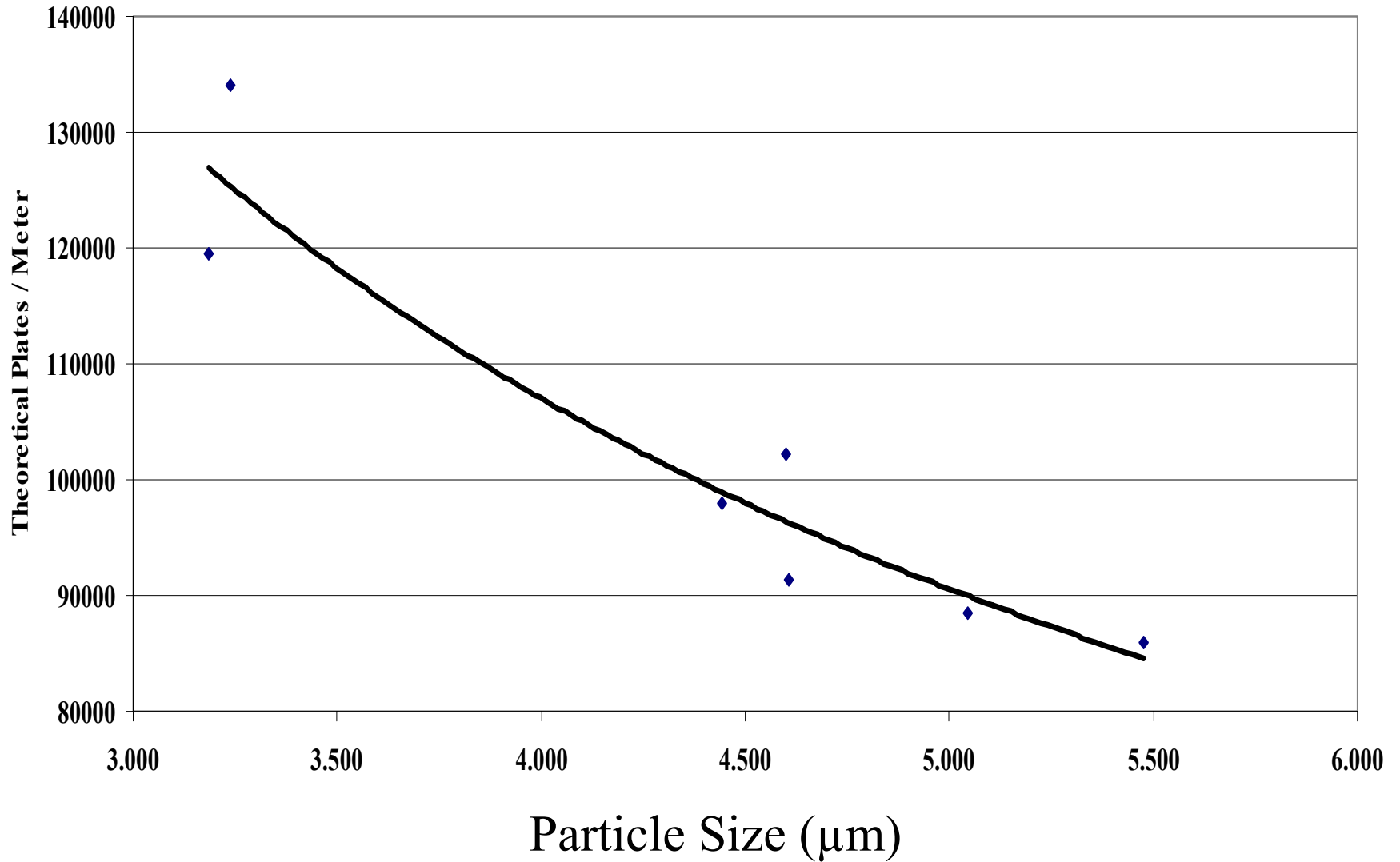
Particle Size and Distribution

- Silica for analytical chromatography is typically available in 3, 5, and 10 μm particles sizes.
- The mean of these particle sizes and the distribution ranges vary between manufactures.
- The smaller the particle size the higher the number of theoretical plates a column can produce.
- Typically columns produced with smaller particles give greater theoretical plates at the cost of higher pressures.
- When using silica of the same pore volume, smaller particles produce a linear increase in k' .

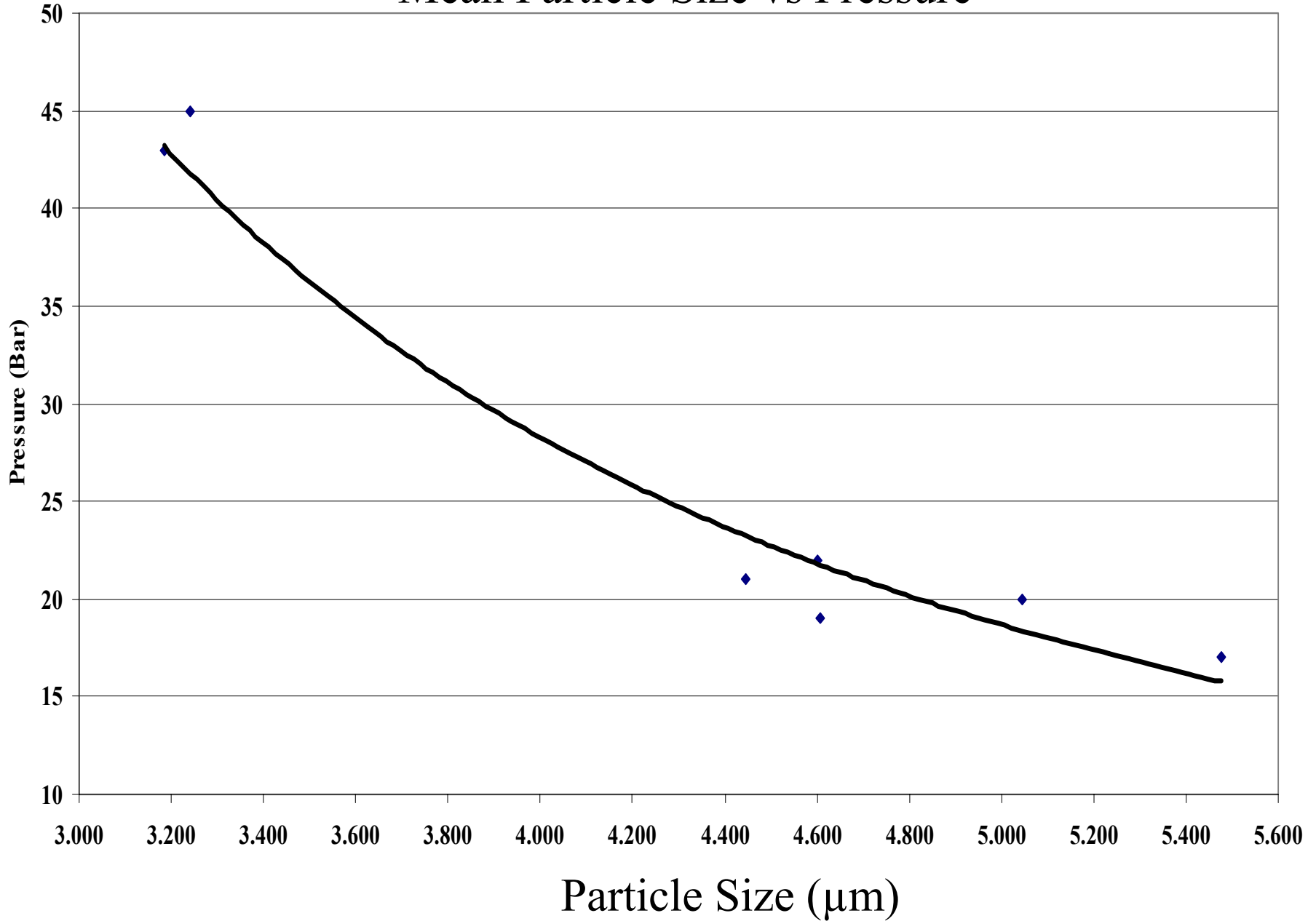
Particle Size and Distribution

- Typically a 3 μm particle can produce 135,000 plates/meter while a 5 μm particle can produce 85,000 plates/meter minimum.
- The pressure increases exponentially when a smaller particle size is used.

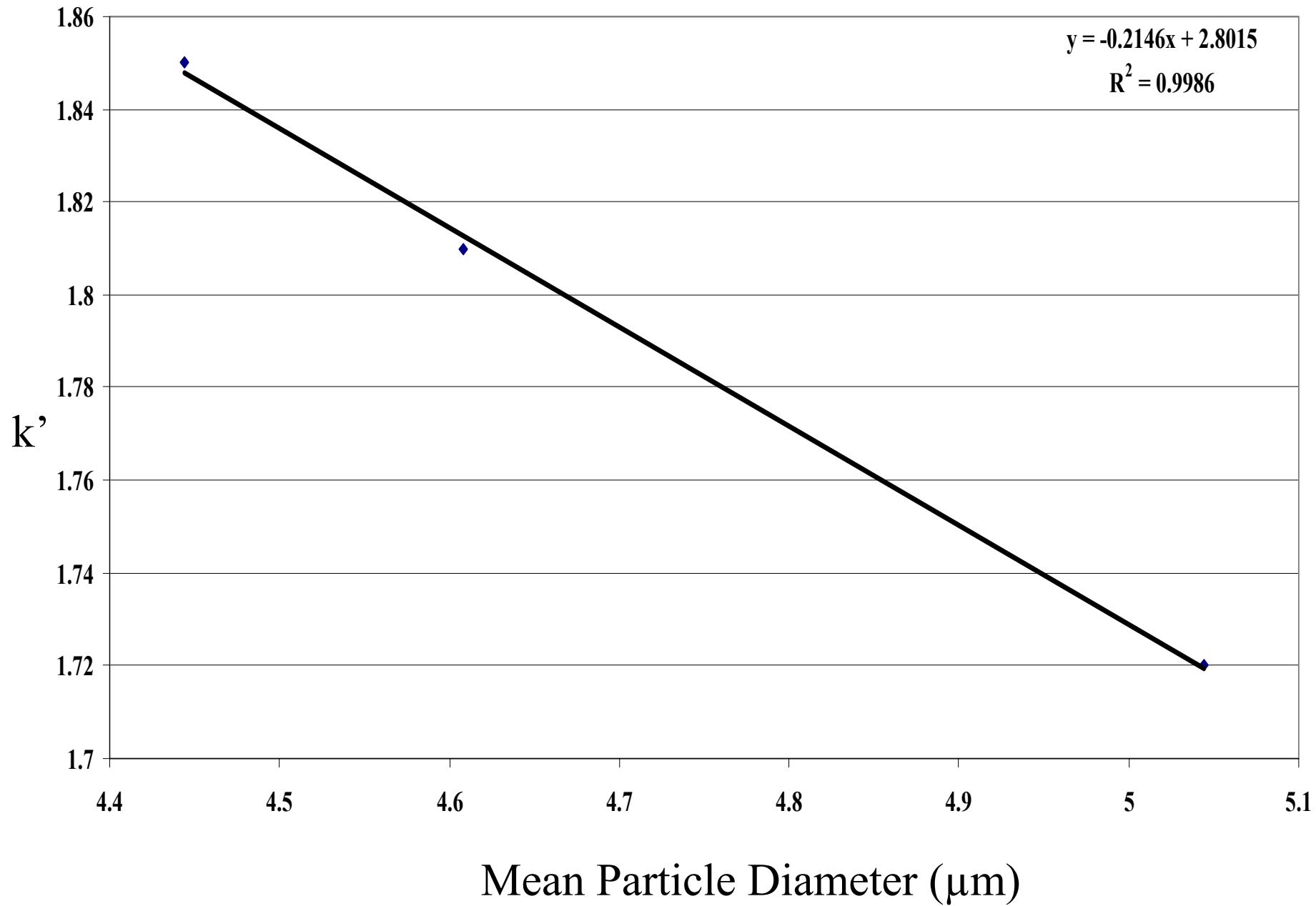
Theoretical Plates/Meter vs Particle Size



Mean Particle Size vs Pressure



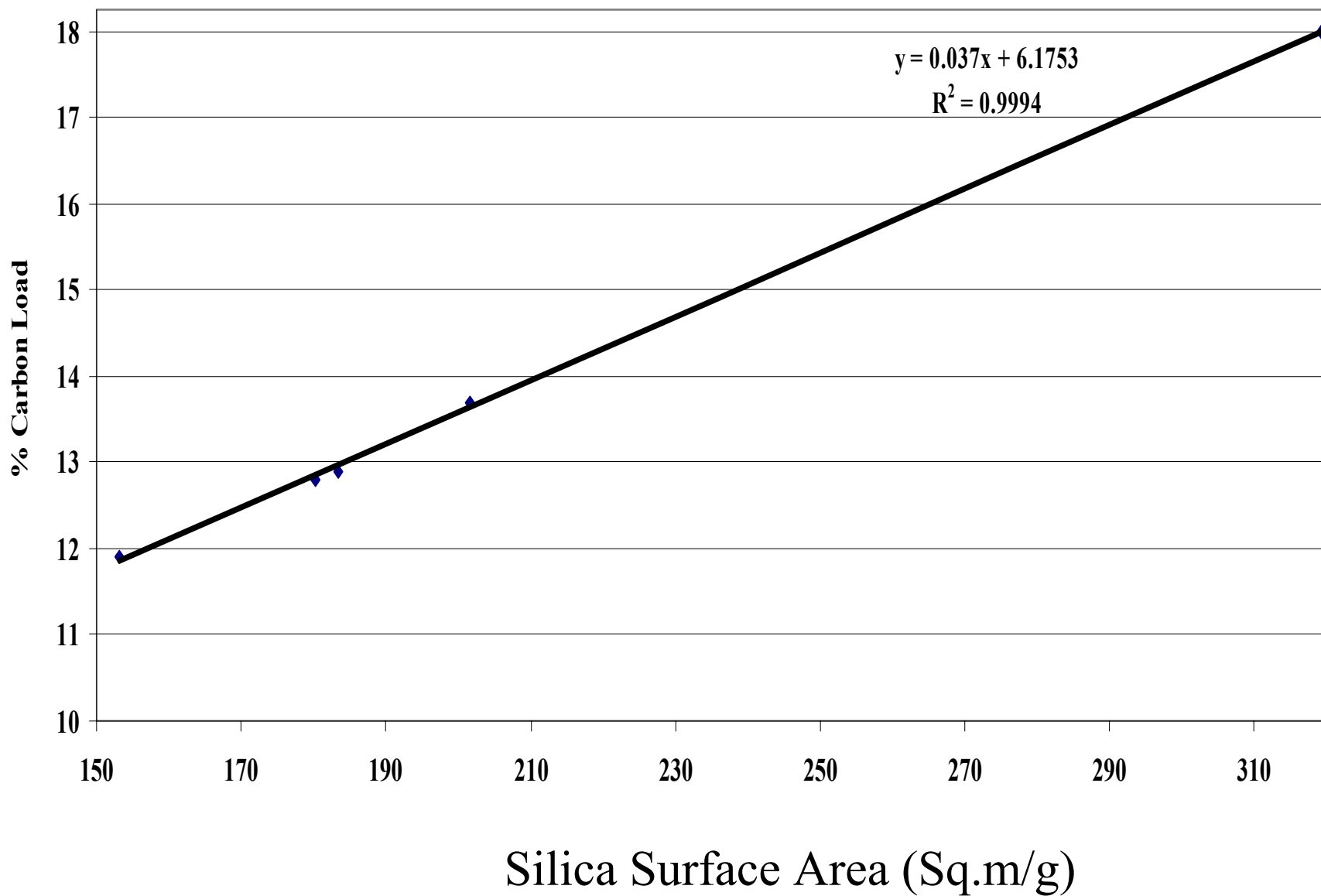
Mean Particle Diameter vs k'



Silica Surface Area

- The surface area of the silica is one of the factors limiting carbon loading of a particular phase (as C18).
- The lower the surface area, the lower the carbon load. Carbon load is directly proportional to surface area if bonding technique provides consistent surface coverage.
- Increases/Losses in carbon load will cause a shift in the k' of analytes.

Silica Surface Area vs Maximum C-18 Loading



Conclusions

- No two silica support lots are exactly the same.
- Variations may be great from manufacturer to manufacturer.
- Simply recommending a column to perform a legacy separation based on phase type (C-8, C-18, etc) is not enough.
- These factors are very important when selecting alternate columns for methods under development or making substitutions.
 - Particle Surface Area
 - Particle Pore Volume
 - Mean particle size and Distribution
 - Mean Particle Pore Size
 - Metals content

Conclusions

- Some base deactivated silicas can provide better peak shape than type B materials.
- Base deactivated silica typically has lower surface area than type B. This results in a lower carbon load for the material.
- To find a phase that will give similar retention characteristics, carbon loading and surface area must be compared.

Acknowledgments

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