SAMPLE BRADFORD ASSAY

BRADFORD.XLS
FR 1-122

The Bradford protein assay is a more recently developed protein assay that is commercially available.* 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 as shown by the equation below.

Volume = 1000 mL

DATA FOR STANDARD CURVE

TEST TUBE	mL BRAD Dil 1:1*	mL Water	mL TRIS	mL STD	mg STD 1.80 mg/mL	ABS_{595}	ABS_{595} corr	ABS_{596} [1] CALC	1/mg STD	$1/ABS_{595}$ corr	$1/ABS_{596}$ [2] CALC
0	200	700	100	0	0.0	0.448	0.000	0.000			
1	200	700	99	1	1.8	0.576	0.128	0.117	0.556	7.800	8.57
2	200	700	98	2	3.6	0.669	0.222	0.223	0.278	4.513	4.48
3	200	700	97	3	5.4	0.770	0.322	0.321	0.185	3.108	3.12
4	200	700	96	4	7.2	0.869	0.421	0.410	0.139	2.375	2.44
5	200	700	95	5	9.0	0.948	0.500	0.493	0.111	2.000	2.03
6	200	700	94	6	10.8	1.004	0.556	0.569	0.093	1.798	1.76
7	200	700	93	7	12.6	1.083	0.635	0.640	0.079	1.576	1.56
8	200	700	92	8	14.4	1.157	0.710	0.706	0.069	1.409	1.42
9	200	700	91	9	16.2	1.222	0.774	0.768	0.062	1.291	1.30
10	200	700	90	10	18.0	1.249	0.802	0.826	0.056	1.248	1.21
									0.000		0.39

SUMMARY OUTPUT Inverse A_{595} vs inverse STD (μg^{-1})

Regression Statistics			*Coef*	*Std Err*
Multiple R	0.99848	***b*** =	0.3935	0.837
R Square	0.99697	***m*** =	14.721	3.8272
Adj R Square	0.99659			
Standard Error	0.11981	*units of*	***m*** =	µg
Observations	10	*or*	14.721	µg

[1] ABS_{595} calc = µgSOD/(***m*** +µgSOD*b*)

[2] $1/ABS_{595}$ calc = ***m***/µgSO) + ***b***

*** BIORAD Cat. No. 500-0001**

Branford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding Anal. Biochem 72, 248-254.

DATA FOR UNKNOWN PROTEIN SAMPLES

[3] **UNK** = A_{595} * ***m*** / (1 - A_{595} * ***b***)

CONTROL	mL BRAD Dil 1:1	mL Water	mL TRIS	mL UNK	ABS_{595}	ABS_{595} corr	mg UNK		mg UNK[3] ave	dev mg	ave dev mg	% DEV
11	200	700	98	2	0.637	0.189	3.01	1.51		0.0557		
12	200	700	98	2	0.654	0.206	3.31	1.65	1.56	0.0915	0.05	3.0
13	200	700	96	4	0.812	0.364	6.25	1.56		0.0016		
14	200	700	96	4	0.804	0.356	6.10	1.52		0.0374		
TREATED												
15	200	700	98	2	0.604	0.157	2.46	1.23		0.3337		
16	200	700	98	2	0.627	0.180	2.84	1.42	1.35	0.1395	0.0525	3.9
17	200	700	96	4	0.776	0.329	5.56	1.39		0.1731		
18	200	700	96	4	0.772	0.324	5.47	1.37		0.1939		
PRO_{50}												
19	200	700	98	2	0.638	0.191	3.03	1.52		0.0462		
20	200	700	98	2	0.641	0.193	3.08	1.54	1.52	0.0212	0.04	2.9
21	200	700	96	4	0.799	0.351	6.00	1.50		0.0632		
22	200	700	96	4	0.802	0.354	6.06	1.51		0.0478		
PRO_{100}												
23	200	700	98	2	0.615	0.168	2.64	1.32		0.2413		
24	200	700	98	2	0.661	0.213	3.42	1.71	1.52	0.1475	0.11	7.5
25	200	700	96	4	0.801	0.353	6.04	1.51		0.0518		
26	200	700	96	4	0.808	0.360	6.18	1.55		0.0170		

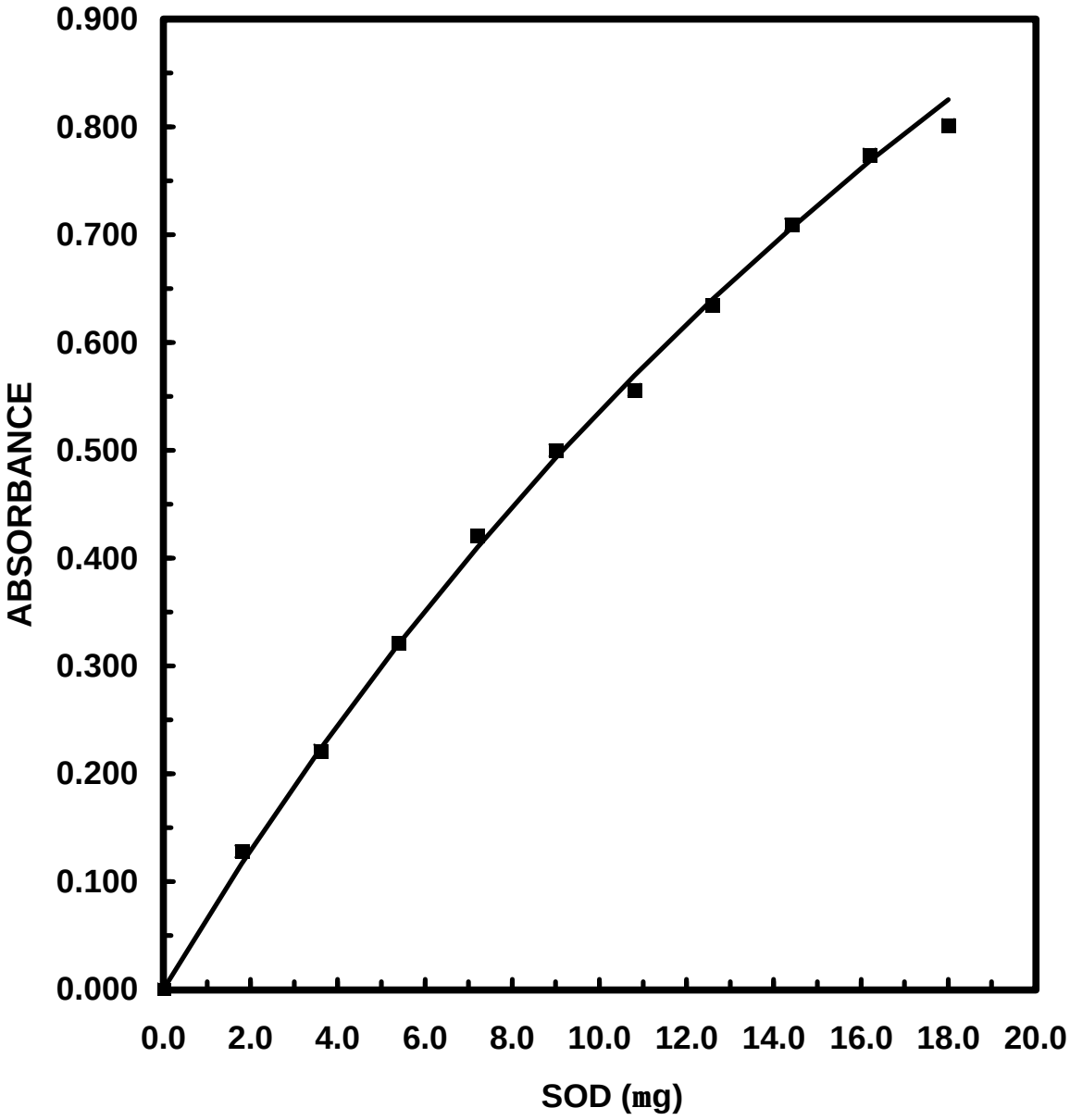

Figure 1a. **Hyperbolic behavior of the Bradford protein assay at 595 nm. The line is calculated from the slope (m) and y-intercept determined from a double reciprocal plot of these data.**

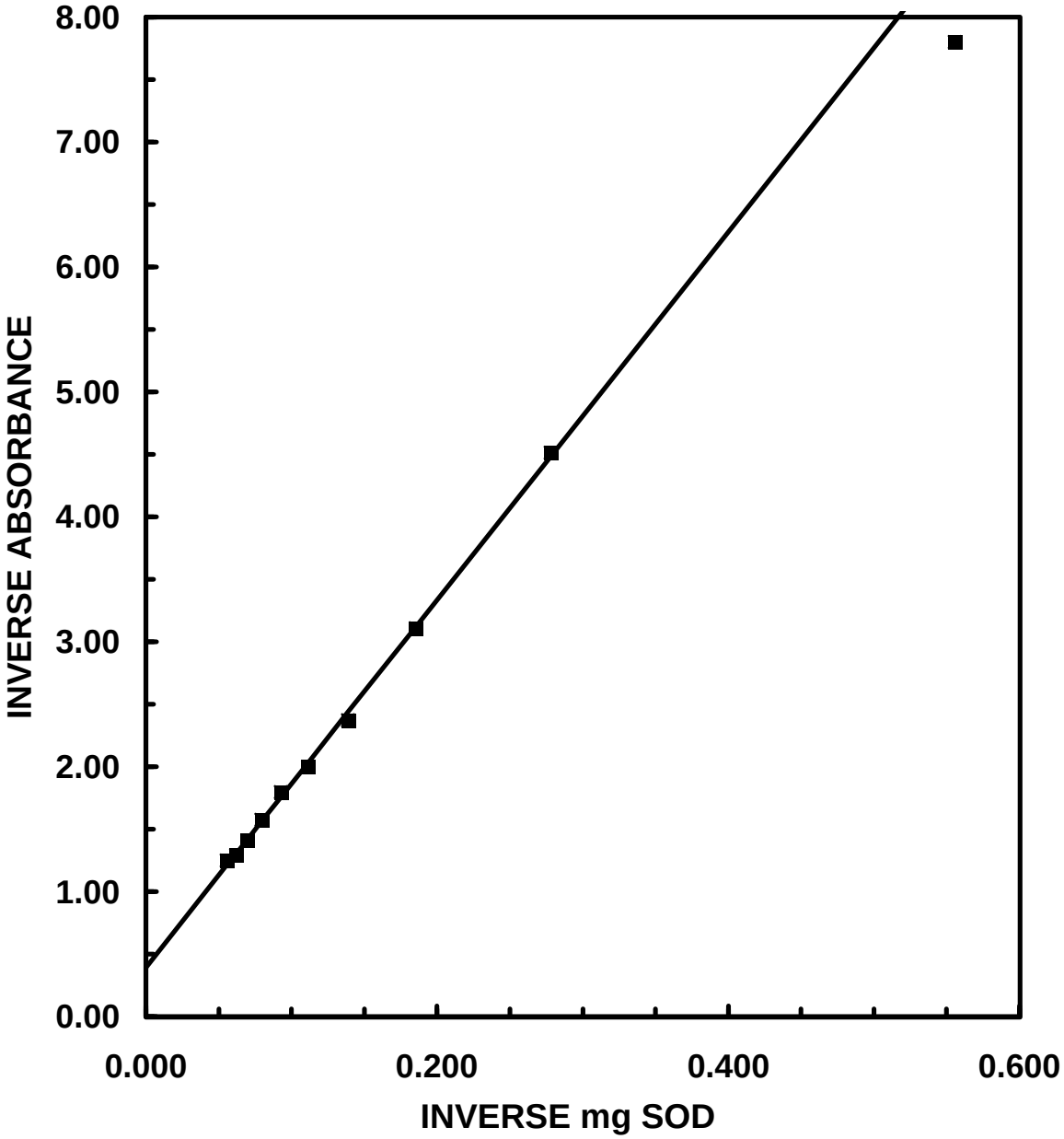

Figure 1b. **Double reciprocal plot of data from Figure 1a. The line is calculated from the slope (m) and y-intercept (b) determined from a linear regression analysis of the data.**