Does an autosampler glass vial influence the pH of your valuable sample?

The simple answer is: yes!

Introduction

Type 1 grade glass (defined by the hydrolytic class norm ISO 719 and all current Pharmacopeias, chapter: glass containers for pharmaceutical use) is nominally neutral pH and should have no effect on the solution stored in it. However, this activity can vary by glass source and expansion type as well as production lot.

Differences in the glass surface activity or level of hydrolytic extraction from the glass surface would be expected to produce a pH change in a stored aqueous solution. After manufacture of the glass it is possible to determine the surface activity by monitoring the pH shift of de-ionized water.

In this study we investigate how glass quality affects the solution pH by measuring pH shift in 18MΩ water pH stored in autosampler vials at room temperature, after an equilibration time. This is compared to a control water sample in a polypropylene container. A broad range of autosampler vial products were tested and the pH shifts measured. In theory the highest quality of vials is expected from 33 type vials, as the coefficient of mean linear thermal expansion is the lowest for the basic tubes used for vial manufacturing, as well as the hydrolytic resistance acc. to ISO 719, acid resistance acc. to DIN 12116 and alkali resistance acc. to ISO 695. Aside of 33 type vials we tested vials of the 51 and 70 type, in order to have broad overview of the quality of finished vials, independent from the basic tube used.

Background

Why is pH Important?

pH can have a dramatic effect on the ionization state of certain analytes. For example, a basic compound with a pKa of 8.5 would be 99% ionized at pH 6.5 and 99% non-ionized at pH 10.5 using the 2 pH unit ‘rule’.

Inconsistent chromatography may be observed when analytes are near their pKa and steps should be taken to ensure that an appropriate ionization state is achieved by modulating pH. To ensure that the chosen pH is maintained, the use of a buffer may be desirable.
What is a Buffer?
A buffer is an aqueous solution containing a weak acid (HA) and its conjugate base (A\(^{-}\)). Ammonium acetate is a good commonly used example. They achieve their resistance to pH change due to the equilibrium between the acid and the base according to the formula below.

\[
HA \rightleftharpoons H^+ + A^{-}
\]

When a strong acid is added to the solution, the equilibrium shifts to left according to Le Châtelier’s principle; though the hydrogen ion concentration increases less than the amount expected.

This ‘buffering’ only helps to maintain the chosen pH at approximately 1 pH unit away from the pKa weak acid in the buffer system. For example an ammonium acetate buffer system would be effective between the pH range of 3.8-5.8.

Choosing the Right Buffer/Buffer Capacity
Many factors influence the choice of buffer, but major considerations tend to be; What is the required pH of the mobile phase? In what pH range is the buffer effective? What buffer capacity is required and does the buffer need to be volatile? These are dictated by the physicochemical properties of the analyte, physicochemical properties of the buffer and the detection technique. For example, if the analysis uses mass spectrometric detection, choosing a volatile buffer is important.

Buffer capacity relates to the efficiency of a buffer to resist pH changes. A high capacity buffer is required where there is significant amount of acidic or basic material added to the solution.

So why does glass quality matter?
Given we can use buffers to mitigate any differences in the glass surface activity why is the glass quality and consistency important? There is an increasing demand for very low buffer concentration in samples and mobile phases to maximize performance of modern ultra-trace chromatography detectors while maintaining physicochemical parameters suitable for successful reversed phase LC

Method
The following protocol was used to assess pH shift in autosampler glass vials from different sources. In this case 1st hydrolytic class tubes have been used with examples of 33, 51 and 70 expansion type raw glass tubes.

- 1 mL 18MO HPLC water (Thermo Scientific™ Smart2Pure™ system (P/N 50129845)) was added to the 2 mL vial and capped using Thermo Scientific™ Chromacol 9mm Open Top Short Screw Cap with soft Blue Silicone/PTFE Septum (P/N 9-SCK(B)-ST101)
- 3 vials have been tested from each vial type and the average has been taken for comparison
- Vials were left at room temperature for 8 hours
- For the pH measurements the Thermo Scientific™ Orion 3-Star Bench Top pH Meter (P/N 1112106) was used

Vials tested as follows;
- Thermo Scientific Chromacol GOLD (P/N 2-SVG)
- EU Type 33 expansion (Thermo Scientific National (P/N C5000-1W))
- US Type 33 expansion (Thermo Scientific National (P/N C4000-1))
- EU 51 expansion (Thermo Scientific Chromacol (P/N 2-RV))
- China 70 expansion from competitor A
Comparison by glass expansion type

Figure 1 shows average delta pH results by glass type. By far the lowest pH shift (0.32) can be seen from the Thermo Scientific™ Chromacol™ GOLD Grade™ vials, which is less than half of the average of the other vials. EU 33 expansion glass gave the next lowest shift in pH, followed by EU 51 expansion and US 33 expansion, which exhibited very similar pH shift. As expected 70 expansion glass gave the largest shift.

In Figure 1 vials made of different glass types have been compared.

In general results were as expected, with Chromacol™ GOLD Grade™ vials exhibiting best results and 70 expansion glass the worst.

One result of note is the very similar performance of EU 51 expansion glass and US 33 expansion glass. Expectation would be that, based on raw glass quality the 33 expansion product should out-perform the 51 expansion product, however, it is often noticed that this is not the case.

Manufacturing conditions have a significant effect on the final product quality, with time, temperature and speed during production damaging/changing the raw glass surface. It is critical to note that a “crude” and highest speed manufacturing process damages this original benefit. The temperature range for the forming of a vial bottom and the closing type version (crimp, snap, screw thread) can vary in a wide range of 50–60°C up or down. The optimum for a surface preserving process is at the bottom level of usable temperature (normally >500°C, with differences for 33, 51 and 70 type glass).

Nevertheless, this corresponds with a longer time the flame needs to “form” the vial bottom and the e.g. the screw thread. This leads to a lower output of vials/hour and for that increases the costs of the product.

Chromacol GOLD vials are manufactured from 33 type glass tubes, in a surface securing process at lowest possible temperatures and enough time to keep the excellent hydrolytic class features “alive”.

Comparison by manufacturer

Additionally we looked at a range of widely available competitor vials on the market to determine if pH shift due to glass is an endemic issue. As the glass types of some of these vials are unknown, they are not highlighted here, however, all products tested were manufactured in the US or EU.

Figure 2 shows these results. These vials show a pH shift between 0.58 and 0.89 so in average 0.73 DpH. This corresponds with the type investigation and average value of figure 1 for common vials of 33 and 51 expansion.

Vials tested as follows:

- US Type 33 expansion 9mm Screw Thread (WMS)
- US Type 33 expansion 9mm Screw Thread (WTV)
- US 9mm Screw Thread (AMS)
- US 11mm Crimp (F)
- EU 9mm Screw Thread (R)
- EU Type 51 expansion 9mm Screw Thread (RS)
Results / Conclusion

- When adding aqueous sample to a glass vial the vial causes a pH shift in the sample.
- This pH shift may affect the ionization state of ionizable compounds which in turn is likely to affect their chromatographic performance.
- pH shifts are the result of glass surface activity caused from raw glass material or manufacturing process.
- Even vials made from high quality, 1st hydrolytic grade glass tubes can cause pH shifts in samples.
- In general 33 expansion glass affects solution pH least.
- The 70 expansion glass, although classified as Type 1, shows a more marked shift in pH than any of the 33 expansion and 51 expansion glass types tested here.
- Addition of buffer can mitigate some effects but may not be appropriate for all analysis, especially where high sensitivity is required.

Due to the high grade raw material and well defined production process used, Chromacol™ GOLD Grade™ vials exhibit least pH shift. They enable the user to receive highest result reproducibility and accuracy independent from the analyte, concentration or time the analyte remains in the vial.