Measurement of Extreme pK_as

The determination of pK_as outside of the range 3 – 11 using potentiometric methods can prove problematic, due to the high buffer capacity of water and the relatively poor performance of pH electrodes at the extremes of the pH range. Capillary electrophoresis methods also perform poorly in this range.

It is also true that predicted pK_a values are less reliable at the extremes of the pK_a range. This application note describes some experimental designs for the PionT3 which may be used to overcome these problems, giving reliable pK_a values between 0.7 and >13.

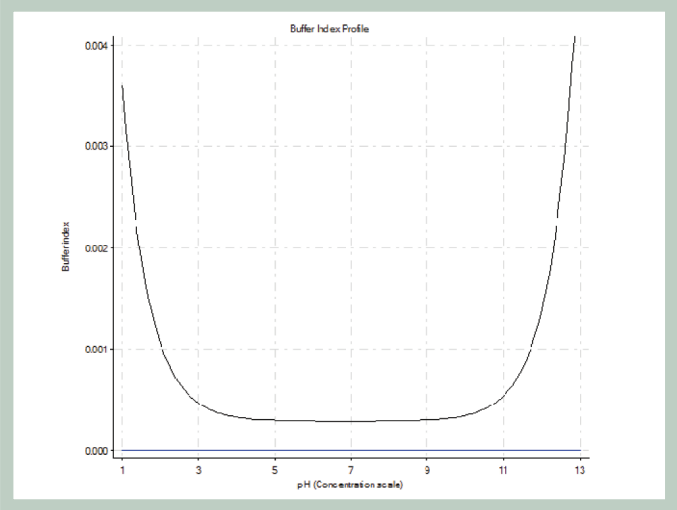

Figure 1. The minimum buffer index needed for reliable potentiometric pK_a measurement as a function of pH.

Experimental

Weak acids and bases

The potentiometric measurement of extreme pK_as is very challenging. Figure 1 shows that the buffer capacity of water increases considerably at the extremes of the pH range. As a consequence, very high sample concentrations are required for pK_as in these regions. Concentrations of several millimolar may be needed, compared to as low as 0.4 mM for pK_as in the range 3 – 11. Many drug-like compounds are poorly soluble in water and it may not be possible to achieve the required concentrations. Additionally, the use of cosolvent to enhance the solubility is usually inadvisable, since the pK_as of weak acids and bases tend shift to even more extreme values in cosolvent mixtures.

UV-metric assays

Extreme pK_as may be more readily measured using the UV-metric method, which is described in detail elsewhere[1]. This avoids the need to overcome the buffer capacity of water.

Only one titration per vial is possible, due to the very large titrant volumes added during titrations at the extremes of pH. It is advisable to use the titrant pre-dose function to adjust the pH in advance. This allows the widest possible pH range to be investigated.

Some suggested assay settings are listed in Table 1.

Parameter	Recommended Setting
Number of titrations in vial	1
ISA water volume	1.2 mL
Titrant predose	Acid or base as appropriate
Titrant predose volume	0.3 mL
Adjust to start pH	No

Table 1. Suggested assay settings for UV-metric pK_a methods for measuring extreme pK_as.

Strong acids and bases

For low acid and high base pK_as, an additional approach is available. In these cases, a cosolvent can be used to shift the pK_as toward the measurable range. However, it is relatively uncommon for drug-like molecules to fall in this category.

Examples and Discussion

a) Dipyridamole: low basic pK_a

The structure of dipyridamole is shown in Figure 2. Three UV-metric titrations from pH 1.2 were carried out under aqueous conditions using 5 µL aliquots of a 10 mM stock solution in DMSO. Figure 3 shows a typical pH-UV profile. Each line represents absorption at a different wavelength. The spectroscopic changes indicate where the sample is undergoing ionisation changes. The average pK_a results were 6.20 ± 0.01 and 0.89 ± 0.01.

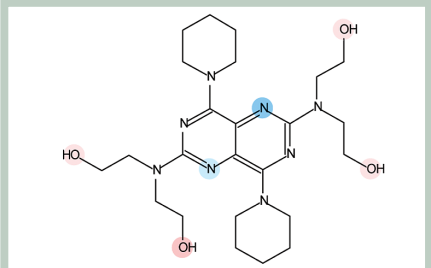

Figure 2. Structure of Dipyridamole

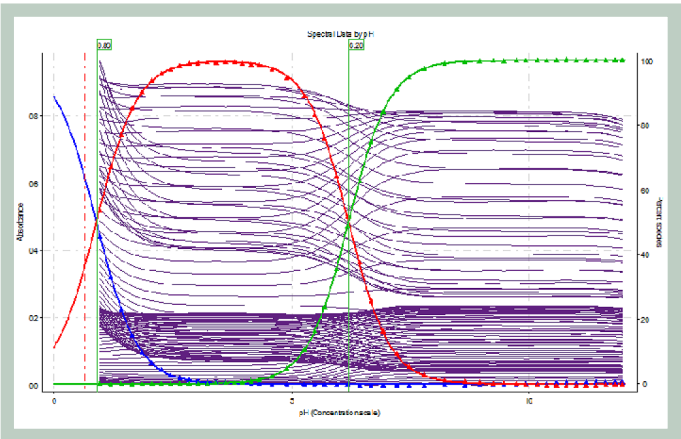

Figure 3. pH-UV profile for dipyridamole

b) Salicylic acid: high acidic pK_a

The carboxylic acid pK_a of salicylic acid (2.78) is relatively easily measured by pH-metric or pH-UV methods but the pK_a associated with the phenol is difficult to measure by standard techniques. In this experiment, the starting solution was adjusted to pH 12.8 by adding strong KOH solution and the solution was then titrated with 0.5M HCl. Figure 4 represents absorbance vs. pH data obtained at each wavelength.

Accurate measurement of pK_as as low as 0.82 is achievable on PionT3 if an appropriate assay design is used

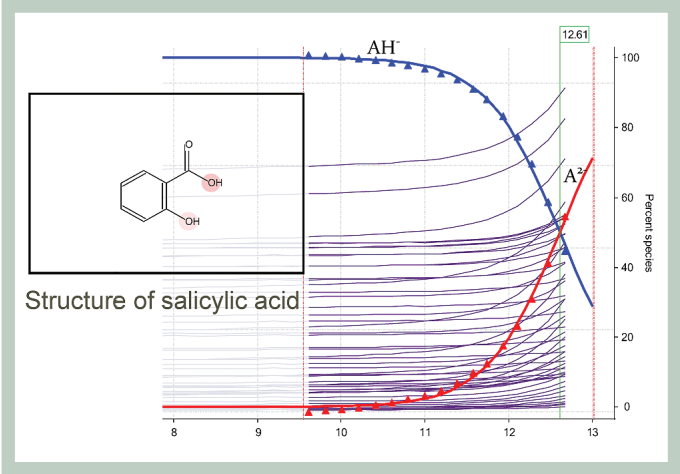

Figure 4. pH-UV profile for salicylic acid

Again, the spectroscopic changes observed as crossover points at high pH indicate that the sample is converting between different ionisable forms. The pK_a result of 12.61 was obtained by target factor analysis applied to the pH-wavelength-absorbance data.

Conclusion

Accurate measurement of pK_as of less than 1 and greater than 13 is achievable using the PionT3 if an appropriate assay design is used. Although the potentiometric method can be used to measure extreme pK_as, high sample concentrations are needed. It is more viable to use the UV-metric method as highlighted in this application note where possible.

References

[1] Tam, K. Y.; Takács-Novák, K. Anal. Chim. Acta **2001**, 434, 157-167

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