Liquid Chromatography Problem Solving and Troubleshooting

Question:

I was using normal phase and then switched to an analysis using a reversed-phase system. When I installed the reversed-phase column, I observed a very noisy, spiked baseline. I then had to go to a meeting and unfortunately let the system run overnight. The next day, things were considerably improved, but I occasionally noticed small blips on the baseline. Was I losing stationary phase? Should I continue my analysis if my column has lost phase?

Answer:

From what you describe, the cause of your problem was not a loss of phase but probably a solvent miscibility mismatch. You mentioned that this HPLC had been running normal-phase separations using hexane as the mobile phase before you converted over to the reversed-phase method using a mobile phase of methanol–water. You also did not mention that you had flushed the pumping system with an intermediate polarity solvent that would be miscible with both hexane and water, so I have assumed that you did not. If you did not clean out the previous solvent by flushing the pumping system with a wash solvent that is also miscibile with the new solvent, it is highly probable that what you observed as spikes on the baseline was in fact small amounts of hexane that had remained in the system. The hexane would have been immiscible with the new mobile phase, so the hexane would have caused baseline blips because, as it exits the column, it has different properties than the mobile phase.

When switching an HPLC system between a variety of uses in both reversed-phase and normal-phase applications, the primary consideration is that the solvent system must be miscible with the new solvent. If not, immiscible pools of solvent will exit in the pumping system, and it is highly likely that the pump will deliver tiny "slugs" of different solvents with the possible net result of one or more of the following observations: erratic flow rate, noisy baseline, baseline drift, and high pressure. All of these observations appear to the operator to be a major troubleshooting and repair problem.

However, this problem is easy to remedy by flushing the system with an "intermediate" solvent that is soluble in both old and new mobile phases. To choose an intermediate solvent, use your chemical background when dealing with commonly used solvents and, if unsure, test compatibility in a small flask. For your situation, I would use tetrahydrofuran (THF) as the intermediate solvent because it is very soluble with hexane and water and is a commonly used HPLC solvent. Acetone is another solvent that could be used if THF is not available, although acetone can be impure and may introduce contaminants into the system if not flushed sufficiently with the new mobile phase.

When you know nothing about the miscibility of the solvents you are using, refer to a "miscibility chart;" these charts are available from a variety of sources. If a chart is not available, use a table of miscibility numbers (1). Prior to changing from one mobile phase to another, refer to the table or chart to determine which solvents to use. For instance, if changing the HPLC from chloroform to water, the miscibility number would suggest that either methanol or THF could be used as the intermediate solvent.

Reference

1. B.A. Bidlingmeyer. *Practical HPLC Methodology and Applications,* John Wiley & Sons, New York, NY, 1993, pp. 244–46.

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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