

Spherical Silica Shows Increased Loading Capacity

Compared to Irregular Silica in MPLC



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Abstract

The use of spherical silica gel for purifying compounds with medium pressure liquid chromatography has several advantages compared to irregular silica. One advantage is an increase in loading capacity compared to silica of the same particle size range. The increased loading capacity leads to fewer runs required to purify compounds with consequent time and solvent savings. Loading capacity is demonstrated by measurements of peak widths at differing loading levels. Spherical silica exhibited at least twice the load capacity compared to irregular silica of a similar particle size range.

Background

There is a tendency towards smaller particle sizes in medium pressure liquid chromatography (MPLC). Smaller particle sizes improve resolution by improving the efficiency (number of theoretical plates) of the column. However smaller particle sizes tend to increase the backpressure for a given column size which limits flow rate and increases the time to purify compounds.

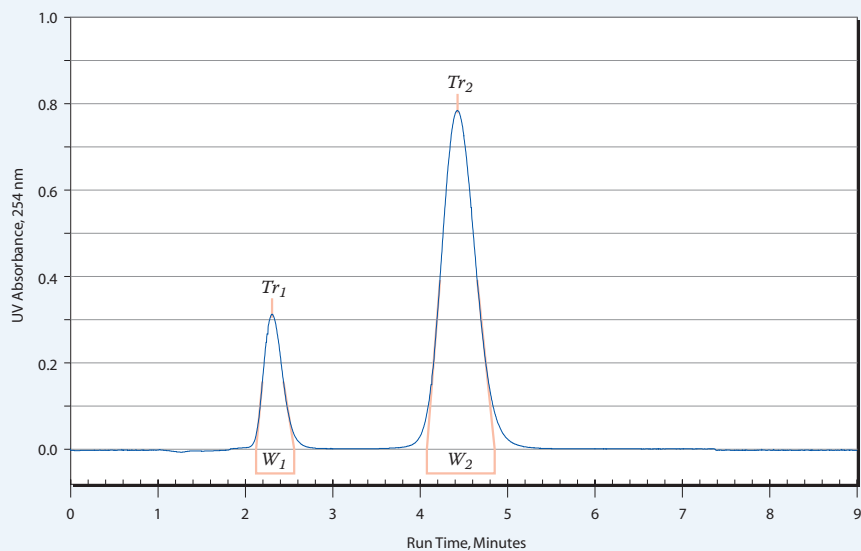


Figure 1:
Purification of acetophenone and 4'-methoxyacetophenone showing peak width and retention time

Equation 1:

$$\frac{W_1}{2} + \frac{W_2}{2} < Tr_2 - Tr_1$$

Peaks are considered resolved if they meet the criterion in equation 1. As the sample load is increased on a column, peak width is increased. If a column can generate narrower peaks, a larger sample can be loaded compared to a column with similar chemistry with wider peaks.

Columns packed with spherical silica are compared to columns packed with irregular silica with a similar particle size range.

Experimental

A sample mixture was prepared from 10.0 g of 4'-methoxyacetophenone (TCI, Portland, Oregon) and 10.0 g of acetophenone (Aldrich, St. Louis, MO) dissolved in hexanes in a volumetric flask for a total volume of 1,000 mL.

The sample mixture was purified on 12 g RediSep Rf Gold columns (spherical silica, 20–40 μ particles, PN 69-2203-344, Teledyne Isco, Lincoln, NE) and 12 g columns from a competitor packed with irregular silica (15–40 μ particles) under isocratic conditions (10% ethyl acetate in hexanes). The sample described above was injected at various injection volumes as described in the results. The same injection volumes were used for each column. Experiments were run on a CombiFlash Sq 16x system with autosampler. Injection volumes ranged from 1.0 mL (0.020 g, 0.2% sample load) through 40.0 mL (0.8 g, 7% sample load).

The sample was dissolved in hexane rather than the mobile phase to concentrate the sample at the head of the column upon injection. Concentrating the sample reduces changes in peak width caused by the increased injection volume from the larger sample.

Peak width was measured by extrapolating the trace from a line between $\frac{1}{2}$ the peak height and $\frac{1}{4}$ peak height to the baseline on both sides of the curve and calculating the difference in x-intercept. The choice of lines tracked well along both the rising and falling parts of the peak on both small and large sample loads.

For the gradient experiments, 1.0 g each of catechol and resorcinol (Sigma-Aldrich, St. Louis, MO) were dissolved in ethyl acetate. A slurry was made by adding 8.0 g of silica (Teledyne Isco, PN 60-3874-091) to the mixture. The solvent was removed with a rotary evaporator to make a free-flowing powder. 2.0 g of this sample (0.4 g total sample, 1.0% column load) was run on 40 g columns packed with either spherical or irregular silica. Both columns were run at 40.0 mL/min using the gradient shown in Figures 4 and 5 on a CombiFlash Rf 200 system (Teledyne Isco, PN 68-5230-006). Absorbance was measured at 270 nm.

Results and Discussion

The column packed with spherical silica showed narrower peaks at all sample loads compared to the column packed with irregular silica (Figures 2 and 3).

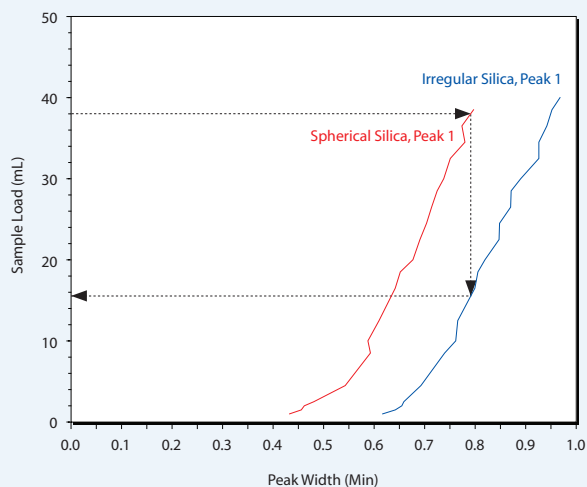


Figure 2:
Acetophenone sample load as a function of peak width

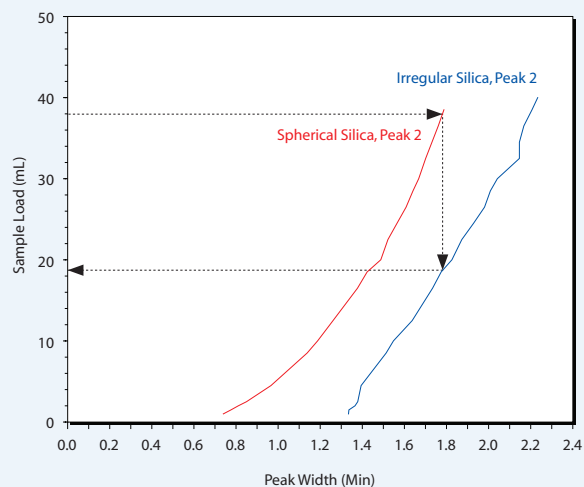


Figure 3:
4'-methoxyacetophenone sample load as a function of peak width

For both compounds, the spherical silica used in the RediSep Rf Gold columns allowed at least twice the sample load for a given peak width in irregular silica. In Figure 2, the spherical silica showed a peak width of 0.8 minutes with a 38 mL injection volume; the irregular silica at the same peak width could load only 16 mL sample. A similar ratio is seen for peak 2 (Figure 3). Extrapolation of the data suggests that the 1:2 ratio would hold up to at least a 20% load.

This advantage in peak width is also seen in gradient elutions (Figures 4 and 5) where the gradient tends to sharpen the peaks. The reduced peak width allows improved purity of fractions when peaks are closely eluting. On these runs, the spherical silica showed a backpressure of 17 psi while the irregular silica exhibited a back pressure of 28 psi.

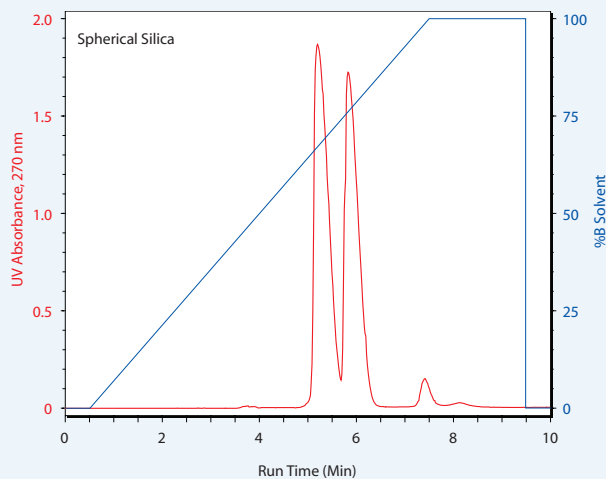


Figure 4:
Purification of catechol and resorcinol on spherical silica

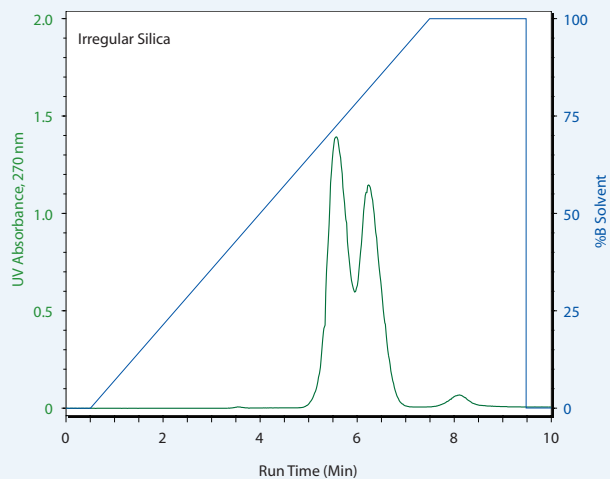


Figure 5:
Purification of catechol and resorcinol on irregular silica

Conclusion

Spherical silica exhibits reduced peak width (Figures 6 and 7) compared to irregular silica of a similar particle size range because of tighter packing and tighter distribution. The reduced peak width creates additional resolution which can be used to increase the sample loading. The additional resolution can also be used to increase the gradient resulting in shorter run times and reduced solvent usage. Spherical silica also results in reduced backpressure allowing higher flow rates.



Figure 6:
Dyes on spherical silica



Figure 7:
Dyes on irregular silica

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