



In this LabNote we will review the findings that were published by Kirkland, Claessens and co-workers in the 1990s regarding the factors that can affect the stability, performance, and lifetime of silica-based RPLC columns from mid to high pH. In our February LabNote, we'll describe a new product addition to the ACE product family, and specify its features, benefits, and applicability for method development and LC-MS analyses. Many experts in the field of liquid chromatography have recommended that reversed-phase method development should begin using low pH (1–4) mobile phases, whether the analytes are acidic, neutral or basic. The reasoning behind this view is that, at low pH, acidic analytes will be un-ionized, and residual silanols on the silica surface of the packing will be protonated. Therefore, there will be fewer secondary interactions between acidic and basic analytes and the silica surface. Unfortunately, many basic compounds, which carry a full or partial positive charge at low pH, will often be poorly retained, have poor peak shape (especially as loading goes up), or may even be excluded (that is, elute before the “void volume”) on most modern, type B silica-based packings. As a result, it is an advantage to be able to use mobile phases with pHs at or above 7.0 for basic compounds or for their mixtures with acidic and neutral compounds. Some other advantages of working at mid pH or high pH are when:

- analytes are insoluble or insufficiently soluble at low pH
- analytes are unstable at low pH
- analytes are not adequately retained or cannot be separated from closely-eluting compounds at low pH
- improvement in response and signal-to-noise ratio is needed for analysis of basic analytes by LC-MS vs. low pH analyses

The separation of many basic pharmaceuticals with relatively high pKa values at pHs 3–10 is often desirable, as they exist as free bases (un-ionized) at those pHs. Under those conditions, interactions of such basic solutes with then-fully-ionized silanols (pKas ranging from 3–6) on the silica support are minimized, resulting in improved retention and peak shape, and alternate selectivity compared to separations at low or mid pH. However, there are a number of findings that are important to know and to remember, before working with mid- or high-pH mobile phases. Historically speaking, it has been known that many silica-based columns have reduced lifetimes at a pH above 6.0, and especially at above-ambient temperatures, due to silica dissolution. J. J. Kirkland and co-workers and H. A. Claessens published a series of excellent articles in the Journal of Chromatography in the 1990s, which covered RPLC column stability at mid and high pH. Some of their important published results are summarized below.

COLUMN STABILITY

- Degradation of silica-based columns at intermediate (and higher) pH is largely a function of dissolution of the silica support, rather than a loss of bonded phase due to hydrolysis.
- Rate of silica degradation due to dissolution is affected by:
 - choice of mobile phase organic modifier
 - the type of silica support
 - the type and bonding density of bonded phase
 - type and concentration of buffer salts in mobile phase
 - column temperature
- Silica support solubility in the pH 6–8 range is greatly increased in the presence of phosphate buffers, particularly at higher temperatures and higher buffer concentrations.
- Silica-based columns are rapidly degraded when carbonate and phosphate buffers are used at pH 10, and phosphate buffers can cause rapid degradation even as low as pH 6–8, especially at higher temperatures and higher buffer concentrations.
- Silica dissolution for untreated silica at pH 9–10 was reduced in high concentrations of organic modifier, especially when ammonia was the source of hydroxyl ions.
- Pre-columns of unmodified silica greatly extend the lifetime of columns used at high pH (e.g., phosphate buffer, pH 11); bonded-phase pre-columns were much less effective.
- Longer chain bonded phases (C18, C8) are more stable than shorter chain phases (phenylpropyl, phenylethyl, C4, CN)
- Densely bonded, endcapped phases are much more stable than unendcapped phases (especially those with sterically protected ligands).
- Certain silica-based, bonded-phase columns can be used routinely for long periods to pH 9–10, if particular operating conditions are used.

Our next Lab Note will review some of the advantages of and potential pitfalls with working at mid to high pH, and will highlight the features and benefits of the newest addition to the ACE column portfolio (available for purchase now, official launch at PittCon 2013 in Philadelphia) for LC method development, LC-MS analyses, and use at mid- to high pH with ammonium hydroxide-containing mobile phases.

REFERENCES:

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