Beer's Law Is Not a Straight Line: Amplification of Errors by Transformation

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Abstract: Beer's law plots are commonly straight-line graphs in which absorbance is plotted versus concentration. The errors in such a plot are accentuated in the high-absorbance region due to the logarithmic transformation. This paper discusses the errors inherent in all Beer's law plots and the choices the student has available: the use of either linear or nonlinear plotting methods.

An experiment is described in which student Beer's law data are treated in several ways to find out if transformation errors are significant. The conclusion is that if the absorbance values obtained for the calibration data exceed $A = 1$, then nonlinear regression or weighted linear regression is indicated.

When using radiation absorption instruments (spectrophotometers) to determine concentrations, the straight-line relationship between absorbance and concentration seems a simple analytical tool. It is not that simple, however, for the statement that absorbance is proportional to concentration is a mathematical transformation of the exponential nature of the transmittance–concentration relationship. This transformation, $A = -\log T$, has a striking influence on the errors that occur in experiments, particularly in regions of high absorbance. We set out to determine if these errors make much difference in the typical student Beer's law determination of an unknown concentration.

Instruments used in chemistry to measure the concentration of a species of interest fall into two categories. Either the instrument response (called the signal) is directly proportional to the concentration of the species, or it is not. If it is not, then some known function relates the signal to the concentration. When using instrumentation to determine an unknown concentration, it is important to know which type your instrument is because it affects the error analysis of the result. Electrical conductance meters, fluorometers, and osmometers are three examples of instruments of the first type. Their signal is, at least approximately, proportional to concentration. Any nonlinearities can be taken into account by suitably small calibration ranges or by curve fits to the calibration points.

Many students are surprised to find out that Beer's law instruments, which means all absorption spectrophotometers, fall into the second category of instruments. The origin of the surprise is that students think that the Beer's law signal is absorbance, and we all know that absorbance is linearly related to concentration. But the signal is transmittance, not absorbance; therefore, because of the transformation to a logarithmic function, error analysis for Beer's law experiments should not be done using the usual straight-line error analysis equations [1–5].

The mathematical transformation, $-\log T = A$, is commonly performed either by the analyst or by the instrument itself, and *A is* proportional to concentration. Thus, the familiar $A = abc$ form of Beer's law is better known than the true Beer's law equation, the exponential decay equation.

Beer's law, more correctly called the Beer–Lambert law, is a statement that the intensity of light, *I*, exiting from an optical absorption cell is exponentially related to the intensity entering the cell,

$$I = I_0 \times 10^{-kC} \tag{1}$$

Here, I_0 is the intensity of the entering light, I is the intensity of the exiting light, C is the concentration of absorbing species in the solution, and k is a decay constant. This exponential decay is far from a straight line (Figure 1a).

Most of us think of this law as a straight line because of a transformation of the variables. The ratio I divided by I_0 is defined as T, the transmittance, and $-\log T$ is defined as A, the absorbance. Thus $A = kC$. Plot A versus C and a straight line results, one that in theory passes through zero. By plotting the data transformed, our eyes are able to envision the straight-line data fit (Figure 1b) much easier than the decaying-exponential one.

One more change is usually made to the equation. The constant k depends upon the absorbing species (its optical properties at the wavelength chosen) *and* on the cell path length. It is usual to write

$$A = abC \text{ or } A = \varepsilon bC \tag{2}$$

In these two forms b is the path length, usually in centimeters, and a or ε are decay constants characteristic of the absorbing species. When ε is used, the concentrations are in moles per liter, and ε is called the molar absorptivity. In older texts ε is called the molar extinction coefficient. If a is used the concentration units can be anything convenient, including mol L^{-1}, and a is usually simply called the absorptivity. In our linear plots the ab in equation 1 is combined into k, the slope of the line as determined by regression.

To illustrate the errors that can arise in spectrophotometry, we generated a set of perfect Beer's law data with a spreadsheet. Using the equation

$$T = T_0 \times 10^{-1 \times k \times C} \tag{3}$$

Table 1. PERFBEER Data from $T = T_0 \times 10^{-1 \times k \times C}$ with k = 15 L mol^{-1}, T_0 = 1

Concentration	T	A
mol L^{-1}	unitless ratio	–log T
0	1.000000	0.000
0.004	0.870964	0.060
0.008	0.758578	0.120
0.012	0.660693	0.180
0.016	0.57544	0.240
0.020	0.501187	0.300
0.024	0.436516	0.360
0.028	0.380189	0.420
0.032	0.331131	0.480
0.036	0.288403	0.540
0.040	0.251189	0.600
0.044	0.218776	0.660
0.048	0.190546	0.720
0.052	0.165959	0.780
0.056	0.144544	0.840
0.060	0.125893	0.900
0.064	0.109648	0.960
0.068	0.095499	1.020
0.072	0.083176	1.080
0.076	0.072444	1.140
0.080	0.063096	1.200
0.084	0.054954	1.260
0.088	0.047863	1.320
0.092	0.041687	1.380
0.096	0.036308	1.440
0.10	0.031623	1.500
0.104	0.027542	1.560
0.108	0.023988	1.620
0.112	0.020893	1.680
0.116	0.018197	1.740
0.120	0.015849	1.800
0.124	0.013804	1.860
0.128	0.012023	1.920
0.132	0.010471	1.980

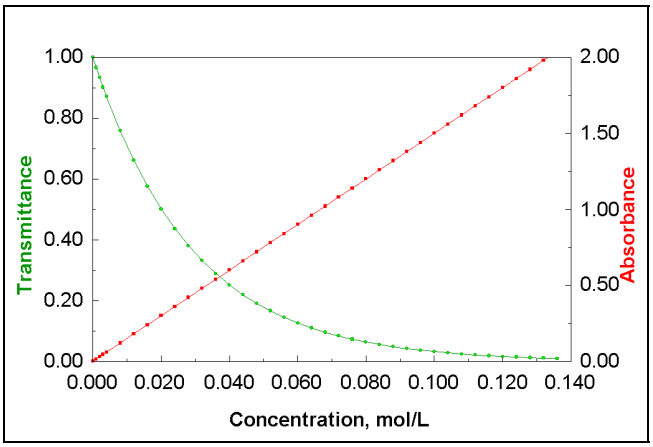


Figure 1. "PERFBEER" data from $T=T_0 \times 10^{-1 \times k \times C}$ with K=15 L mol^{-1}, T_0=1 and C as shown. A). Plot of T vs. C in green. B). Plot of -log$T$ vs. C in red.

with k =15 L mol^{-1}, T_0 = 1, and C values from 0.004 to 0.132 mol L^{-1}, we generated the data table PERFBEER (Table 1). T_0 stands for an adjustable parameter, which in ideal data sets will always equal 1, but in real data sets will not necessarily be 1, but a parameter adjusted by the nonlinear regression program. See Figures 1a and 1b. The intercept, i, plotted linearly will be –log T_0. For plotting purposes we use the symbol i as the intercept of a linear plot and k as the slope of that plot. Again, the parameter k is in reality ab in the commonly seen form of Beer's law, $A = abc$, and the parameter i is the y intercept of the linear plot. When we do nonlinear plots we call the pre-exponential factor T_0 and the decay constant k because it is numerically the same as the line slope in the linear form.

Errors in Exponential Decay Curves and Distortion of This Error by Transformation

If instruments actually measured A rather than T, then a constant error in measurement of A would mean a constant error in deducing the concentration from that A reading. Because T is what the instrument measures, a look at the exponential decay curve (Figure 1a) shows that a constant error in T, call it ΔT, would *not* mean a constant error in concentration. The varying slope of the line shows that the resulting error in concentration, ΔC, would be small at low concentrations, high at large concentrations.

The inverse of the slope times the constant error in T would be the resulting error in C, the concentration error. This error would be small (but not zero) at low concentration values, and with increasing concentration it would become very large.

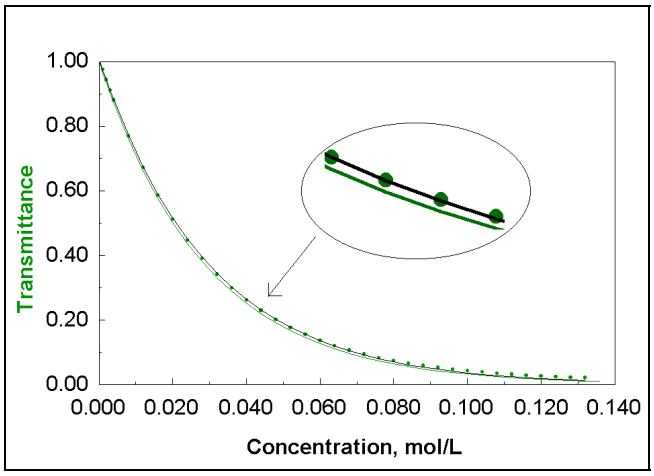
The relative error in concentration would be this error divided by the concentration (relative error = $\Delta C/C$). The percent error is, of course, this times 100. The division by C results in a relative error that is more favorable at some concentration values than at others. This can be illustrated by taking some "perfect" Beer's law data and introducing a constant transmittance error into each point.

Constant Error in *T*

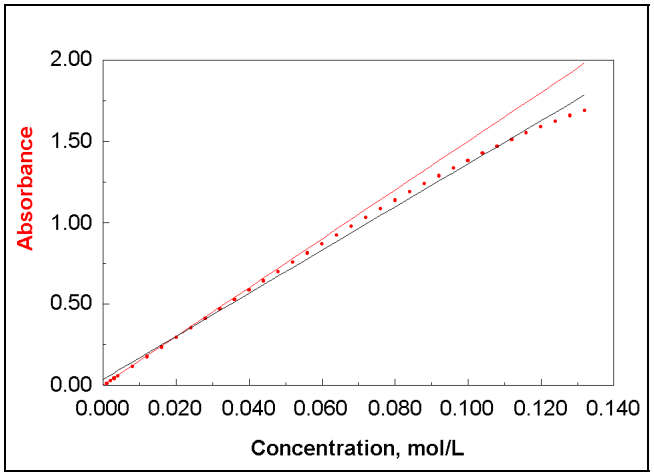
As previously described, the plots in Figures 1a and 1b were created by generating perfect Beer's law data using a spreadsheet. By simulating error in this data it is easy to see the different ways that errors show up in the two kinds of plots, exponential and linear. After seeing these plots with constant simulated error, we became convinced that nonlinear regression on the T versus C plot is to be preferred over the usual linear regression on the A versus C plot. In the computer age, nonlinear regression is no more difficult than best-straight-line fits (Please see Endnote 1 located in the supporting material, 43jb1897.pdf).

We introduced a constant error of +0.01 into each data point. It is important to realize that the error is introduced into T, not A. Instruments measure T, not A. If an instrument gives a read-out of A, it is because the instrument converted from transmittance to absorbance by taking the negative logarithm of T. The resulting plot (Figure 2a) looks much the same as the perfect data plot (Figure 1a). After introduction of this error, the parameters reported by nonlinear regression were T_0 = 1.0026 and k = 14.5. Note that this constant error made about a 3% difference in the k parameter.

Next, we plotted the same data in the transformed (linear) way (Figure 2b). At high concentrations the small constant error in A. The linear regression parameters for this set of

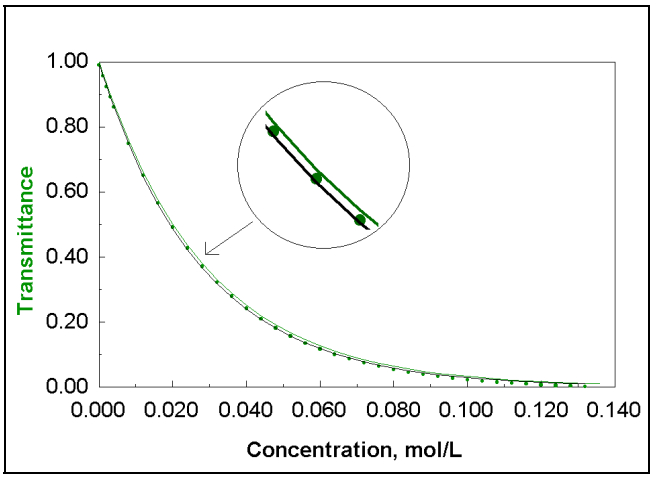


A



B

Figure 2. A). Plus 0.01 error in *T*. ●●●●●● Data with 0.01 error, —— Perfect line before error, —— Nonlinear regression line. B). Plus 0.01 error in *T*. ●●●●●● Data with 0.01 error, —— Perfect line before error, —— Linear regression line.



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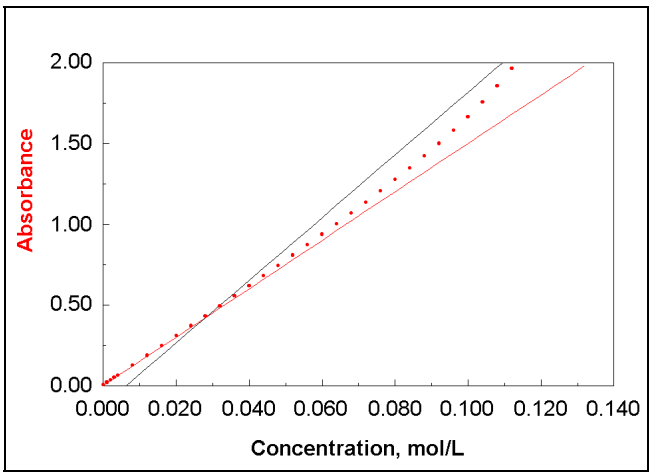


B

Figure 3. A) Minus 0.01 error in T. ●●●●●● Data with 0.01 error, —— Perfect line before error, —— Nonlinear regression line. B) Minus 0.01 error in *T*. ●●●●●● Data with 0.01 error, —— Perfect line before error, —— Linear regression line.

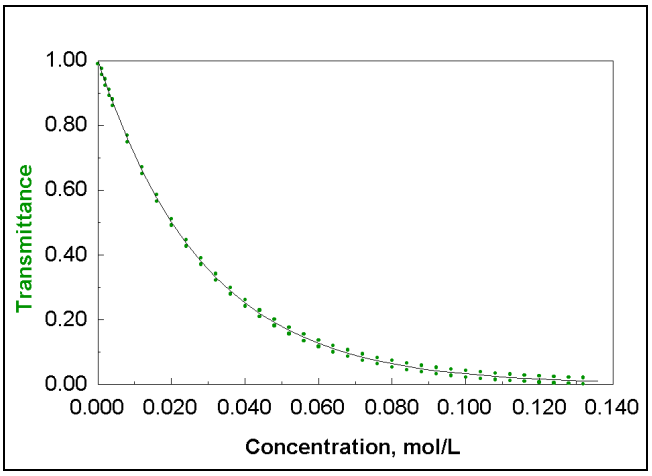
error in *T* gets transformed into a surprisingly large, negative points are intercept, *i*, equal to 0.0471 and slope, *k*, equal to 13.12. The slope of the regression line is 12.5% too low.

Repeating the process nonlinearly with a –0.01 error results in small changes (Figure 3a), but an even more striking deviation from straightness is obtained when plotted with a transformed variable (Figure 3b). Here the intercept is –0.1626 and the slope is 19.83. The slope of the regression line is 32.2% too high.

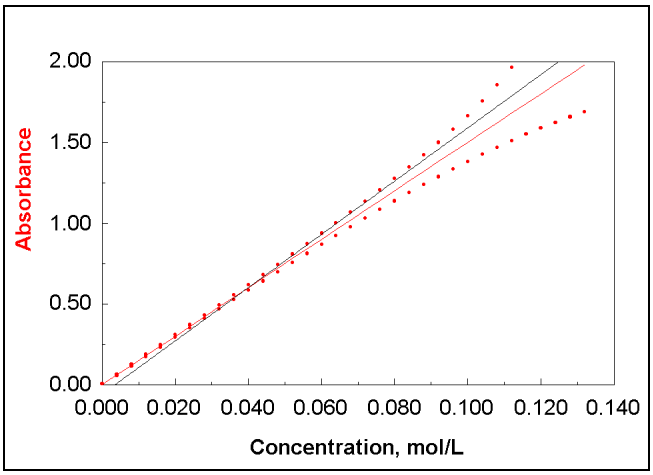
Plotting both $T \pm 0.01$ data in one graph and doing nonlinear regression on the combined set of data points yields the correct result (Figure 4a). The result is correct because the errors have canceled. But plotting combined plus and minus errors linearly is a very different story. See Figure 4b. Here, because the transformed errors do not cancel, the best straight line is not good at all. In all fairness we should point out that such gross errors do not result until the absorbance exceeds unity.

A Closer Look at the *T* Versus *C* Data Before Transformation

The *T* versus *C* plot does not exhibit these gross errors because it was the transformation to *A* which caused them. Still, there are errors before transformation because of the curvature of the *T* versus *C* plot. If we inspect the middle region of this plot (Figure 5), we see that the slope is intermediate here, and that the steeper this slope the more accurate the determination should be. What can we say about the error in concentration introduced by our 0.01 errors in *T*? We can see that the error in concentration, ΔC, gets worse as the concentration increases, because the slope of the line is decreasing. But analytical chemists are interested in the relative error, ΔC divided by *C*. What happens to the relative error in moderate, high, and in low concentration regions? Remember that relative error is inversely proportional to *C* and also inversely proportional to the slope of the line.



A



B

Figure 4. A) Both plus and minus 0.01 error in T. Perfect line and nonlinear regression line are the same line. B) Both plus and minus error in *T*. ●●●●●● Data with ±0.01 error, —— Perfect line before error, —— Linear regression line.

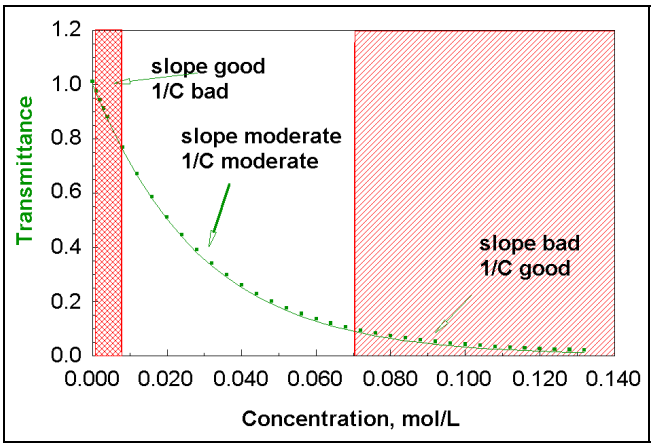


Figure 5. Three regions of the T versus C plot. ■■■■■■ Data with 0.01 error, —— Perfect line before error.

Error at Moderate Concentrations. Because *C* is moderate and $\Delta T/\Delta C$ is moderate, the relative error is a minimum in this region.

Error at High Concentrations. In the high C region the relative error would seem to get smaller still because of C itself getting larger, except that the slope of the line gets extremely small. This smaller slope increases ΔC for a given ΔT and increases the error faster than can be compensated for by the increasing C in the denominator. Thus, the relative error goes up in this region.

Error at Low Concentrations. Inspecting the low C region, we see that the slope is favorable to yield a small ΔC for a given ΔT, but the C itself is small, and because C is in the denominator of the relative error expression the result is that the smallness of C overrides the bigness of the slope, and the relative error becomes increasingly large at these small concentrations.

The Twyman–Lothian Plot

F. Twyman and G. F. Lothian recognized all of this in 1933, and published [6] a simple derivation of the relative error versus transmittance equation:

$$\frac{1}{C}\frac{dC}{dT} = \frac{0.434}{T \log T} \qquad (4)$$

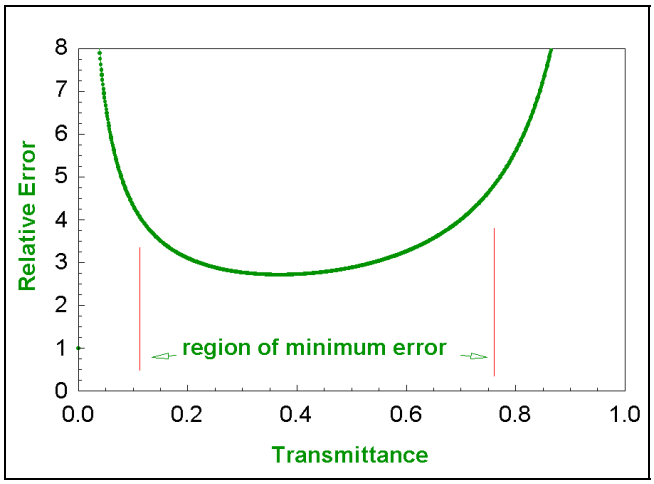
(Please see Endnote 2 located in the supporting material, 43jb1897.pdf.)

Because dC/dT is always negative, plotting $-1/C\ dC/dT$ versus *T* gives a minimum relative error plot that is familiar to most instrumental analysis students [7]. (See Figure 6A.) The *y* axis of a Twyman–Lothian plot is the relative fractional error in the concentration for each fractional error in measurement of *T*. Put another way, it is the relative standard deviation of the concentration answer for each relative standard deviation of the *T* values.

Errors are minimized when *T* is in the range of 0.1 to 0.75, which corresponds to *A* values between 1 and 0.12. Outside of these ranges we expect greater error. Figure 6b is the same plot of relative error, but plotted versus *A*. These Twyman–Lothian plots show probable relative error in the unknown for a given experimental error, Δ*T*. This error will be most pronounced outside the regions shown because of the exponential nature of *T*. It is important to note that amplification of these errors by conversion to logarithms is not shown on these plots, and that this extra error is most pronounced in the high *A* (low *T*) region.

Real Data

A recent paper in the literature [8] titled “The Remarkable Resilience of Beer’s Law” seems to suggest that Beer’s law can be used in these regions of very low and very high absorbance, despite Twyman–Lothian errors and transformation errors. This data set of Muyskens and Sevy is not typical Beer’s law data as students might collect it. It is data on very-low-pressure gas samples collected using very sophisticated laboratory equipment. The light source was a laser, and the detector was a special time-averaging pulse detector. Such results may mislead students into thinking that Beer’s law should work fine for their solutions at all values of absorbance (Please see Endnote 3 located in the supporting material, 43jb1897.pdf).



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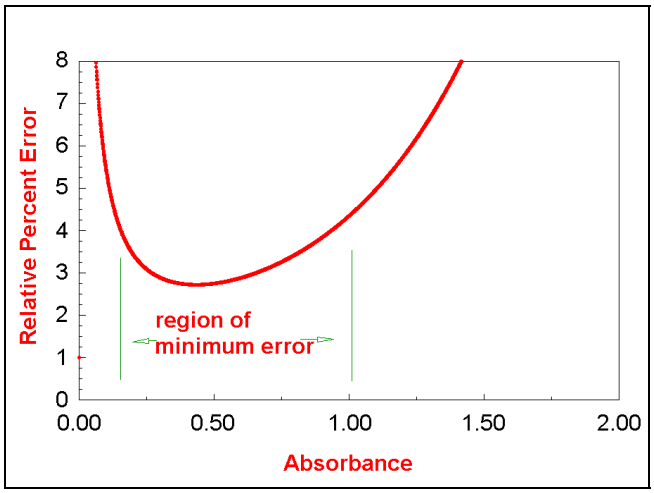

B

Figure 6. A). The Twyman-Lothian plot of relative error versus *T*. B). The Twyman-Lothian plot of relative error versus *A*.

Student Data

We hope to answer the question, "Should students plot Beer's law data nonlinearly (*T* versus *C*) and do nonlinear regression, or is it satisfactory to plot *A* versis *C* and do linear regression, as probably most do?" Real student solution data are expected to show errors, particularly outside of the recommended concentration range. In order to answer the question posed above, "Does it make any difference?," we analyzed student data taken on an inexpensive spectrophotometer. The instructor is presumed to have the right answer to the "unknowns." Students A, B, C, and D took data (Table 2) on a Milton Roy Company Spectronic 20D spectrophotometer at 395 nm using standard solutions, which they prepared by serial dilution from a 1.00 M nickel(II) nitrate stock solution [9]. The path length of the cells was 1.165 cm. Two of the data sets were kept in the recommended range, but the other two sets were extended intentionally beyond this region to answer the question about which plotting method is superior. The students were also given an unknown solution which fell in the range 0.01 to 0.10 M nickel(II) nitrate.

Table 2. Student Percent *T* Data Taken on a Spectronic 20D for Nickel(II) Nitrate Solutions

Concentration mol L^{-1}	Student A %*T*	Student B %*T*	Student C %*T*	Student D %*T*
0.004	95.2			
0.01	89.2	88.0	88.2	88.2
0.02	79.0	78.8	78.6	77.8
0.03	70.0	68.5	69.7	68.5
0.04	62.3	61.0	61.0	60.5
0.05	55.8	54.5	54.8	54.2
0.06	49.6	47.5	48.0	47.4
0.07	43.4	42.0	42.5	41.7
0.08	39.2	38.0	38.0	37.1
0.09	34.5	33.0	33.1	32.8
0.10	30.6	30.0	29.6	29.9
0.11	26.8			27.4
0.12	24.2			24.8
0.13	21.6			21.5
0.14	19.6			19.4
0.15	17.9			18.7
0.16	16.0			16.9
0.17	13.8			13.2
0.18	12.4			12.4
0.19	11.2			11.4
0.20	10.2			10.6
0.30	4.8			4.6
0.40	2.1			2.9
0.50	1.0			1.1
Unk. soln.	67.0	67.0	67.0	64.9

What to Do with Beer's Law Data

We recommend that when a student has finished taking data, the next step should be to make a hand-drawn graph of these data on graph paper. For the graph, absorbances should be calculated from transmittance readings and a plot of *A* versus *C* prepared. The graph should have points only, no lines, and certainly not lines connecting each point. Inspection of the graph should lead the student to one of five courses of action as follows:

1) The points seem to fall on a line, and the unknown is within these calibration points. Conclusion: Use a computer to do standard linear regression on the calibration points and deduce the concentration of the unknown from the parameters *i* and *k* for this least-squares line.
2) The points do not fall on a straight line. The deviations are in the high *A* region. The unknown absorbance falls within the straight region. Conclusion: Do standard linear regression, ignoring (that is not entering) the high *A* outliers.
3) The points do not fall on a straight line. The deviations are in the high *A* region. The unknown absorbance falls in or near the curved region. Conclusion: Do one of the following procedures.
    a) Perform a nonlinear regression analysis on the *T* vs. *C* data. Use the T_0 parameter to calculate *i* from the equation $-\log T_0 = i$. Use *i* and *k* as you would with linear regression to calculate the unknown concentration.

Table 3. Student Results (from Table 2) Treated Two Ways

Student Results: Nonlinear Data Analysis				
	Student A	Student B	Student C	Student D
Parameters returned	i = 0.0882	i = 0.0034	i = -0.00029	i = 0.1207
	k = 4.095	k = 5.26	k = 5.298	k = 3.868
Parameter confidence	i = ± 0.3	i = ± 0.003	i = ± 0.35	i = ± 0.002
limits	k = ± 0.018	k = ± 0.051	k = ± 0.021	k = ± 0.029
Equation of best line	A = 0.088+	A = 0.003+	A = 0+	A = 0.12+
	4.1 × C	5.26 × C	5.298 × C	3.8 × C
Unknown answer	0.022 ±	0.032 ±	0.033 ±	0.017 ±
reported	0.01	0.0009	0.0007	0.02
Percent error	-33%	-3%	0%	-48%
	Student Results: Nonlinear Data Analysis			
	Student A	Student B	Student C	Student D
Parameters returned	T_0 = 0.997	T_0 = 0.996	T_0 = 0.999	T_0 = 0.973
	k = 5.069	k = 5.29	k = 5.288	k = 5.045
Parameter confidence	T_0 ± 0.008	T_0 ± 0.01	T_0 ± 0.0007	T_0 ± 0.017
Limits (95%)	k ± 0.07	k ± 0.09	k ± 0.07	k ± 0.138
Equation of line	$T = 0.997 \times 10^{-5.069 \times C}$	$T = 0.996 \times 10^{-5.29 \times C}$	$T = 0.999 \times 10^{-5.288 \times C}$	$T = 0.97 \times 10^{-5.04 \times C}$
Unknown answer	0.034 M ±	0.033 M ±	0.033 M ±	0.035 M ±
reported	0.001	0.001	0.0002	0.003
Percent error	3%	0%	0%	6%

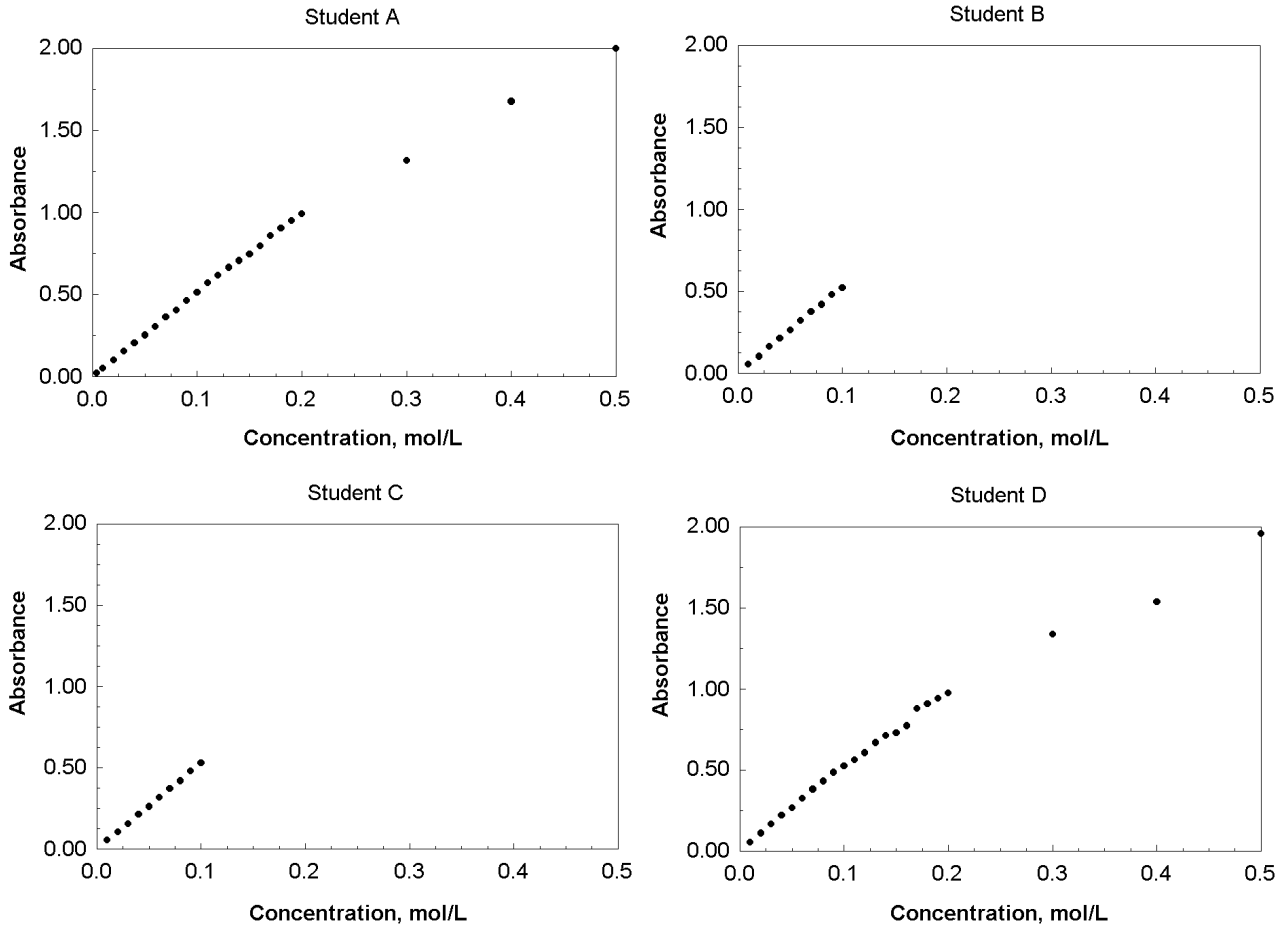

Figure 7. Student preliminary plots from data in Table 2.

Table 4. Five Methods Applied to Student Data. Numbers are the Percent Error Using Each Method on the Unknown Solution (Concentration 0.033 M)

Student	A	B	C	D
Nonlinear (exponential)	3%	0%	0%	6%
Nonlinear (quadratic)	-5%	-2%	0.6%	4.2%
Linear (weighted)	6.7%	-1.5%	-0.6%	11.2%
Linear (cropped)	3%	4.8%		
Linear (regular)	-33%	-3%	0%	-48%

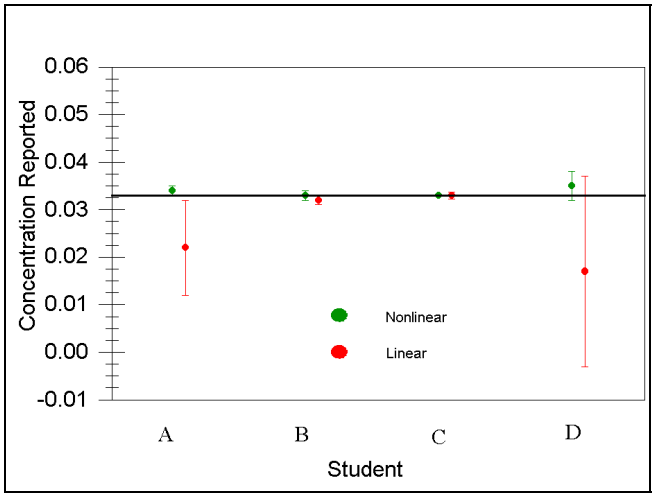

Figure 8. Error Bars for Linear and Nonlinear Regression.

b) Use nonlinear regression to fit a quadratic equation to the *A* vs. *C* data. Solve the quadratic equation to find the concentration of the unknown. This technique is inferior to 3(a) above and 3(c) below, but it is better than simple linear regression.

c) Perform a weighted linear regression on the *A* vs. *C* data. Use a weight factor of T^2 [16]. Use the parameters reported to calculate the unknown concentration.

(Please see Endnote 4 located in the supporting material, 43jb1897.pdf)

Taking Our Advice

Table 2 contains the student data. Figure 7 shows the student data plotted linearly. Students B and C could see that the short data sets did not need elaborate techniques. Their use of a simple *A* versus *C* plot gave good answers (Table 3). Students A and D had data sets that were definitely curved. They chose correctly to chop off the high *A* data (above 0.10 M) and thus got reasonable results (Table 4, cropped). We then took the four data sets without truncation to test the techniques of nonlinear, weighted linear, and quadratic fit. We wished to see how effectively each of these could compensate for the curvature in two of the data sets. Table 4 shows the percent by which the student would miss the unknown concentration (0.033 M) using all the methods.

Several things are clear from the results of the student's data sets. If students stayed in a small range within the Twyman–Lothian limits they had no difficulty getting good results. The inexpensive spectrophotometer was quite sufficient for the experiment. Using nonlinear data analysis was only slightly better than linear regression for the short data sets. Because analysis of the confidence limits for the answer is somewhat easier for linear plots [12], it may not be worth the trouble to go to nonlinear regression when the data do not exhibit curvature.

In the two cases of the extended data ranges, the transformed data is definitely not linear, and the simple linear-least-squares data analysis gives awful answers (Figure 8). The students were able to see that the exaggerated curvature (Figure 7) caused the poor results by linear analysis. These are the data sets that needed cropping, since the unknown falls in the linear region.

Conclusion

Beer's law is not a straight line. Better fits to data with serious errors are found by nonlinear regression on *T* versus *C*, rather than by transformation to *A* followed by simple linear regression on the *A* versus *C* plot (Table 3). If the data are kept within the Twyman–Lothian limits of $T = 0.1$ to 0.75 ($A = 1$ to 0.12) then the differences between the two methods are small, even with student data from an inexpensive instrument. If there is obvious curvature in the high absorbance region of a linear plot**,** *and if these points cannot be discarded because the unknown is also in this region*, the student would be well advised to use nonlinear regression of *T* on *C*. What seem gross errors on a linear plot are not always bad points, unless they are well beyond the Twyman–Lothian limits. State-of-the-art instrumentation will allow experimentation in the higher absorbance region [7], but with ordinary spectrophotometers the student is advised to avoid the region beyond $A = 1$, either by dilution of the samples or by changing the cell path length if that is an option. If these options are not available, then nonlinear regression will minimize the errors in the low *T* region.

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