Liquid Chromatography Problem Solving and Troubleshooting

Question:

I am analyzing glycerol on an ion exclusion type column with a refractometer and am observing "ghost" peaks which are negative deflections. Unfortunately, I am not able to determine the cause of these. The column dimensions are 7.8 × 150 mm and the mobile phase is water at 70°C. The glycerol elutes at 4.2 min and the ghost peaks elute at approximately 5.9 and 6.2 min. Any suggestions?

Answer:

Since the location of the ghost peaks do not effect the analysis accuracy or precision, you are in an enviable position of having a problem that is mostly of an academic interest rather than a serious drawback to the method. However, it is always nice to explain the chromatographic behavior (if there is an explanation) so that the method is more believable to those who might not appreciate the value of the chromatographic technique. My first response is that these peaks are real, not ghost peaks, and are indeed representative of the individual samples. Do not panic; these peaks are not interfering with your analysis. I suggest that, because the location of the two ghost peaks are at the total volume of the column, they are due to dissolved air in your sample.

Why do I suggest this? First, because you are using a refractive index detector that is a bulky property detector, it measures any difference in the refractive index of the mobile phase. Furthermore, it is well known that dissolved air in a liquid has a different refractive index from the liquid without air. Also, it is known from the early literature in gel permeation chromatography that two "system" peaks occur after the total column volume. Thus, these two peaks are always observed when injecting real samples and only a solvent "sample". Therefore, its source is not sample dependent. In GPC, one of these two peaks can be diminished by degassing using nitrogen, while at the same time the other peak is increased in size. Both peaks can be increased in size by bubbling air through the sample solvent. Many gel permeation chromatographers (GPCers) tend to refer to the peaks as the "air" peaks, and other GPCers refer to the peaks as nitrogen and oxygen (the nitrogen peak is approximately 4 times greater than the oxygen peak).

Since you are using a column that is very similar to the type used in GPC, I believe that what you are observing as ghost peaks are indeed what the GPCers have become familiar with in the 1960's: these peaks are due to dissolved air in the solvent that was injected, one peak nitrogen and the other oxygen. To test this hypothesis, compare the effect upon the ghost peaks by using (*a*) fresh solvent alone, (*b*) fresh solvent in which air has been bubbled thoroughly, (*c*) fresh solvent in which nitrogen has been bubbled thoroughly, (*d*) fresh solvent in which helium has been bubbled thoroughly, (*e*) fresh solvent which has been degassed by heating (however, it will re-equilibrate with air quickly). All of these should be chromatographed and the results compared. The hypothesis is supported if (*a*) when the solvent is saturated with air, the two peaks grow in comparison with the degassed sample, (*b*) when the nitrogen saturated solvent is analyzed, one peak increases and the other is diminished, and (*c*) when the helium degassed solvent is analyzed, it shows two diminished peak heights in comparison with those observed in the air saturated sample.

Ghost peaks such as these could also be observed using low UV wavelength detection, because many UV detectors at low wavelength (below 220 nm) will observe responses due to the refractive index of the eluting region bending the light away from the photodetector. This effect is often referred to as "a refractive index effect" in UV detection.

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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