Application of Structure-based \( pK_a \) Prediction to Reversed-Phase Chromatographic Method Development

**Introduction—pH and HPLC**

One of the strengths of reversed-phase high performance liquid chromatography is the large number of parameters that are available for optimization of the separation. Analyte retention can be altered by changing the organic mobile-phase modifier, mobile-phase pH, gradient, temperature, or modifier concentration, among other things. Selectivity is often described with reference to the mobile-phase pH. The mobile-phase pH affects the ionization of the analyte and consequently the retention of the analyte. Mobile-phase pH also affects the ionization of the counterion and consequently the selectivity of the separation. Mobile-phase pH is a key parameter in regulating the retention of the analyte.

**Ionization and Hydrophobicity**

Analyte hydrophobicity determines its retention characteristics. The measured log value of the analyte hydrophobicity is the log parameter, which is essentially the logarithm of the equilibrium distribution coefficient of the analyte between organic and aqueous phases accounting for ionic and nonelectrostatic forces. Logarithmic hydrophobicity is a function of the analyte’s ability to interact with the aqueous mobile-phase solvent, and the analyte’s retention is a function of the analyte’s log parameter. The log parameter describes the analyte’s hydrophobicity by normalizing the log parameter of the neutral form of the analyte.

**Ionization and Retention Time**

As a first approximation, it can be assumed that the hydrophobicity of the compound and the retention time have a linear dependence in general for a given compound.

\[
	ext{Log } K_w = a \log X_b + b
\]

**Effect of Organic on pH Modifier and Analyte Ionization**

In order to determine the true ionization state of an ionizable analyte at a particular mobile-phase pH in reversed-phase HPLC, the effect of the organic modifier on the analyte ionization and pH modifier ionization must be taken into account. Both \( pK_a \) values of the analyte and the effective pH of the buffer are affected. The effect of the concentrations of methanol and acetonitrile on the spread of the ionization of carboxylic acids and bases has been studied. Systems have been demonstrated to be well-behaved to \( pK_a \) values to 6.0. Lower values of \( pK_a \) are difficult to work with.

**Retention Capacity**

Retention capacity is defined as the relative concentration of organic modifier that will allow the analyte to achieve a certain level of retention. This is defined as the ratio of the organic modifier concentration to the aqueous phase concentration.

\[
\text{Retention Capacity} = \frac{C_{	ext{Organic Modifier}}}{C_{	ext{Aqueous Phase}}}
\]

**Selecting the Optimal Starting pH:** Practical Example

If the \( pK_a \) of an analyte is not known, it may be estimated by commercial software such as ACD/LC Simulator (v. 6.0). The interaction of the analyte with the column is considered to be in the ionized or un-ionized state. For example, a \( pK_a \) of 6.7 indicates that the analyte is 50% ionized and 50% un-ionized.

**Conclusion**

Mobile-phase pH is a critical variable in reversed-phase chromatography. The effective selection of pH based on structures of known compounds can speed the process of method development, and improve the robustness of the resulting method. Taking into account the stability of the analyte in the mobile phase, the selectivity and retention time are not the only factors to consider when selecting the pH. The selection of a pH that is too low may cause the analyte to be retained for too long, while a pH that is too high may cause the analyte to be lost.

**References**