High-throughput automated pH measurements for biologics formulations on Big Kahuna and Junior

Introduction

One of the major challenges in biologic drug development is the need to characterize formulations of drug candidates. One significant bottleneck in this process is pH measurement of formulations. Measuring pH is ubiquitous in the laboratory and critical for preparing buffers, analyzing formulations, monitoring stability, and numerous other applications during formulation development.

Manual pH measurements are labor intensive, time consuming, and error prone. Some buffers are more challenging when measuring pH, particularly tris buffers and buffers with excipients. Biologics also tend to be formulated at low buffering capacity, which adds challenges to the measurement reliability. Manual pH measurements require frequent, lengthy calibrations of the pH probe and are usually performed one at a time. Probes must be thoroughly washed between measurements to prevent cross contamination, and dried to prevent subsequent sample dilution. More accurate pH measurements for formulations are performed with glass pH probes, which are delicate and require proper handling and storage for continued accuracy.

Automating pH measurements with Big Kahuna or Junior alleviates many of the pain points in measuring pH for biologic samples. A new arm element from Unchained Labs (Figure 1) uses four highly-sensitive glass pH probes at a 27 mm pitch to automatically measure the pH of four samples simultaneously, significantly reducing the time and labor required. For single measurements or for samples at a different pitch, one probe is extendable, making the pH arm flexible enough for use with most samples.

In this application note, a Big Kahuna with the pH arm element was used to measure the pH of 16 buffer formulations with and without protein. In total, 30 replicate measurements were collected for each of the 32 conditions and compared to manual pH measurements with a typical benchtop pH meter.

Figure 1: The pH arm for Big Kahuna and Junior allows four pH measurements to be performed at a time and can analyze a 96-well plate in 45 minutes.

Methods

Automated pH arm

The automated pH arm on Big Kahuna has four glass pH probes (Orion ROSS Micro pH electrode, Thermo Scientific) spaced 27 mm apart, with one extendable probe for single pH measurements. The pH probes are maintained in the manufacturer's reference solution in a storage rack before and after each run. The storage rack is automatically unloaded from the arm at the start of a run and loaded onto the arm for short-term storage after the experiment is completed.

The probes are washed between measurements. Deionized (DI) water and an on-deck wash station wash the probes by dunking the probes multiple times then submerging them in flowing DI water. Following washing, the pH probes are dried with an on-deck air knife drying station.

Samples

For the following experiments, 16 buffers were manually prepared. The buffers were composed of 20 or 100 mM acetate, citrate, phosphate, or tris, with or without 300 mM NaCl, and spanned a pH range of 4.5 – 8.0 (Table 1). Buffers were tested without protein or supplemented with 10 mg/mL human IgG (hIgG).

	Buffer	Excipient	Target pH
1	20 mM acetate	None	4.5
2	20 mM acetate	300 mM NaCl	
3	100 mM acetate	None	
4	100 mM acetate	300 mM NaCl	
5	20 mM citrate	None	5.5
6	20 mM citrate	300 mM NaCl	
7	100 mM citrate	None	
8	100 mM citrate	300 mM NaCl	
9	20 mM phosphate	None	6.5
10	20 mM phosphate	300 mM NaCl	
11	100 mM phosphate	None	
12	100 mM phosphate	300 mM NaCl	
13	20 mM tris	None	8.0
14	20 mM tris	300 mM NaCl	
15	100 mM tris	None	
16	100 mM tris	300 mM NaCl	

Table 1: The 16 buffers used in this experiment spanned a pH range of 4.5 - 8.0, 20 or 100 mM buffering capacity, with or without 300 mM NaCl.

Manual pH comparison

Manual pH measurements were collected with the same Orion ROSS Micro pH electrodes. A Consort multi-parameter analyzer C3011 benchtop pH meter was used according to the manufacturer's instructions to collect pH measurements.

Calibration

A fully automated calibration was performed daily on the pH arm before experiments. The arm was calibrated using pH 4.000 ± 0.002, 7.000 ± 0.002, and 10.000 ± 0.005 Ricca standards. Triplicate voltage measurements were recorded from each of the four pH probes. A successful calibration was one where triplicate measurements were reproducible within ± 1 mV.

Automated pH measurements

The 16 buffers without protein were each manually pipetted into six wells of a 96-well plate (300 µL/well) and placed on the deck of a Big Kahuna. To test robustness, the plate was laid out such that 1) all probes were in a different buffer at any given time, 2) pH changed significantly between consecutive measurements, 3) buffering capacity changed between consecutive measurements, and 4) 300 mM NaCl was present in alternate measurements (Figure 2). Probes were washed and dried as described between measurements. The 96-well plate was measured five times for 480 data points, comprising 30 measurements for each of the 16 buffers.

The 16 buffers with 10 mg/mL hIgG were manually pipetted in a similar pattern into a 96-well PCR plate (80 µL/well, Thermo Scientific) and placed on the deck of a Big Kahuna. The 96-well PCR plate was also measured five times, totaling 30 measurements for each of the 16 buffers with protein.

The total time to read a 96-well plate was approximately 45 minutes.

	Probe 1			Probe 2			Probe 3			Probe 4		
	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	3	3	3	15	15	15	13	13	13
B	8	8	8	6	6	6	10	10	10	12	12	12
C	9	9	9	11	11	11	7	7	7	5	5	5
D	16	16	16	14	14	14	2	2	2	4	4	4
E	2	2	2	4	4	4	16	16	16	14	14	14
F	7	7	7	5	5	5	9	9	9	11	11	11
G	10	10	10	12	12	12	8	8	8	6	6	6
H	15	15	15	13	13	13	1	1	1	3	3	3

Figure 2: Buffer plate layout and operation. Probes are at a 27 mM pitch. Measurements start at A1, A4, A7, and A10 simultaneously and move down the column, then continue measurements at A2, A5, A8, and A11.

Results

Buffer pH measurements

Table 2 shows the average pH of each buffer measured by the automated arm on Big Kahuna compared to the manual measurement. Each automated pH measurement was within ± 0.05 pH units of the manual measurement, showing the high precision and accuracy of the 30 repeated automated measurements.

Buffers with protein pH measurements

Table 3 shows the average pH of buffers with 10 mg/mL hIgG measured by the automated arm on Big Kahuna compared to the manual measurement of each buffer with protein. Each automated pH measurement was within ± 0.05 pH units of the manual measurement, showing the precision and accuracy of the 30 repeated automated measurements.

The results showed internal consistency as each of the four probes performed similarly. There was no dependence on the buffer condition itself, or the previous sample measured that impacted the accuracy of the readings. There was no change between the measurements from the first run to the fifth.

Conclusion

The automated pH arm for Big Kahuna and Junior is a highly accurate and precise way to measure pH with minimal hands-on time. The system can be used to measure the pH of buffers across a wide range of aqueous buffer types and concentrations, excipient types and buffering capacities, and pH values. The pH of protein formulations is easily measured by the automated pH arm, with thorough washing by the on-deck wash station to prevent cross contamination.

Samples in this experiment spanned pH and salt concentrations consistent with biologics

Buffer	Manual pH measurement	Automated pH measurement n = 30
1	4.51	4.51 ± 0.007
2	4.48	4.49 ± 0.003
3	4.52	4.51 ± 0.006
4	4.48	4.47 ± 0.007
5	5.50	5.46 ± 0.009
6	5.54	5.54 ± 0.008
7	5.51	5.49 ± 0.011
8	5.55	5.56 ± 0.011
9	6.56	6.53 ± 0.009
10	6.52	6.52 ± 0.009
11	6.55	6.53 ± 0.006
12	6.52	6.51 ± 0.006
13	8.05	8.04 ± 0.007
14	7.98	7.96 ± 0.014
15	8.04	8.04 ± 0.005
16	7.99	8.00 ± 0.005

Table 2: Buffer pH comparison of manual vs. automated pH measurements with the same pH probe type. The first column is manual pH measurements from a benchtop pH meter. The second column is average ± standard deviation automated pH measurements from Big Kahuna.

Buffer with protein	Manual pH measurement	Automated pH measurement n = 30
1	4.65	4.68 ± 0.013
2	4.68	4.70 ± 0.014
3	4.52	4.54 ± 0.009
4	4.49	4.53 ± 0.018
5	5.52	5.51 ± 0.009
6	5.59	5.61 ± 0.009
7	5.50	5.49 ± 0.010
8	5.55	5.56 ± 0.006
9	6.54	6.52 ± 0.013
10	6.52	6.52 ± 0.005
11	6.53	6.53 ± 0.008
12	6.51	6.50 ± 0.005
13	7.93	7.95 ± 0.010
14	7.84	7.83 ± 0.008
15	7.99	8.01 ± 0.008
16	7.95	7.97 ± 0.006

Table 3: Buffer with protein pH comparison of manual vs. automated pH measurements with the same pH probe type. The first column is manual pH measurements from a benchtop pH meter. The second column is average ± standard deviation automated pH measurements from Big Kahuna.

formulations. The pH arm performed equally as well across a range of buffers and protein formulations, even those at high pH levels that can be difficult to analyze. The new pH arm alleviates the challenges of high throughput pH measurements and provides accuracy and reproducibility consistent with manual one-at-a-time measurements.

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