

LOWRY PROTEIN ASSAY

LOWRY.XLS

The Lowry protein assay has been used by biochemists since the original work was published in 1951*. When a large number of points are used to define the standard plot, the data is not linear, rather it is **hyperbolic**. A double reciprocal plot is constructed from the standard data and the slope (m) and y-intercept (b) of the standard plot are used to determine the concentration of the unknown by the equation shown below.

TOTAL 1.00 mL 800 mL 1st reagent / 10 minute incubation
VOLUME 100 mL 2nd reagent / 1.5 hours at RT

DATA FOR STANDARD PLOT

mL BSA	mL H ₂ O	mg BSA	A ₆₆₀	A ₆₆₀ corr	A ₆₆₀ CALC ¹	1/mg BSA	1/A ₆₆₀	1/A ₆₆₀ CALC ²
0.400								
mg/mL								
0	100	0	0.032	0.000	0.000			
10	90	4	0.099	0.067	0.071	0.250	14.93	14.04
20	80	8	0.172	0.140	0.140	0.125	7.14	7.13
30	70	12	0.241	0.209	0.207	0.083	4.78	4.83
40	60	16	0.302	0.270	0.272	0.063	3.70	3.68
50	50	20	0.364	0.332	0.335	0.050	3.01	2.99
60	40	24	0.426	0.394	0.396	0.042	2.54	2.53
70	30	28	0.480	0.448	0.455	0.036	2.23	2.20
80	20	32	0.553	0.521	0.512	0.031	1.92	1.95
90	10	36	0.606	0.574	0.568	0.028	1.74	1.76
100	0	40	0.655	0.623	0.622	0.025	1.61	1.61
						0.000		0.23

$$^1 A_{660} = \mu\text{g BSA} / (m + b * \mu\text{g BSA})$$

$$^2 1 / A_{661} = m / \mu\text{g BSA} + b$$

DATA AND ANALYSIS FOR SAMPLES

$$\text{UNK} = A_{660} * m / (1 - A * b)$$

CONTROL	A ₆₆₀	A ₆₆₀ corr	UNK mg	mg/mL
mL				
10	0.164	0.132	7.52	0.752
10	0.153	0.121	6.87	0.687
20	0.247	0.215	12.5	0.624
20	0.243	0.211	12.2	0.612

TREATED	A ₆₆₀	A ₆₆₀ corr	UNK mg	mg/mL
mL				
10	0.153	0.121	6.87	0.687
10	0.151	0.119	6.76	0.676
20	0.250	0.218	12.7	0.634
20	0.269	0.237	13.8	0.692

For double reciprocal plot: SUMMARY OUTPUT

Regression Statistics		
Mult R	0.99989	
R Square	0.99977	
Adj R Sq	0.99974	
Std Error	0.02921	
Observ	9	
	Coef	Std Error
b	0.22555	0.0195
m	55.2632	0.3164

SUMMARY AVE mg/mL %

CONTROL	0.669	100
TREATED	0.672	100

*Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951)
Protein Measurement With The Folin Phenol Reagent. J. Biol. Chem. 193, 265-275.

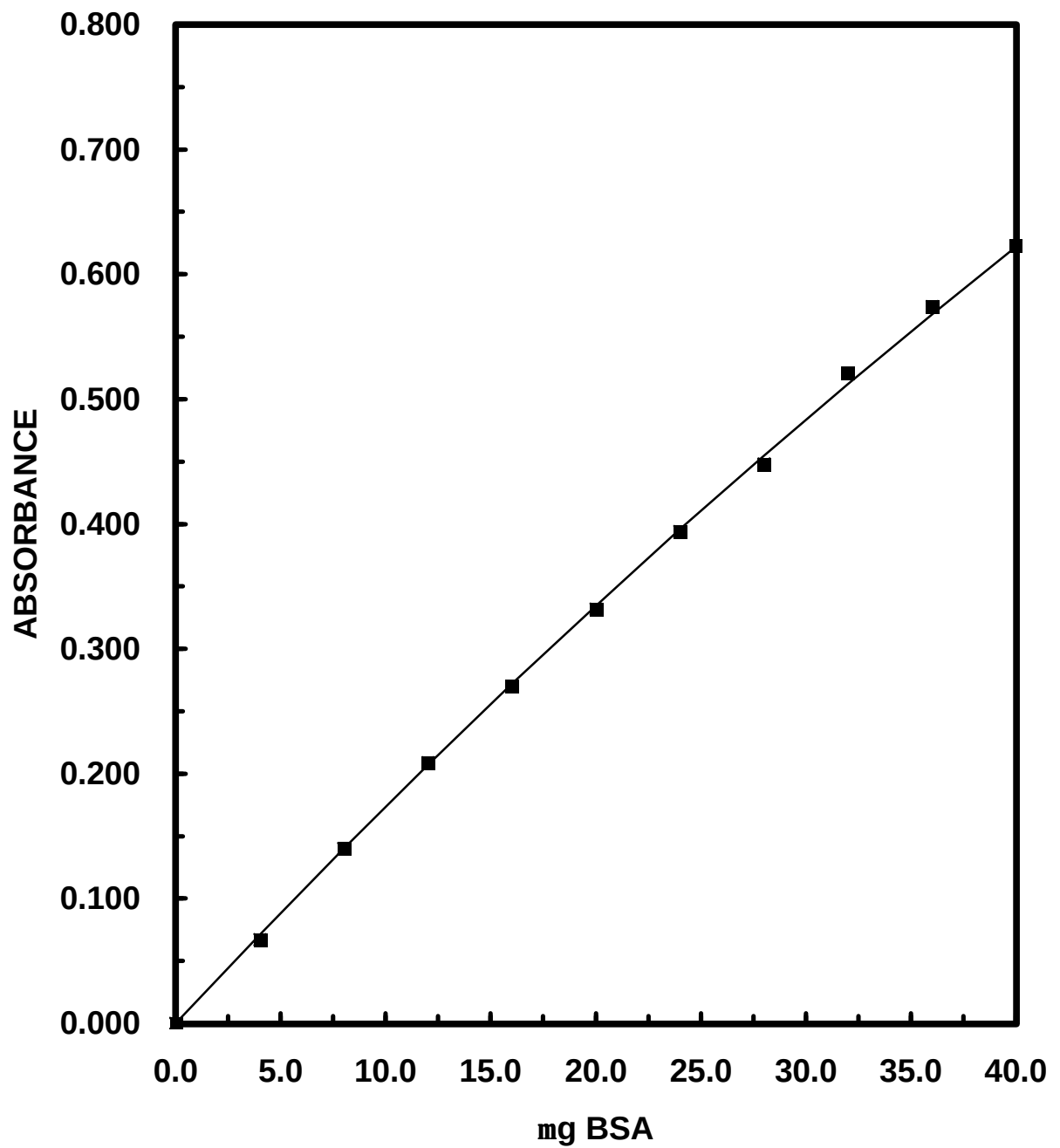


Figure 1a. **Hyperbolic behavior** of Lowry protein assay. The line is a fit to the slope (m) and y-intercept (b) of the double reciprocal plot in Figure 1b.

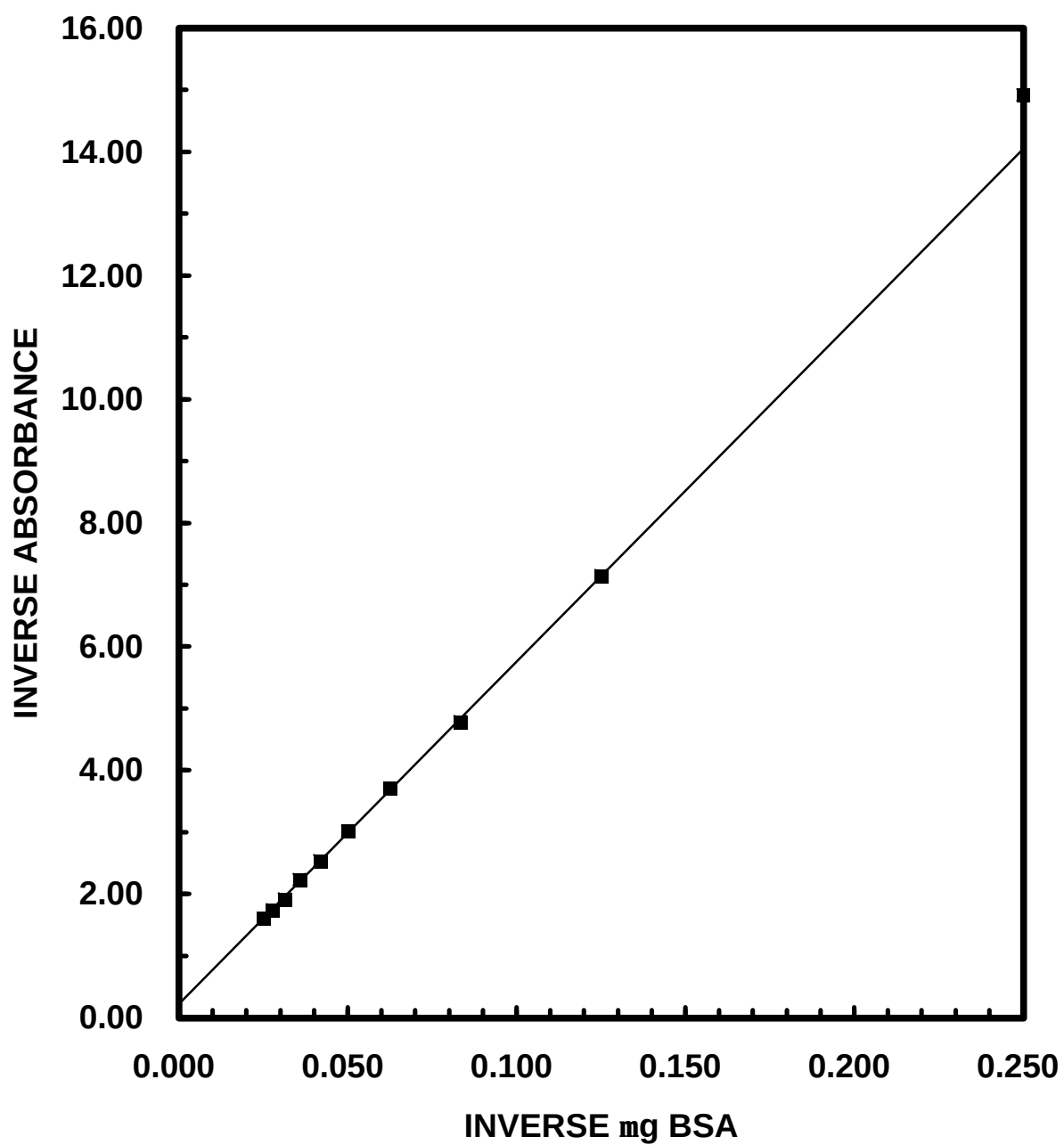


Figure 1b. Double reciprocal plot for the Lowry protein assay. The line is calculated from the slope (m) and y-intercept (b) from a regression analysis of the data.