

Liquid Chromatography Problem Solving and Troubleshooting

Question:

My understanding is that the carbon load determines peak retention (i.e., the greater the load, the longer the retention). However, I recently encountered a situation in which I was using C₁₈ column packing with a 7% carbon load that had more retention than C₁₈ with a 10% carbon load. Why is this? Is my understanding correct? If my assumption is incorrect, why do manufacturers report carbon load?

Answer:

In concept, your understanding is correct. However, there are many exceptions to this guideline, and in reality, this "rule" is false. If the pore size and surface area of the base silica particles are identical, the rule holds. Thus, within a family of packings, the carbon load relationship is true. This guideline lingers as the result of a hold-over from the developing years when there were only a few columns, and many were made from silicas of similar density, pore size, and surface area. Predicting retention is a complex task that chromatographers are always attempting to simplify. As a guideline, the carbon load helps in choosing an appropriate packing. With the wide variety of families of chromatographic packings currently available, the use of the carbon load as a guideline is qualitative at best.

Carbon load is expressed as the percentage of carbon per weight of silica. This is determined experimentally by observing the weight loss of a bonded phase as the carbon is burned off (thermal analysis). A higher percentage of carbon corresponds to a greater amount of bonded phase that is attached to the packing. Therefore, if the density of the silica is constant, the greater carbon load will have greater retention of a neutral molecule. If the carbon load is the same, the packing with the higher density (smaller pore volume) is more retentive. This could explain your observation.

Carbon load is reported because it is one of many physical tests that manufacturers use to insure that a consistent amount of the alkyl chain is bonded to the silica. Batch-to-batch reproducibility is important in making a reproducible stationary phase, and the percent carbon load is one test that can be used to monitor the bonding. Some critics have suggested that the ability to measure the percentage of carbon by thermal analysis is not sufficiently precise to be a sensitive test of the bonded phase coverage. These critics suggest that a better, more representative test of the packing-to-packing reproducibility is the generation of a chromatogram of a neutral molecule. This debate will probably continue for a while. It is important to understand that a guideline for the retentivity of the packing is available by using the reported value of the carbon load and the silica density together. Remember, this is just a guideline for relative retention of a neutral compound. It should also be noted that the chain length will affect the carbon load. In other words, C₄ will have less carbon load than an equivalent molar amount of C₁₈ bonded onto the same silica.

There has been a recent trend to report the surface coverage (sometimes called the ligand density) in terms of micromoles per centimeter squared. For a given bonded alkyl chain, if the surface coverage of two packings is the same, the material with the larger surface area will have more retention. For two bonded phases with the same surface coverage on the same silica, the longer alkyl chain length will have the greater retention.

The above discussion refers only to neutral compounds. The retention of polar compounds is more complex. Polar compounds often have a mixed mode of retention; some retention depends on the nonpolar bonded alkyl chain, and some retention is due to the attraction of the polar silica support. As a result, retention of polar compounds on short chain bonded phases may be greater than anticipated.

Table I is exemplary of this discussion. Anthracene on C₁₈ with a 20% load exhibited an approximately proportional increase in retention compared with C₁₈ with a 12% carbon load, but the C₁₈ with a 20% load exhibited more than twice the retention observed on a 12% carbon load phenyl phase. The retention of a more polar solute, diethyl phthalate, had a proportional increase in retention for the two C₁₈ columns (20% versus 12%) but, surprisingly, had essentially equivalent retention on the phenyl (12%) and C₁₈ (20%) columns.

In summary, carbon load can be a useful guideline under appropriate boundary conditions, but do not be surprised if the guidelines are violated in some situations.

Reference

1. A.P. Goldberg. Comparison of columns for reversed-phase liquid chromatography. *Anal. Chem.* **54**: 342–45 (1982).

Packing	Carbon load	Capacity factor*	
		Anthracene	Diethyl phthalate
C ₁₈	20	5.0	3.0
C ₁₈	12	3.3	1.8
Phenyl	12	1.4	2.9

* Data calculated from reference 1.

The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC-MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward to JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting*, P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

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