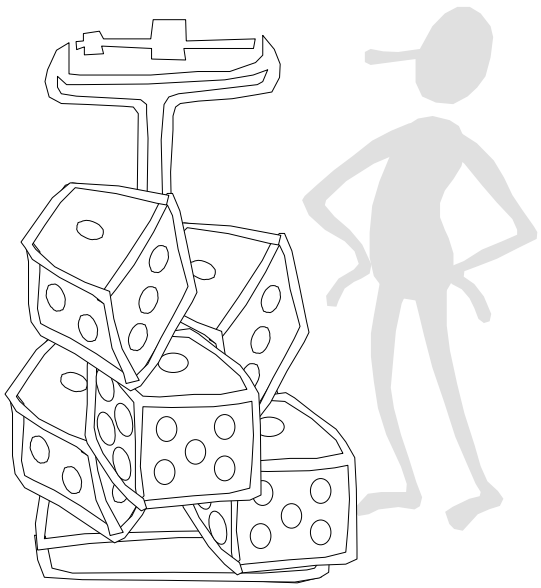
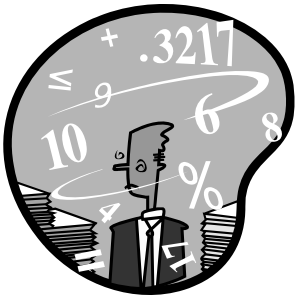


More on Calibration

Statistics 101

There are three kinds of lies -
lies, damned lies, and
-Benjamin Disraeli



Statistics are like a bikini.
What they reveal is suggestive,
but what they conceal is vital
-Aaron Levenstein

"Statistics show:
every two minutes
another statistic is created."
-Anonymous

Calibration Basics

Calibration: Linear Regression is best. Hand Drawn acceptable but discouraged.

levels: Minimum of 3 plus a blank

Frequency: Daily is best; minimum quarterly

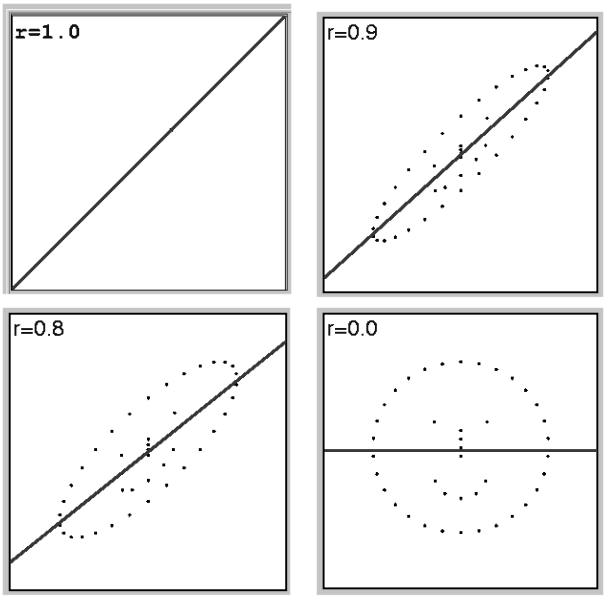
Range: Appropriate for samples

Spacing: Standards should be evenly spaced

Low standard: near but > the LOD; at the LOQ

High standard: Within linear range, set to cover the highest concentration expected.

The Correlation Coefficient



… doesn't always tell the tale!

To Force or not to Force (the Intercept)

- This is science folks!
- All instruments and analytes are associated with background signal noise which is expected to be non-zero
- Therefore science would indicate that an intercept passing through the origin is UNlikely

So…then why would you do it?

How is the line "forced"

Manual (old school)

Iterative inclusion of fictional (0,0) data points until

a) the intercept is "effectively" zero

b) the residuals are minimized

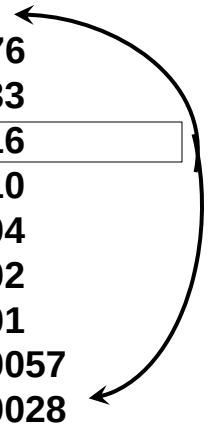
Mathematically

Mathematic regression formula yielding a slope with intercept = 0

$$\frac{\sum(X_{obs}Y_{obs})}{\sum X^2_{obs}} \quad \textbf{vs.} \quad \frac{N\sum(X_{obs}Y_{obs}) - \sum X_{obs}\sum Y_{obs}}{N\sum X^2_{obs} - (\sum X_{obs})^2}$$

Manual Force Example

	Slope	Intercept
Zero Yint	**1.1988**	**0**
Calc Yint	**0.7466**	**0.76**
10 (0,0) data points	**0.7580**	**0.33**
25 (0,0) data points	**0.7603**	**0.16**
50 (0,0) data points	**0.7611**	**0.10**
100 (0,0) data points	**0.7618**	**0.04**
200 (0,0) data points	**0.7621**	**0.02**
400 (0,0) data points	**0.7623**	**0.01**
800 (0,0) data points	**0.7623**	**0.0057**
1600 (0,0) data points	**0.7624**	**0.0028**



Forced Origin & Residuals

$$X_{residual} = [(Y - Intercept) / slope] - X_{true}$$

Back-calculated concentrations…….……..

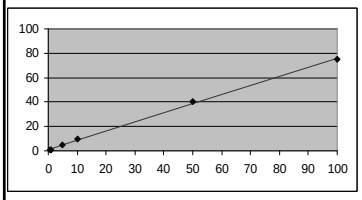
X	Y	Calc. Int.	Zero Int	25 θ s'	1600 θs'
0.5	0.5	-0.94	0.66	0.45	0.65
1	1	-0.27	1.31	1.11	1.31
5	5	5.09	6.56	6.37	6.55
10	10	11.79	13.12	12.94	13.11
50	40	51.97	52.47	52.40	52.46
100	75	98.85	98.37	98.44	98.37

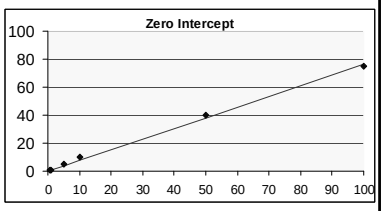
Back-calculation= First step in the calculation of residuals. The predicted concentration for a particular response based on the calibration algorithm.

Residual = The difference between True value and the value predicted by the algorithm used.

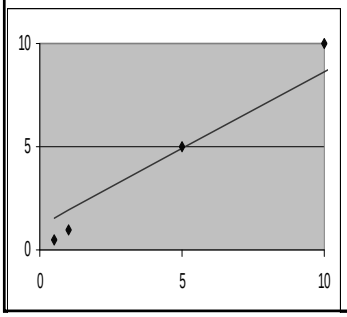
Calculated vs. ZERO Intercept

X	Y
0.5	0.5
1	1
5	5
10	10
50	40
100	75

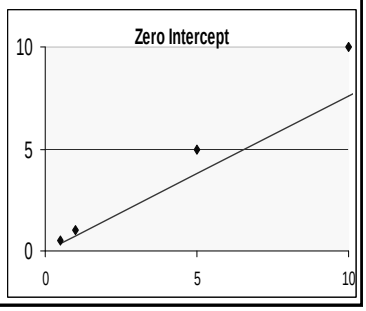




r^2 =0.998
r =0.999
slope=0.7466
Y-int=0.76



r^2 =0.998
r =0.999
slope=1.1198
Y-int=0



Summary: Forcing thru the origin

Generally not recommended unless…

- Calibration performed at very low levels.
- Calibration function has been well documented between "zero" and the first level calibration standard.
- There is no change in slope over the range (no break in the curve).

NOTE: Linearity through zero is a statistical assumption and not a rationale for reporting results below the calibration range demonstrated by the analysis of the standards

If you DO decide to use….

- Monitor residuals over the WHOLE range.
- Consider limiting the useable range to that area over which residuals agree very well with known.

Brief discussion of Non-Linear Functions

Concentration	Response
1	65000
2	140000
5	365000
10	680000
50	2250000

Average CF = 64200

%RSD = 17.3

r = 0.9950574

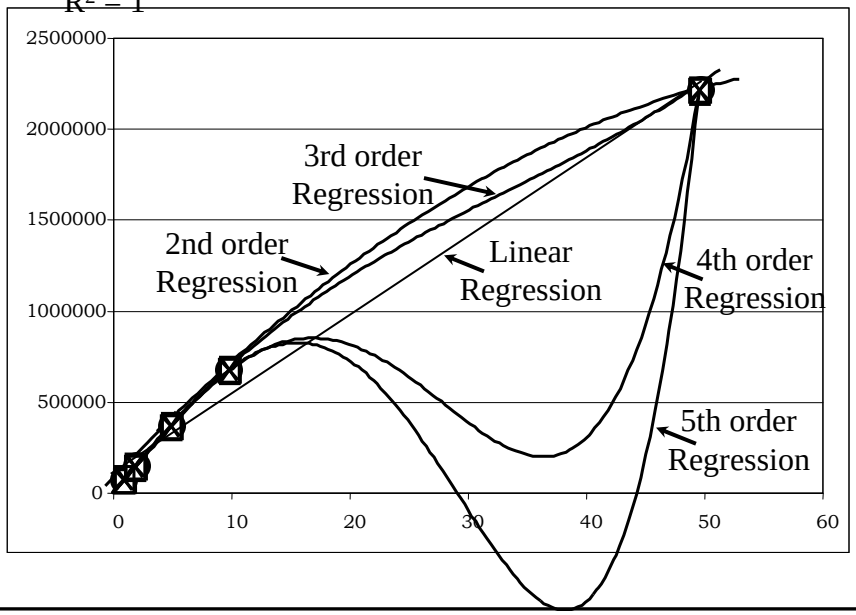
Non-Linear Functions Example

$y = 43320x + 110842$

$R^2 = 1$

$y = 0.059x^5 - 0.1991x^4 - 176.48x^3 + 1406.5x^2 + 72017x - 8247$

$R^2 = 1$



Construction & Verification of Non-linear algorithms

Number of Standards

SW-846 8000B:

...employ five standards for a linear model,
a quadratic (2nd order) model requires six standards,
3rd order polynomial requires seven standards.

Verification

- Ensure that calibration verification levels are designed to check regions of non-linearity
- Include one additional calibration verification check standard for each order beyond a linear regression
  - 2nd order polynomial - 2 check levels
  - 3rd order polynomial - 3 check levels
- Use more standards to characterize the curve!

Higher Order Calibrations Summary

- ☞ Make sure you understand the implications of high-powered statistical tools before using them.
- ☞ Availability of options does not make them appropriate
- ☞ The correlation coefficient is not above reproach!
- ☞ Forcing through the origin is generally not appropriate
- ☞ Always use the lowest order calibration curve possible
- ☞ Use of higher order curves should be justifiable based on the analysis particulars
- ☞ Consider altering calibration range to allow a linear fit
- ☞ Consider using more standards to characterize a curve
- ☞ Include appropriate tools to evaluate the calibration