You can determine the concentration of an unknown concentration Cx by fluorescence using the method of standard addition. To a volumetric flask of volume Vt you add (i) a volume Vx of the unknown concentration Cx and (ii) volume Vs of a solution with a known concentration Cs.

#### For example:

Make five solutions by addition of Vs where Vs =  $n\Delta$  n= 0 to 4;  $\Delta$  = 5 mL

For each solution, the # of moles is CxVx + CsVs and volume is always Vt.

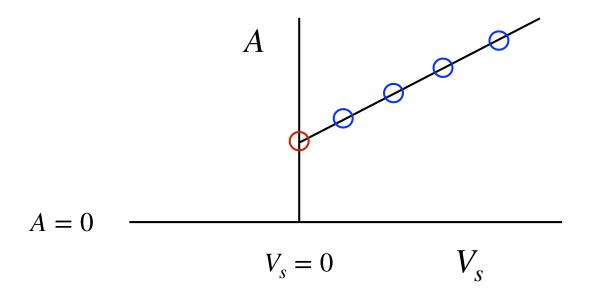
The concentration is: 
$$\frac{C_x V_x + C_s V_s}{V_t} = \frac{C_x V_x}{V_t} + \frac{C_s V_s}{V_t}$$

The Absorbance is: 
$$A = \epsilon d \left( \frac{C_x V_x}{V_t} + \frac{C_s V_s}{V_t} \right) = b + m V_s$$

Plot Absorbance vs Vs and fit with a straight line to get m & b.

$$A = \epsilon d \left( \frac{C_x V_x}{V_t} + \frac{C_s V_s}{V_t} \right) = b + m V_s$$

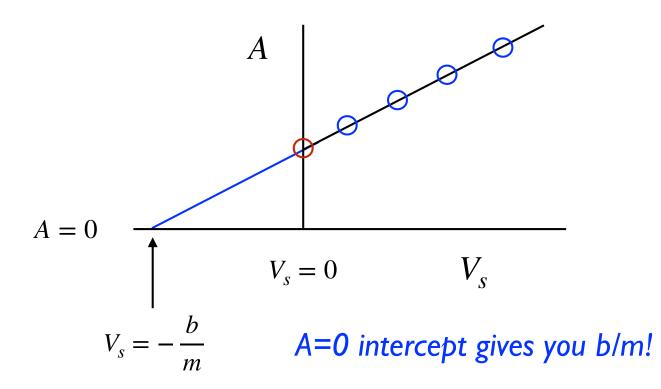
$$\frac{b}{m} = \frac{C_x V_x}{C_s} \qquad \longrightarrow \qquad C_x = \frac{bC_s}{mVx}$$



We can calculate Cx using b/m!

$$A = \epsilon d \left( \frac{C_x V_x}{V_t} + \frac{C_s V_s}{V_t} \right) = b + m V_s$$

$$\frac{b}{m} = \frac{C_x V_x}{C_s} \qquad \longrightarrow \qquad C_x = \frac{bC_s}{mVx}$$



$$A = \epsilon d \left( \frac{C_x V_x}{V_t} + \frac{C_s V_s}{V_t} \right) = b + m V_s$$

$$\frac{b}{m} = \frac{C_x V_x}{C_s} \qquad \longrightarrow \qquad C_x = \frac{bC_s}{mVx}$$

#### Standard deviation for Cx is sc:

$$s_c = \frac{s_r}{m} \sqrt{\frac{1}{N} + \frac{(\bar{y})^2}{m^2 S_{xx}}}$$

Book has different incorrect equation.
See handout & paper.

95% confidence interval:

$$C_x \pm t_{N-2} s_c$$

	Α	В	C	D	E	F	G H	1 .	J K	L
1 !	Standard #	x: Added Vol. (mL)	y: Reading	xi^2	yi^2	xi*yi	N	5	Standard Conc. (ppm)	50
2	1	0	14	. 0	196	0	xbar	2	Volume Unknown (mL)	5
3	2	1	25	1	625	25	ybar	36.6	Total Volume (mL)	25
4	3	2	37	4	1369	74	Sxx	10		
5	4	3	47	9	2209	141	Syy	1301.2	-x-intercept (mL or ppm)	1.210526316
6	5	4	60	16	3600	240	Sxy	114	xc (volume as axis,mL) (ppm)	12.10526316
7	SUM	10	183	30	7999	480			xc (conc. as axis, ppm) (ppm)	6.052631579
8	(SUM xi)^2	100					m	11.4		
9	(SUM yi)^2	33489					b	13.8	Note: LINEST can calculate some of these numbers for you	
LO									Linest Output	
L1							sr	0.73029674	m	b
L2							sm	0.23094011	sm	sb
L3							sb	0.56568542	r^2	sr
L4							sc	0.07106881	F	N-2
.5										
.6							t (N-2)	3.182		
.7									Linest Output	
18							95%CI		11.4	13.8
19							slope (m)	0.73495449	0.230940108	0.565685425
20							Intercept (b)	1.80026349	0.998770366	0.730296743
21							x-intercept	0.22617268	2436.75	3
22									1299.6 1.6	
23										
24									matches what we calculated!	
25										

Please check out our spreadsheet to help you make these calculations!\*

\*M3LC students: see Spreadsheets Folder on the Canvas site.