PURITAN PRODUCTS

Down The Rabbit Hole of Uncertainty: A Position Paper on Mathematical Uncertainty and Tolerances in the Manufacture of pH Buffers and Conductivity Standards

Through coincidental requests by two major Fortune 500 pharmaceutical and biopharmaceutical companies, Puritan Products was engaged to begin manufacture of cGMP manufactured pH buffers and conductivity standards. During the development phase of our line of these products our chemists and engineers pondered the subject of mathematical uncertainty and its impact on determining the tolerance to which these materials can be manufactured. In other words, how accurately can pH buffers and conductivity standards be measured? One of our Senior Chemists, Bill Wolf, performed the research and wrote this paper to make a definitive statement about what the industry should expect from all manufacturers of these products vis-à-vis claims made about uncertainty.

Puritan Products draws its scientific line in the sand with this paper. We do it to unite scientific thinking and to define the “achievable” state of the art. Theory and practice do not always co-exist peacefully in the real world. We offer readers a product line that can scientifically exist in the real world where scientists demand real, defensible data.

Down the Rabbit Hole of Uncertainty

I have to say, when I first heard about the subject of mathematical uncertainty, I thought to myself, "It's just another name for error analysis." Well, yes and no. The more I read about uncertainty, the more my eyes glazed over. As Puritan Products ventured into the manufacture of pH buffers and conductivity standards and began its assessment of what "uncertainty" or tolerance the company could support in producing these materials, one thing was certain - the company's position had to be logical, scientific, and defensible.

I am a product of the twentieth century. That includes my education, and in particular, my knowledge of statistics. But sometime in the 1990's, a change occurred and the "uncertainty" movement began.

> As Alice said," Dear, dear! How queer everything is today! And yesterday things went on just as usual. I wonder if I've been changed in the night. Let me think: was I the same when I got up this morning?

This particular change had its roots in the Age of Enlightenment and the Industrial Revolution. Science flourished and the concepts of modern measurements were conceived. Some names that may come to mind are: Laplace, Gauss, Galton, and Shewhart. Some concepts that stand out are: least squares, standard deviation, the "bell curve", and the control chart.

First published in 1912, John Keenan Taylor's book *Quality Assurance of Chemical Measurements* mentions the term "uncertainty" many times. But during the twentieth century, and from one author to another, terms and concepts were not used consistently.

> "When I use a word," Humpty Dumpty said in a rather a scornful tone, "it means just what I choose it to mean – neither more nor less."

In 1993, the GUM (Guide to the expression of Uncertainty in Measurement) was published. In 1997, the JCGM (Joint Committee for Guides in Metrology) was formed from the seven international organizations that wrote the GUM. Like the First Council of Nicaea, its purpose is to come to agreement, and define, what we believe.

> "Who are YOU?" said the Caterpillar

What is uncertainty? It is a metrology, which is the science of measurement. Where traditional error analyses are expressed as ±, uncertainty in its pure form is non-negative. Traditional error methods ask the question, "what is the worst that could happen and what is that maximum consequence?" Uncertainty asks, "What could probably happen and what outcome could I reasonably expect?" Also, there are two underlying and fundamental questions that drive the uncertainty calculations: quantitatively speaking, how does each and every variable affect the parameter being measuring, and while analyzing these variables, how can each be traced back to an accepted and recognized unit of measure?

But uncertainty is not a cruel master. If a variable can be shown to have a miniscule and inconsequential effect on the measurand, then a simple statement can express that thought with the accompanying statement that says, “Ignored”.

After the GUM, the scientific community, through NIST, IUPAC and others, quickly published guidelines to assist in interpreting the GUM. It followed that with detailed studies and explanations of how the GUM applies to conductivity and pH measurements.

“Read the directions and directly you will be directed in the right direction.” Doorknob

After reading (and rereading) the IUPAC paper *Measurement of pH. Definition, Standards, and Procedures*, Pure Appl. Chem., Vol. 74, No. 11, pp 2169-2200, 2002, it becomes clear that if one wishes to prepare certified pH buffer solutions expressed with the same decimal precision and with the same uncertainty as NIST, then the Harned cell apparatus is required. If this is impractical, and traditional pH glass electrodes are to be used, then a multi-point calibration utilizing NIST purchased buffers would be the next best thing. This would sacrifice a decimal point, but makes the u_c of the Bates-Guggenheim Convention insignificant (and ignored).

“Would you tell me, please, which way I ought to go from here?” asked Alice. “That depends a good deal on where you want to get to,” said the cat.

If one wanted to learn how to perform the required uncertainty calculations, the first step might be to simply try to reproduce the results from the example IUPAC calculations. A spreadsheet application performs this task handily.

An Excel worksheet was structured with the necessary equations so that when any set of the IUPAC example parameters were entered, the resultant expanded uncertainty value (U) would be displayed. All the IUPAC examples were confirmed in the Excel worksheet, except one. The very critical example of Cell V (section A-5.3 Table-5C: for use with glass electrodes and a multipoint calibration) did not return the same result as the IUPAC paper. It returned twice the IUPAC value. Also, there was a question about two sensitivity coefficients.

After contacting a few of the members of the IUPAC committee that penned the paper, it was confirmed that there was an error. However, it was not clear whether it was typographical or mathematical. The IUPAC document was, by this time, ten years old. The committee had disbanded. The paper trail had gone cold. If we were going to use this template to report uncertainty, we decided to use only what we could support and prove.

The IUPAC paper recommends a 5-point calibration for glass electrodes. By using SRM buffers from NIST, and following the NIST directions for preparation, much of the critical work will have been done. Nearly all traceability to national and international standards will have been established. Nearly all the uncertainty budget in the measurement of a test sample can be explained from examining the linear regression analysis of the calibration (standardization). Most of the remaining uncertainty will come from temperature control and voltmeter specifications. Unlike conductivity solutions, pH buffers are less affected by temperature, so the temperature sensitivity coefficient is minor. It is implied that the user utilizes proper laboratory equipment which is calibrated and traceable to NIST.

A warning: some commercially available pH and conductivity meters may offer multi-point calibrations, but will only use the two nearest ones to bracket the unknown value. In essence, it would be a two point calibration, and will always be a perfectly straight line. With multi-point calibrations, all points must be used for the regression analysis.

Below is a screen-shot of an Excel spreadsheet designed to display the calibration graph, the uncertainty budget chart, and to show the linear regression statistics of the NIST SRM. It is interactive, and cell entries for the voltage values will update the graph and charts immediately. The NIST pH values stay constant until new SRM lots are used, while their corresponding voltage values are updated at each calibration. The voltage value of the sample under investigation is entered into the cell labeled “volts of sample”. The slope and y-intercept of the linear calibration equation are used to calculate a pH value in the cell labeled “pH of sample”. The various standard deviation values from the regression analysis are utilized in the uncertainty budget chart. The regression statistics chart and the uncertainty budget chart are color coded to show which standard deviation is used in the budget.

It is beyond the scope, here, to derive and explain uncertainty calculations. However, the example below shows how the partial derivatives were calculated for the required sensitivity coefficients │ci│.

It has been noted that the budget’s expanded uncertainty value for the IUPAC example of Cell V was questioned. Also, the budget item for the sensitivity coefficient of k'/V was found to be different than the published IUPAC value.

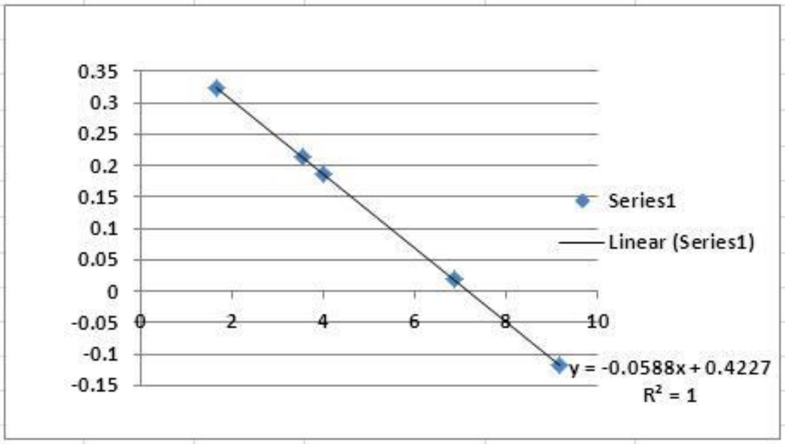
The third item of contention in the IUPAC paper is their uncertainty contribution from temperature. The use of partial derivatives with respect to pH will produce uncertainty contributions in units of pH. The IUPAC temperature uncertainty contribution is not in pH units as written. If 1.98×10^{-4} T is substituted for K in the equation $pH(x) = [E_{vo} – E_v(x)]/k$, then $\partial pH/\partial T$ would be in pH units and its value would be applicable to the budget.

Because of these differences, the expanded uncertainty value is different than it would be if it were calculated using the budget sensitivity coefficients listed in the IUPAC template. Since this discrepancy could not be resolved, it was decided to proceed with what could be proven. Therefore, pH buffers manufactured and tested using these procedures may appear inferior to a manufacturer using the IUPAC factors as published.

“Reeling and Writhing, of course, to begin with, and then the different branches of arithmetic – Ambition, Distraction, Uglification, and Derision.” The Mock Turtle

The following is an example of the uncertainty budget for a pH buffer.

Enter Data Below ↓			cal date→	10/1/2012		
(convert mV to V)			y=mx+b	x=(y-b)/m		
	0.1872	= volts of sample		**4.00775**	pH of sample	
NIST	Buffers		Slope	-5.87588E-02	4.22690E-01	y-intercept
pH (x)	Volts (y)		Std Dev of Slope	5.81678E-05	3.32052E-04	Std Dev of Y-intercept
1.679	0.324		r^2	9.9999706E-01	3.44269E-04	Std Dev y
3.557	0.2137					
4.008	0.187					
6.865	0.0198					
9.18	-0.117					
	↑					
	VOLTS					
r2 =	0.99999706					
% slope =	99.32					
nernst =	-0.059159					



Quantity	Estimate x_i	std uncert $u(x_i)$	sens coeff $\lvert c_i \rvert$	uncert contrib u(xi) * I ci I	$u(xi)^2$	$\lvert ci \rvert^2$	multiply $u(xi)^2 * \lvert ci \rvert^2$
E(m)/V	1.3E-04	1.5E-04	16.9	2.58E-03	2.3E-08	285.61	6.7E-06
E^o/V	4.23E-01	3.3E-04	16.9	5.61E-03	1.10E-07	285.61	3.15E-05
T/K	298.15	0.058	1.98E-04	n/a	n/a	n/a	
E(x)/V	0.1872	3.44E-04	16.9	5.82E-03	1.19E-07	285.61	3.39E-05
K'/V	-0.0588	5.8E-05	68	3.97E-03	3.38E-09	4652.151	1.57E-05
T/V(S)	298.15	0.058	1.34E-02	7.76E-04	3.36E-03	0.000179	6.02E-07
							↓
						sum	0.00009
					sq rt of sum	u =	0.0094
					k = 2	U =	0.019

E(m)= voltmeter accuracy specification(±0.00013V)
E^o = y-intercept
T/k = temperature as it relates to the slope
E(x) = voltage reading from the sample under test
k' = slope of multi-point curve
T/V(s) = temperature as it relates to the pH
u(m)= tolerance from voltmeter / √3 (= 0.00026/1.73)
u(E^o) = standard dev of y- intercept from regression output
u(T/k) = std dev from temperature deviation during measurements (± temp /√3)
u[E(x)] = std dev of y value (sample under test)
u(k') = standard dev. of slope from regression output
u (T/V) = uncertainty from temperature effect on slope during sample testing.
uncertainty due to sample evaporation is equivalent to NIST standards and is ignored
Coverage factor = 2

For most sensitivity coefficients, take the partial derivative of:

$$pH(X) = [E_V{}^{\circ} - E_V(X)]/k'$$

So.. ∂pH/∂k'

With [Ev^0-E(x)] held constant and combining constants into "C", this simplifies to ∂pH/∂k' = Ck'^{-1}

$=-Ck'^{-2} = -C/k'^2$

So.

$\partial pH/\partial k' = [Ev^0 - E(x)]\,(k'^{-1})$

$= -[Ev^0 - E(x)] / k'^2$

Substitute Ev^0= 0.423 and E(x)= 0.1872 into equation→ $-[-0.423 - 0.1872]/0.059^2$

$=0.2358/0.059^2 = 68$

∂pH/∂k' = l ci l =68

∂pH(x)/∂E^0

$=Ev^0/k' - Ev(x)/k'$

$=Ev^0k'^{-1} - Ev(x)k'^{-1}$

$= Ev^0k'^{-1} - 0$

$=k'^{-1} = 1/k'$

=1/.059 = l ci l = 16.95

∂pH(x)/∂E(x)

= same as above

= l ci l =16.95

$$k_{nernst} = \frac{(2.303)\,(R)\,(T)}{(F)}$$

$$k = \frac{(8.3)\,(2.303)}{96500}\;T$$

$$k = 1.98 \times 10^{-4}\;T$$

pH(x) = [Ev° - Ev(x)]/1.98 x 10^{-4}T

∂pH/∂T = {-[Ev° - Ev(x)]/ 1.98 x 10^{-4}}/T^2

= -[0.423-0.1872/.000198] / 88893

=-1192/88893

= -1.34 x 10^{-2}

It can be seen, by the uncertainty contributions in the budget above, that a major contribution to the uncertainty is due to the regression analysis of the NIST SRM. If these NIST standards are prepared properly, their uncertainty contribution can be minimized. A remaining contributor to uncertainty will be the voltmeter and it will be dominant. A high impedance voltmeter with a quality A/D converter and shielded cables is required. Some have 5-1/2 digit displays, and some have 8-1/2. Uncertainty budgets found in some literature do not mention this, or include this in the uncertainty budget.

The above budget example does include the uncertainty from the measurement of the sample under investigation arising from the voltmeter's accuracy. The meter uncertainty while measuring voltage of the NIST buffers is accounted for in the regression analysis.

The temperature control during measurement of the sample under investigation is the same as that of the NIST buffers, and contributes the least uncertainty. Again, the modified pH equation which includes temperature is used to derive the sensitivity coefficient. The uncertainty due to temperature of the NIST buffers is accounted for in the regression analysis.

The White rabbit put on his spectacles. "Where shall I begin, please your Majesty?" he asked. "Begin at the beginning." The King said gravely, "and go on till you come to the end: then stop."