Expanding the Capacity and Performance of Flash Chromatography with Spherical Silica

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Abstract

Modern flash chromatography requires high throughput capabilities that are not currently met by the use of cartridges packed with irregular silica. Cartridges packed with spherical silica provide higher functional capacity, better efficiency, decreased elution volumes, and reduced fraction sizes. This study compares the performance between commercially available cartridges packed with irregular silica and cartridges packed with spherical silica.

Spherical silica has a higher functional capacity; allowing the use of smaller cartridges. The use of smaller cartridges decreases mobile phase usage. Spherical silica has increased efficiency; providing sharper chromatographic peaks and reduced run times. Sharper peaks allow the collection of smaller fractions. Smaller fractions decrease the amount of solvent that must be evaporated. Flash chromatography cartridges packed with spherical silica offer better performance and cost savings.

Introduction

As more chemists spend an increasing portion of their time doing sample preparation and purification, the need for faster, cleaner, and more efficient flash chromatography increases. Many medicinal chemists spend more than 30 percent of their time doing compound purification. Increasing separation efficiency and functional capacity while decreasing separation time will greatly impact throughput in drug discovery labs.

The original flash chromatography work published by Still, et al, used 40-63 µm irregular particle silica gel. VersaPak® flash chromatography cartridges use 20-45 µm spherical silica gel. There are numerous advantages to using small spherical particles. Some of these include higher efficiency, higher functional capacity, and decreased backpressure.

Introduction (contd.)

These advantages translate into several benefits for the researcher including being able to use smaller cartridges, decreasing the fraction volume and total volume of solvent used per run, or decreasing run time by using higher flow rates. These advantages are most evident for difficult separations

Chromatographic efficiency is a measure of peak shape and retention. Multiple factors affect efficiency:

- Bed geometry
- Uniformity of packing
- Extra-column effects
- Particle size
- Particle shape

Introduction (contd.)

The salient factors here are particle size and shape. Flash cartridges packed with small, spherical silica are much more efficient than those packed with larger irregular silica particles.

Spherical particles provide increased efficiency because they pack uniformly and consistently, have less fines, are more stable, and have higher bulk density.

Total cartridge capacity is determined by the effective surface area of the packing material. Effective surface area can be defined as the surface of the packing that can be accessed by analytes. The higher the effective surface area of the packing, the more analyte can be retained by the cartridge. Total cartridge capacity is measured using a loading study as described.

Introduction (contd.)

The total capacity of a cartridge does not completely characterize the capacity in real applications. The ability of the cartridge to separate analytes must also be considered. Functional capacity can be defined as the greatest amount of analyte that can be loaded while maintaining adequate resolution.

Experimental

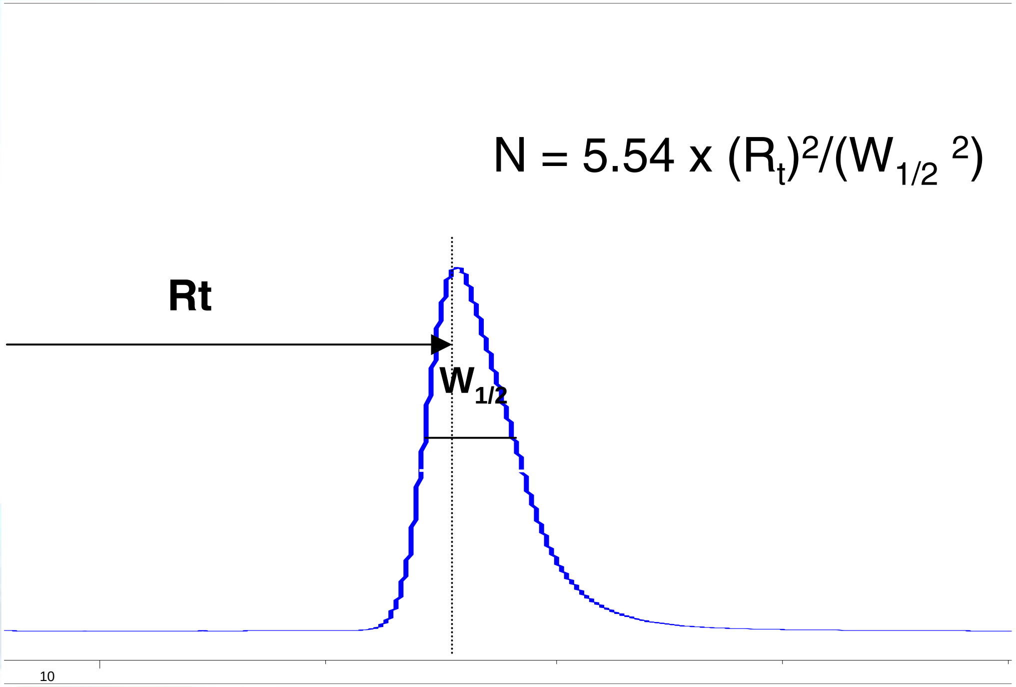
Efficiency

Cartridge efficiency was determined chromatographically using the conditions set forth in Table 1. Cartridge efficiency was calculated using the method described in Figure 1.

Table 1. Chromatographic Conditions

pump:	SciLog ACCU single piston variable stroke volume and rate
mobile phase:	960:39:1, hexane:isopropanol:water
flow rate:	20 mL/min.
det.:	254 nm UV
injection:	1 mL on-column injection
sample:	200 mg toluene:3.75 mg 2,6-dinitrotoluene/mL mobile phase
detector:	Linear UVIS-201 absorbance detector with variable path length prep cell

Figure 1. Theoretical Determination of Cartridge Efficiency



Units of efficiency are "theoretical plates."

$W_{1/2}$ =width of peak at half height in seconds.

R_t= retention time

Capacity

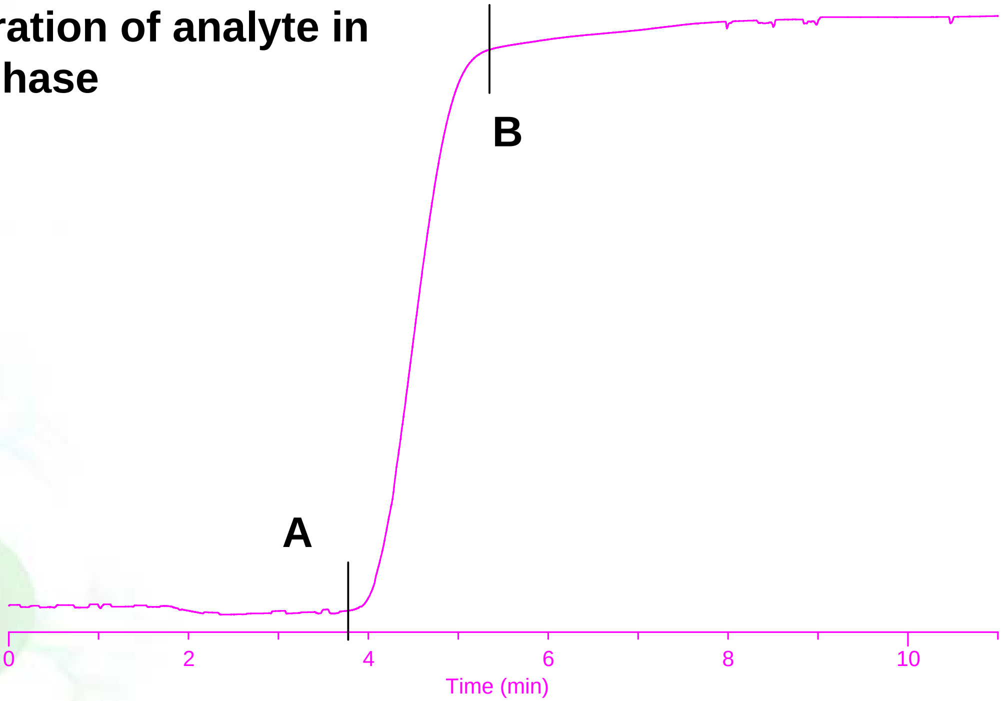
Total cartridge capacity was determined objectively using a loading study. After equilibrating each cartridge with a mobile phase of 80:20 hexane:ethyl acetate, the mobile phase was switched to one of the same composition containing a known concentration of 2,6-dinitrotoluene. The run was started simultaneously with the switch. The total capacity of the cartridge was reached when the analyte broke through the cartridge and was seen by the detector.

Functional capacity was determined by choosing analytes that were barely resolve using a silica gel TLC plate and determining the ability of the flash cartridges to resolve the compounds. Methyl 4-hydroxybenzoate (MHB) and n-propyl p-hydroxybenzoate (PHB) were chosen. TLC was done on Merck Silica Gel 60 F_{254} 2.5 x 7.5 cm with 250 µm layer thickness. Separations on cartridges packed with small spherical silica and larger irregular silica TLC plates were optimized using 80:20 hexane:ethyl acetate mobile phase. The chromatographic parameters are given in Table 2.

Figure 2. Theoretical Determination of Total Cartridge Capacity

Capacity = capacity factor x time to point A

Capacity factor = flow rate x concentration of analyte in mobile phase



The time from point A to point B represents the mass transfer time. Shorter mass transfer times result in sharper peaks and lower solvent consumption

Table 2. Chromatographic Conditions for Capacity Determination

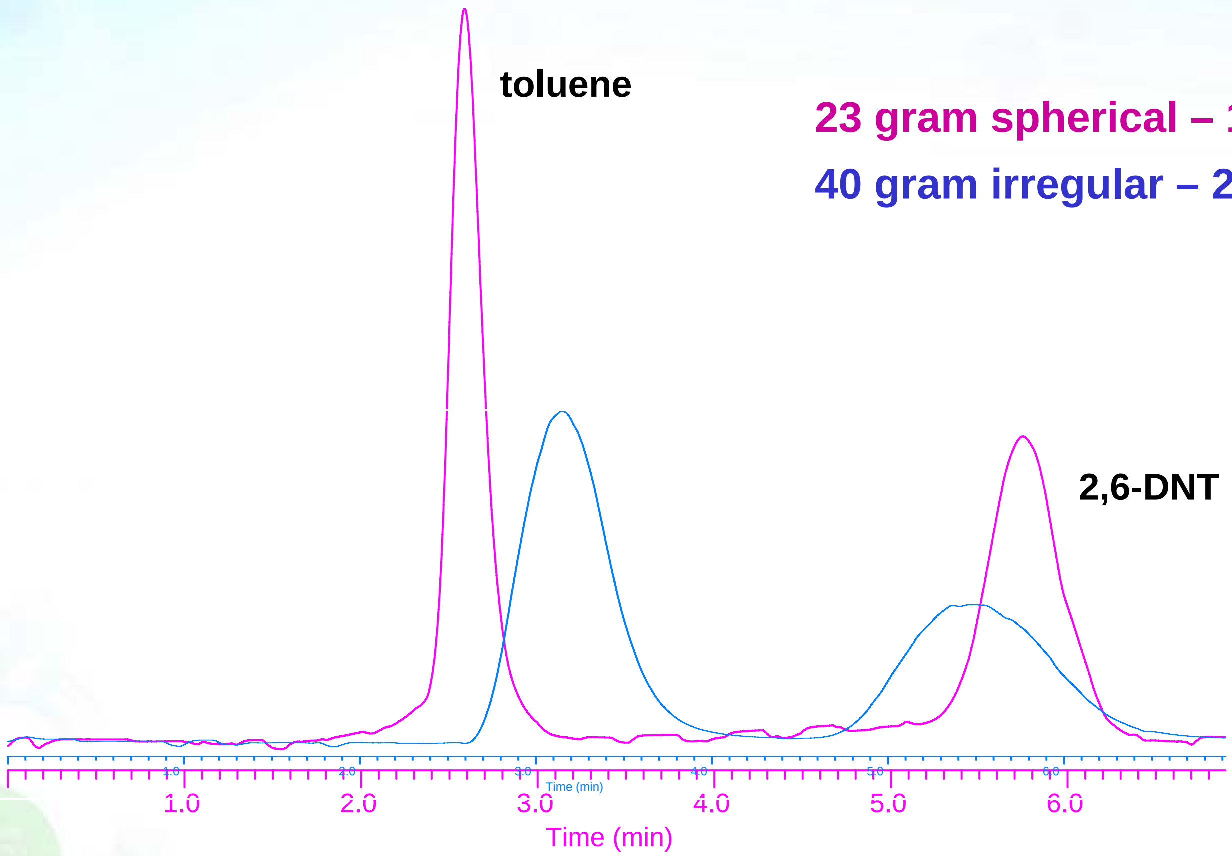
pump:	SciLog ACCU single piston variable stroke volume and rate
mobile phase:	80:20 hexane:ethyl acetate
flow rate:	20 mL/min.
det.:	254 nm UV
injection:	various volumes injected directly on the cartridge
sample:	methyl 4-hydroxybenzoate and n-propyl p-hydroxybenzoate dissolved in mobile phase
detector:	Linear UVIS-201 absorbance detector with variable path length prep cell

Results

Cartridge efficiency for cartridges packed with 20-45 µm spherical silica particles averaged more than two times higher than for cartridges packed with larger, irregular silica particles. Average fraction volume for the 2,6-dinitrotoluene peak from the cartridge packed with 20-45 µm spherical silica particles was 16.95 mL while the average fraction volume from cartridges packed with larger, irregular silica was 34.80 mL. Total solvent use per run was nearly 30% less (93 mL for spherical and 129 mL for irregular) for cartridges packed with small spherical silica.

The total capacity of cartridges packed with 23 grams of 20-45 µm spherical silica is lower than that for cartridges packed with 40 grams of irregular silica. However, when processing difficult samples, the greater efficiency of the spherical silica allows both higher loading and shorter run times.

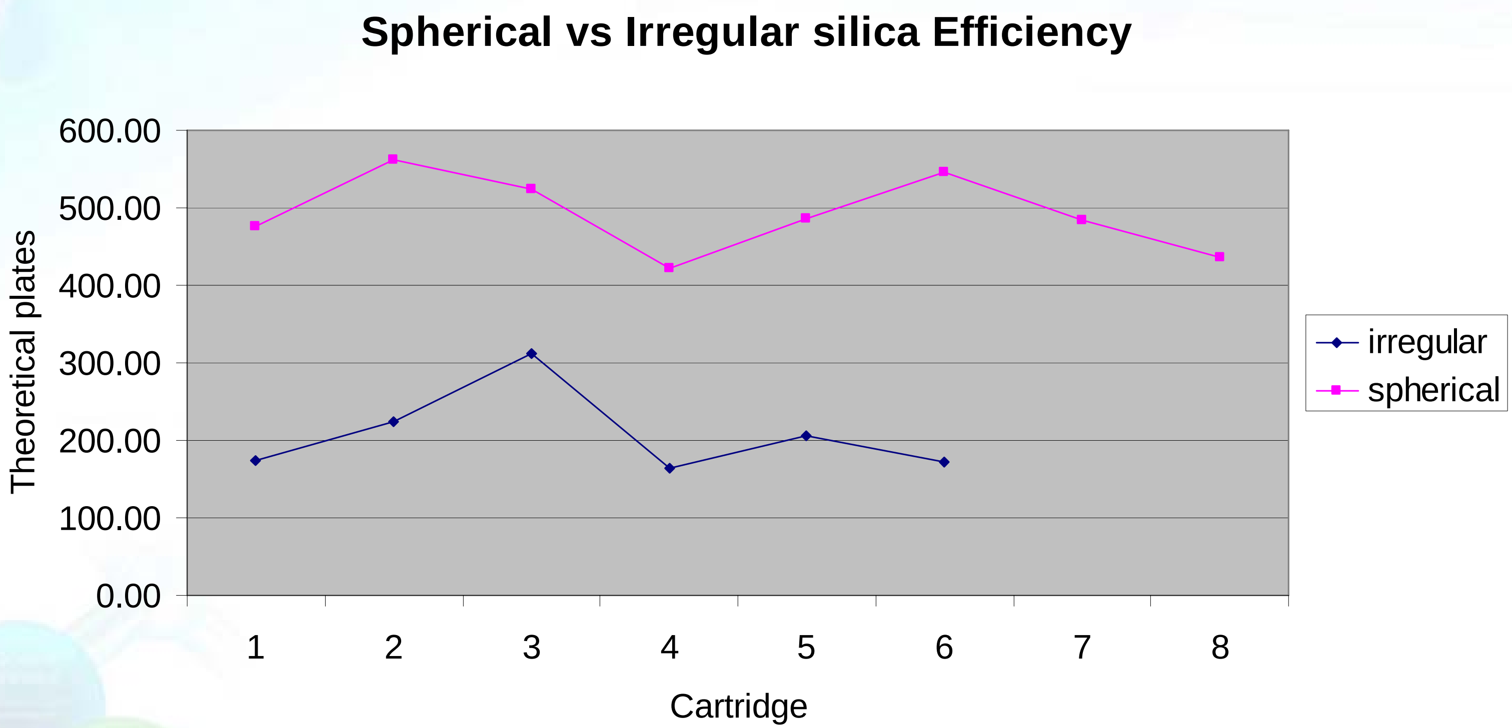
Figure 3. Chromatograms Comparing Efficiency of Cartridges Packed with Spherical and Irregular Silica



0.2 gram toluene on cartridge

3.75 mg 2,6-dinitrotoluene (2,6-DNT) on cartridge

Figure 4. Comparison of Efficiency between Cartridges Packed with Spherical and Irregular Silica



Average irregular – 208.62 ± 55.40

Average spherical – 493.92 ± 52.70

Figure 5. Comparison of Total Cartridge Capacity between Cartridges Packed with Spherical and Irregular Silica

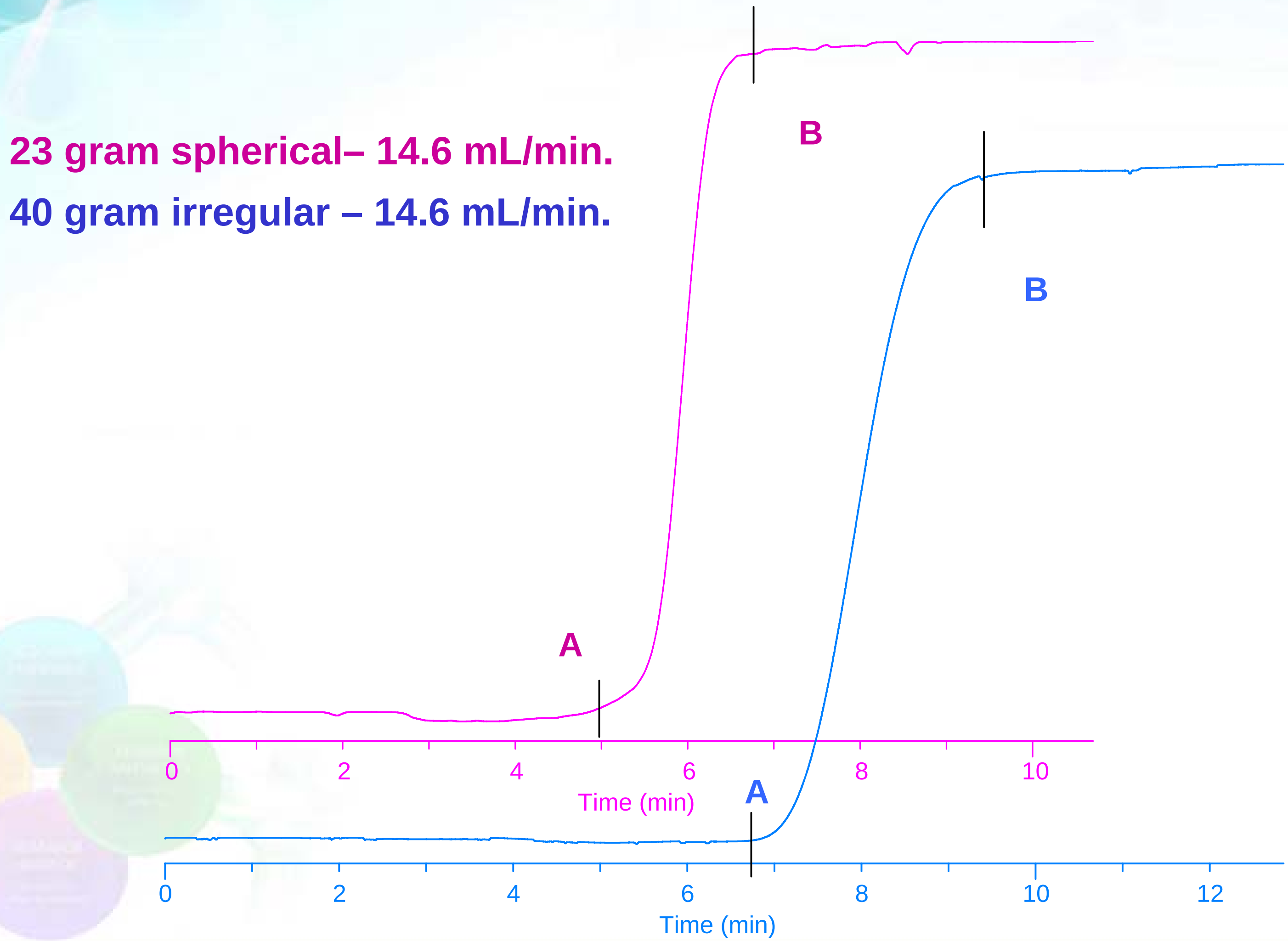
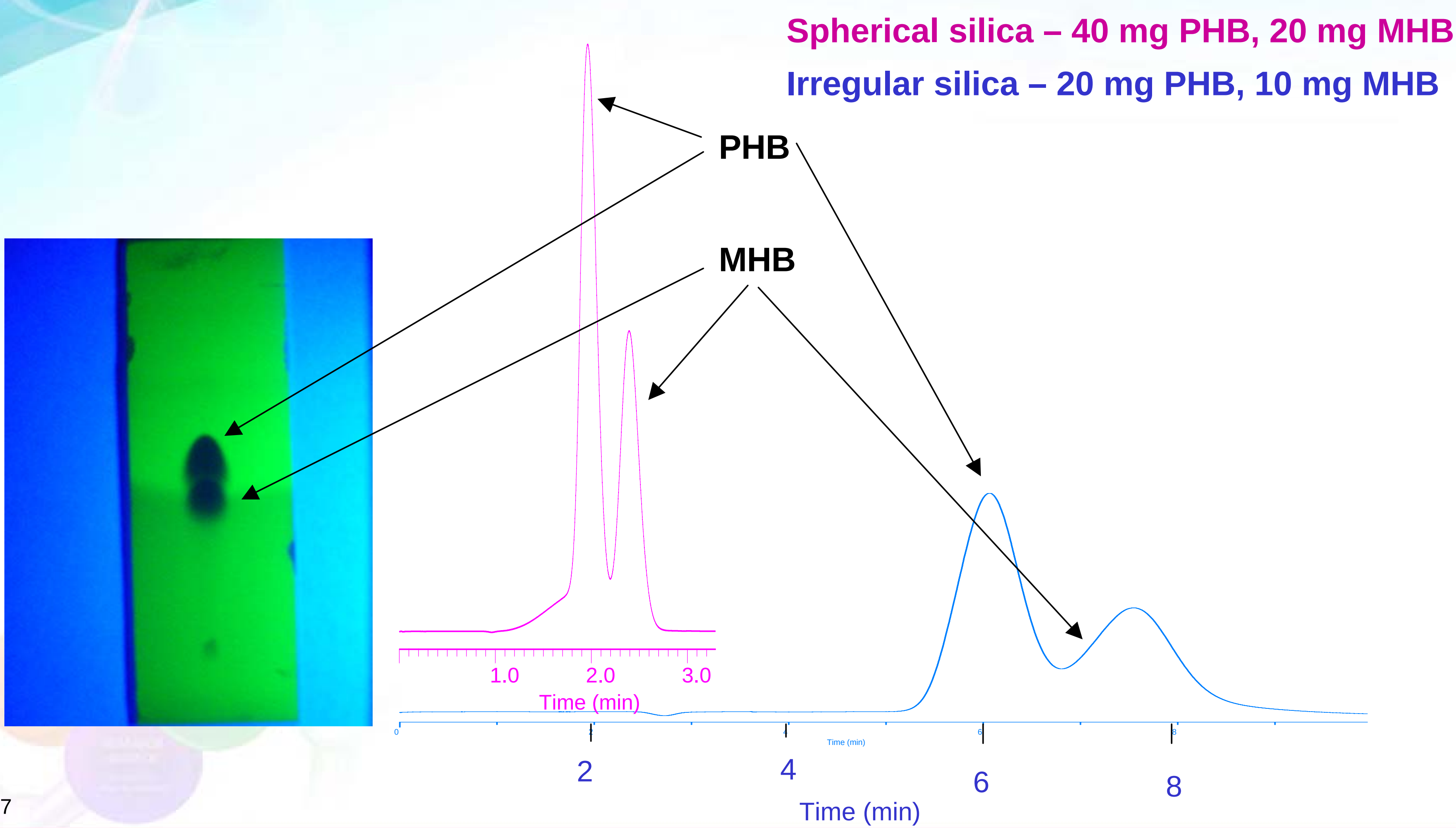


Table 3. Comparison of Total Cartridge Capacity between Cartridges Packed with Spherical and Irregular Silica

	23 gram spherical	40 gram irregular	50 gram spherical
Point A (min.)	5.1	6.7	3.7
Point B (min.)	7.0	9.4	5.6
A -B Segment (min.)	1.9	2.7	1.9
flow rate (mL/min.)	14.6	14.6	40.0
Concentration of DNT (mg/mL)	3.75	3.75	3.75
Capacity factor (mg)	54.75	54.75	150.00
Cartridge capacity (mg)	279.23	366.83	555.00
Capacity per gram of silica (mg)	12.14	9.15	11.10

Figure 6. Comparing the Performance of Cartridges Packed with Spherical Silica and Irregular Silica on a Difficult Separation



Conclusions

The use of flash cartridges packed with small spherical silica particles provides significant advantages over cartridges packed with traditional large irregularly shaped silica particles. Purification of analytes that are not well separated by TLC can be accomplished in less time while loading more analyte if flash cartridges packed with 20 – 45 µm spherical silica particles are used. Additional benefits include less solvent usage and smaller fraction volume, requiring less evaporation time.

Reference

1. W.C. Still, M. Kahn, and A. Mitra, *J. Org. Chem*. 43, **1978**, 2923