DiamondAnalytics
tools for tomorrows chemistry

Guidelines for Understanding the Retention Mechanism of Diamond Analytics Flare Mixed-Mode Column

Chuan-Hsi Hung[1], A.A. Kazarian,[2] Andrew E. Dadson,[3] Brett Paull,[2] Pavel N. Nesterenko,[2] Matthew R. Linford[1]

Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602 USA,[1]Australian Centre for Research on Separation Science (ACROSS), School of Chemistry, University of Tasmania, Hobart, Tasmania 7001, Australia,[2]and Diamond Analytics1260 South 1600 West, Orem, Utah 84058, USA[3]

The Diamond Analytics Flare mixed-mode column is made of diamond, carbon, and polymer to allow anion exchange and/or hydrophobic retention mechanisms to be operative in its separations. As with many mixed-mode columns, it is important to understand both the ionic (charged) state of one's analytes, as well as that of the stationary phase to best predict retention. Both of these charged states may depend on the mobile phase pH. While the retention mechanism of a mixed-mode column is generally more complex than that of a C_{18} phase, this added complexity can allow separations that may not be possible on traditional C_{18} columns. This application note explains the protonation state of both the Flare mixed-mode column and a series of common analytes/functional groups as a function of pH. These general guidelines should help the user best select conditions for chromatographic separations on the Flare column.

The following tutorial assumes some understanding of acid-base chemistry. If the reader is not familiar with this chemistry, he/she may wish to refer to the Diamond Analytics application note: '*A General Tutorial on Acid-Base Chemistry as a Basis for Understanding the Diamond Analytics Flare Mixed-Mode Column*'.

1. The Stationary Phase of the Diamond Analytics Flare Column

In essence, the stationary phase of the Diamond Analytics Flare column contains two important functionalities: hydrophobic octadecyl (C_{18}) chains and mixed primary, secondary, and tertiary amine groups.

The C_{18} chains facilitate at least some degree of hydrophobic interactions with analytes at all mobile phase compositions.

The amine groups allow the protonation state of the column to be varied with pH. The pK_a for a *molecular* amine is about 10 – when we refer here to the pK_a for an amine we really mean the pK_a for the amine in its protonated form. However, the protonation state for an amine-containing *polymer*, as a function of pH, is be a little more complicated than the situation for molecular species. For example, as the pH of the mobile phase is lowered from ca. 10 to 9, we will not find a ca. 10:1 ratio of protonated to deprotonated amines in the stationary phase. This is because the charged amine groups on the polymer are in close proximity to each other and will repel each other. In other words, the

more the column is protonated the more difficult it is to further protonate it so an increasingly lower pH will be required to protonate it completely. In practice this means that as we lower the pH over a number of pH units we will increasingly protonate the column, which is unlike the more limited pH range over which molecular amines change their protonation state. The removal of protons should be more straightforward – because we are reducing charge repulsions as we remove protons from the column, we should find that the column is mostly deprotonated at ca. pH 11.

Thus, at higher pH values the column will be more deprotonated (neutral) and will behave in a more reversed-phase mode. At lower pH values the column will be increasingly protonated, which means it will be increasingly hydrophilic (less retentive in its reversed-phase mode) and more able to interact strongly with anionic analytes.

In general we recommend that a buffer always be present in the aqueous portion of the mobile phase with the Flare mixed-mode column so that the protonation state of the column will remain fixed.

2. Ionization Behavior of Major Classes of Analytes

The following is a list of functional groups found on many analytes with their approximate pK_a values and/or permanent charge states, and a discussion of their expected retention on the Flare mixed-mode column. These functional groups/analytes are placed in one of three categories: a. neutral analytes, b. permanently charged analytes, and c. weak acids and bases with protonation states that depend on pH. By noting the charge states, pK_a values, and hydrophobicities of analytes, one can predict their retention on the Flare mixed-mode column. Note: while the following rules of thumb are generally accurate, actual results may vary somewhat for different analytes and mobile phases – of course better predictions will be possible if the user knows the actual pK_a values of his/her analytes. Thus, we recommend that as much information as possible be gathered about new analytes/analyte mixtures and that they be tested with a few different mobile phases at different pHs. That is, the recommendations below may be considered as a starting point for experimentation.

A. Neutral Analytes (no permanently charged or ionizable moiety)

These analytes, depending on their hydrophobicity, may be retained at any pH on the Flare mixed-mode column. In general, however, retention will increase with increasing mobile phase pH because the stationary phase will be increasingly deprotonated and therefore hydrophobic.

There are a number of functional/structural groups on organic molecules that remain neutral over the entire pH range of the Flare column. These include alcohol (-OH) groups (they are very weak acids – pK_a values of ca. 17 - 19), cyano (-CN) groups, alkyl and aryl halides, aromatic rings, alkyl chains, and perfluorinated alkyl chains. Hydroxyl groups can interact with amine functional groups on the Flare mixed-mode adsorbent by formation of relatively weak hydrogen bonds, but, as a rule, this should not be a dominant type of interaction.

B. Permanently Charged Analytes

i. Quaternary Amine Groups ($-NR_3^+$)

Quaternary amines have permanent positive charges. Unless they are quite hydrophobic, they will not, in general, be well retained in the Flare column at lower pH values because of cation-cation repulsions between the protonated stationary phase and the charged analyte – best retention will be at elevated pH values where the stationary phase is neutral.

ii. Sulfonates (RSO_3^-) and Sulfates ($ROSO_3^-$)

These analytes will be almost entirely negatively charged over the entire pH range of the Flare column. Thus, their retention will increase with decreasing pH as the stationary phase is increasingly protonated (cationic). In addition, the retention of all charged analytes can be regulated by the concentration of the buffer in the eluent, i.e., retention of ionic species will be less with higher concentrations of buffer.

C. Weak Acids and Bases with Protonation States that Depend on pH

i. Aliphatic Amines, pK_a ca. 10, e.g., tricyclic antidepressants

Primary, secondary, and tertiary amines have pK_a values of ca. 10. Retention will be greatest on the Flare column when both they and the stationary phase are deprotonated – above ca. pH 11, where the column is in reversed-phase mode. Below pH 10 one would expect reduced retention because of cation-cation repulsion between the protonated analyte and the protonated stationary phase.

ii. Carboxylic Acids (RCOOH), pK_a ca. 4.0, e.g., various acidic herbicides and benzoic acid

Carboxylic acids are acids. Above ca. pH 5.0 they will be mostly deprotonated (anionic) and will interact strongly with the protonated Flare stationary phase. At higher pH values where the stationary phase is increasingly deprotonated their retention will decrease. Their retention will also decrease at low pH values (below ca. pH 3.0) where they will be mostly protonated (neutral) and the column will be protonated. That is, best retention is expected from ca. pH 3.0 – 7.0.

iii. Phenols, pK_a ca. 10, e.g., phenol (C_6H_5OH)

Phenols are acids. Below ca. pH 9 they will be mostly protonated. At ca. pH 11 they will be mostly deprotonated. Thus, at lower pH values (at or below ca. 9) one will have a neutral analyte and an increasingly cationic Flare column. At higher pH values (at or above ca. 11) one will have an anionic analyte and a neutral column. In both of these cases retention will be less than between ca. pH 9 and 11, where a significant amount of both the analyte and the column will be

charged; retention will be greatest when charge-charge interactions are attractive – between ca. pH 9 and 11.

iv. Aromatic Amines/Pyridinic-Type Species, pK_a *ca.* 5, e.g., aniline ($C_6H_5NH_2$) and pyridine (C_5H_5N)

The protonated forms of aniline and pyridine have pK_a values of ca. 5. Accordingly, at pH values below ca. 4 they will be mostly protonated and poorly retained on the Flare column because of cation-cation repulsions between these analytes and the stationary phase. Above ca. pH 6 they will be deprotonated (neutral). Retention will thus increase as the mobile phase pH increases and the stationary phase is increasingly deprotonated.

Note: For all charged analytes retention is dependent on the type and charge of the ions composing the buffer, along with the concentration and pH of the buffer. An increase of pH increases the eluting ability of the buffer for weak acids and the opposite effect takes place for weak bases.