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An Assay for the Estimation of Organic Content in Unknown Biological Samples

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Abstract

Potassium dichromate, in an acidic solution, will oxidize organic material. The reduction to the chromium ion is associated with a color change, which can be measured as a change in absorbance using a spectrophotometer. The degree of change is linearly related to the total energy contained in a sample and this information can be used to predict the energy content of unknown samples. However, the slopes of these relationships are significantly different for carbohydrates and proteins. It is hypothesized that these variations are due to differences in the reaction kinetics and that these differences can be used to predict the biochemical composition of mixtures of proteins and carbohydrates. Data collected to date indicate changes to the procedure are necessary for accurate estimation of biochemical content.

Introduction

In studying energy in biological systems it is often necessary to quantify total energy content within that system or within other systems with which it interacts. This includes, but is not limited to, the total energy content of an organism and total organic carbon content in environmental studies. This information can help provide clues about an organism's diet, metabolism and life history. In soil analysis it is often useful to determine the amount of organic material and energy present, especially in agriculturally related

studies. Two types of tests have historically been used to estimate the total energy content of a sample. The first is dry-oxidation using combustion or high temperatures with oxygen flow. Energy content is determined by bomb calorimetry or detection of evolved CO_2 respectively, both of which have long and tedious processes, which require relatively large sample sizes.

Dry oxidation techniques have fallen out of favor and been replaced by wet-oxidation techniques, which use reagents such as dichromate or permanganate in an acidic environment to digest organic material. The acid-dichromate oxidation procedure is advantageous because it is relatively easy to perform and results are rapidly obtained. It can be applied in the simplest of laboratories and even in the field. It is also extremely sensitive to small amounts of organic material, which makes it important to keep glassware free from trace organic material, but allows for small samples to be analyzed. The reduction of the dichromate ion to the chromium ion is coupled with a color change allowing for easy observation of changing dichromate levels with a spectrophotometer.

The acid-dichromate test was first described in the late 19th century. Modifications of this test by Strickland and Parsons (1960) and McEdward and Carlson (1987) have been used to estimate egg, larval and juvenile energy content in invertebrates (McEdward *et al* 1988; McEdward and Coulter, 1987). It has also been used to compare energy content to egg size in echinoderms, polychaetes and oysters (Strathman and Vedder, 1977; McEdward and Chia, 1991) along with studies of phytoplankton and other invertebrate eggs (Mullin *et al*, 1966, and Strathmann, 1967 both cited in Gosselin and Qian, 1999).

The basic procedure, as described by Parsons *et al* (1984), calls for the treatment of samples with potassium dichromate dissolved in concentrated sulfuric acid for 30

minutes at 110° Celsius. The oxidation of the organic material is coupled to the reduction of dichromate to the chromium ion via the following generalized reaction:

$$Cr_2O_7^{2-} + C + 10H^+ \rightarrow CO_2 + 2\,Cr^{3+} + 5H_2O \qquad (1)$$

The amount of carbon, and hence energy, present can be determined by monitoring the disappearances of dichromate ($Cr_2O_7^{2-}$) or appearance of the chromium ion (Cr^{3+}). Changes in the amounts of these compounds can be detected colorimetrically using a spectrophotometer. The change in absorbance of dichromate, observed at 440 nm, at low concentrations is linearly related to energy present, which can be converted to an amount using standard conversion factors, via Beer's law. Predictions about total energy content of a sample can be estimated from the amount of dichromate that is reduced. The change in absorbance of dichromate ion is traditionally observed because the extinction coefficient is ten times greater than that of the chromium ion, which leads to greater detection of variability of absorbance values.

Numerous studies have demonstrated that the acid-dichromate test has some shortcomings, which have prevented more widespread use of the assay. Johnson (1949) noted that the test resulted in "a somewhat arbitrary, although reproducible, figure" and that the rate of oxidation for proteins is slower than that for carbohydrates. A number of more recent reports have indicated that proteins are oxidized to a lesser degree than carbohydrates (Gosselin and Qian, 1999; Jaeckle, unpublished data). Estimates of total organic content, using glucose as a standard, underestimate total energy by 10-20% of those determined by dry-oxidation techniques (Paine, 1971). One attempt to account for these differences is to multiply the final value by an experimentally determined conversion

factor. However, proposed conversion factors range from 1.19 to 1.30 (Nelson and Summers, 1982 cited in Grewel *et al*, 1991; Nikolajewski, 1982).

These differences can be accounted for chemically. Equation (1) assumes the oxidation state of carbon to be zero. However, many organic carbons are not in this oxidation state. For glucose, where five of the six carbons are in the zero oxidation state, this is an adequate representation. Carbons found in proteins, though, have varying oxidation states and steric hindrance, due to folding, may prevent some groups from reacting at all. Both factors may lead to incomplete oxidation of protenaceous compounds to carbon dioxide and water. The oxidative differences between glucose and proteins cannot be accurately predicted without knowing the precise chemical composition of each compound. The best that can be hoped for is that a mixture of compounds within each single class can be used to generate standards that approximate what is found in living organisms.

Differences in the oxidation of proteins and carbohydrates imply a difference in the reaction kinetics. Based on the evidence of Johnson (1949), Nelson and Summers (1982, cited in Grewel *et al*, 1991), Gosselin and Qian (1999) and Jaeckle (unpublished data), it is hypothesized that proteins and carbohydrates have significantly different degrees of final oxidation and reaction rates with dichromate in an acidic environment. Using a standard consisting of both proteins and carbohydrates, as opposed to a single compound (typically glucose) as is currently used, when evaluating biological samples containing both types of compounds should result in a better estimation of total organic content of the unknown mixture. The change in absorbance of dichromate, measured by a spectrophotometer, of a

sample containing both types of compounds is equal to the sum of the individual changes in absorbance for each type of compound (equation 2).

$$\text{total } \Delta \text{ abs} = \Delta \text{ abs of carbohydrates} + \Delta \text{ abs of proteins} \quad (2)$$

It has been experimentally shown that the change in absorbance for either type of compound is related to the energy content of each type of compound via the Beer's Law relationship (Gosselin and Qian, 1999; Jaeckle, unpublished data). The Beer's Law slopes generated by individual proteins and carbohydrates are significantly different, implying differences in the reaction rates (Ibid.)

If there is a significant difference in the rates of reduction of dichromate with proteins and carbohydrates, then the amount of change in absorbance of dichromate will be significantly different for each type of compound with respect to time. An analysis of the change in absorbance of dichromate with respect to time for a mixture of both compounds can then be compared to standard series for each compound that relates change in absorbance to time to estimate the amount of each type of compound in the sample. A derivation of this follows for a two compound system:

Starting with equation (2):

$$\text{total } \Delta \text{ abs} = \Delta \text{ abs of carbohydrates} + \Delta \text{ abs in protein} \quad (2)$$

The change in absorbance with respect to time for acid-dichromate treatment of organic material shows a first order rate reaction over time. This can be explained mathematically using the Michaelis-Menton equation:

$$\Delta \text{ abs} = \frac{(\text{time})*(\text{max } \Delta \text{ abs})}{(\text{reaction rate} + \text{time})} \quad (3)$$

Max Δ abs will vary according to the amount of the compound that is present. Because there is a Beer's Law relationship, there will be a linear relationship between max Δ abs and amount of compound present, with a theoretical intercept of zero. A ratio of the change in absorbance of a sample to the change in absorbance of the standard can be established. This equals the ratio of the amount of a sample to the amount in the standard. Multiplying the numerator of the Michaelis-Menton equation by this ratio allows a family of Michaelis-Menton equations to be derived for a specific compound of any amount. In an unknown sample, the ratio is the unknown. The ration can be solved for if the change in absorbance is known at any time *t*. Multiplying the ratio by standard allows one to determine the actual amount in the sample/unknown. This is seen in equation (4).

$$\Delta \text{ abs} = \frac{t*(\text{max } \Delta\text{abs})*(\text{ratio of A})}{(\text{reaction rate} + t)} \qquad (4)$$

Substitution of equation (4) into equation (2) yields:

$$\text{total } \Delta \text{ abs} = \frac{t*(\text{max } \Delta\text{abs})*(\text{ratio of A})}{(\text{reaction rate} + t)} + \frac{t*(\text{max } \Delta\text{abs})*(\text{ratio of B})}{(\text{reaction rate} + t)} \qquad (5)$$

where t is equal to time--the independent variable. Max Δ abs and reaction rates are calculated from standards of the respective compounds, and total Δ abs is measured using the spectrophotometer. Substituting in the appropriate values, equation (5) simplifies to an equation with two unknowns, the ratio of A and ratio of B. Measurements taken at two different times can be used to solve for the unknowns using basic algebra. The ratio of each compound is then multiplied by the amount used to obtain the Δ abs to obtain the actual amount of each compound in the mixture/unknown.

In this way, it is possible to estimate the amounts and energy present of specific types of compounds within a given mixture and the total amount of mixture with increased

accuracy. This test can be expanded to three compounds by a similar manner, assuming there is significantly different reaction rates between the three compounds. Standards can be generated for mixtures of carbohydrates, proteins and lipids allowing biological samples to be assayed for the amount of each component in the overall sample.

This hypothesis was tested in two ways. Standard plots for a protein (bovine serum albumin--BSA) and a carbohydrate (glucose) for change in absorbance with respect to time was generated. From this data, the values of max Δ abs and reaction rates were estimated using linear transformations. Known mixtures of both compounds were assayed and the results evaluated by comparing observed absorbance to estimate absorbance and actual biochemical composition to estimated biochemical composition.

Materials and Methods

This procedure is modified from Parsons *et al.* (1984).

The acid-dichromate reagent was made by dissolving 2.42 g of potassium dichromate in 10 ml of distilled water and slow dilution to 500 ml with concentrated sulfuric acid. Samples of BSA and glucose are made up to a concentration of 12 J/μl (38.53 mg/ml Glucose, 33.33 mg/ml BSA) and treated 20 μl at a time with 2 ml of the dichromate reagent and was heated at 110° Celsius for various intervals of time. At the completion of the heating period the samples were cooled to room temperature in an ice bath to terminate oxidation. They were then diluted with 3.5 ml of distilled water and the absorbance was determined at 440nm using a Shimadzu UV-1601 spectrophotometer. The absorbancies were compared to a control containing 20 μl of distilled water and treated in the same manner described above.

Two glucose standards were generated. The first standard used measurements obtained every minute for 20 minutes. The second standard used glucose that had been dried at 110° C to remove any residual water and measurements were obtained every 10 seconds for the first minute and every minute thereafter for 15 minutes. The standard curve for BSA was generated by taking absorbance measurements every minute for thirty minutes. All standards were done in replicates of five.

An error in the procedure was discovered during the project. All timing began when the reaction mixture was placed in the heating block, not when the dichromate was added to the organic material, a difference of approximately six seconds. Timing was performed correctly for the second glucose standard, but time did not allow for the creation of a second BSA standard.

Known mixtures of glucose and BSA containing 12J/μl in proportions of 1:1, 2:1 and 1:2 were each reacted for 1 minute, 5 minutes and 15 minutes.

All glassware was cleaned with Nochromix cleaning solution and rinsed with distilled water between uses to eliminate residual organic material. All statistical analysis was performed using SPSS 9.0 for Windows.

Results

Graphical depiction of change in absorbance as a function of time for both BSA and glucose indicated first order kinetics over time. These are displayed in Figures 1-3 along with the line of best fit calculated using the Michaelis—Menton equation with the Lineweaver—Burk approximations for max Δ abs and reaction rate. The Michaelis-Menton equation can be used to mathematically represent first order reactions over time and was used as the line of best fit to estimate change in absorbance vs. time. Four

methods were utilized to estimate maximum absorbance and reaction rate using linear transformations of the Michaelis—Menton equation. These methods were the Lineweaver –Burk plot, Woolf-Augustinsson-Hofstee plot, Eadie-Scatchard plot and Hanes-Woolf plot. A short description of the Lineweaver—Burk approximation method can be found in Appendix 1.

Two sets of mixtures were analyzed, one with each glucose standard. The estimates for max Δ abs and reaction rates determined by each of the linear transformations can be seen in Table 1. Also included are the correlation coefficients for the linear fit of each transformation.

Table 1

Estimates for max Δ ABS and Reaction Rates for BSA and Glucose Standards With Correlation Coefficients

BSA	max Δ ABS	Reaction Rate	R^2
Lineweaver-Burk	0.7540	1.316	0.9143
Woolf-Augustinsson	0.7666	1.437	0.8108
Eadie-Scatchard	0.7904	1.772	0.8108
Hanes-Woolf	0.7902	1.846	0.9888
Glucose-First Standard			
Lineweaver-Burk	0.9304	0.1866	0.6809
Woolf-Augustinsson	0.9289	0.1731	0.5834
Eadie-Scatchard	0.9493	0.2968	0.5834
Hanes-Woolf	0.9215	0.1073	0.9986
Glucose-Second Standard			
Lineweaver-Burk	1.014	0.0516	0.6185
Woolf-Augustinsson	1.020	0.0543	0.5915
Eadie-Scatchard	1.049	0.0920	0.5915
Hanes-Woolf	1.065	0.0173	0.9982

The Lineweaver—Burk and Hanes—Woolf estimates provided the best approximations, with correlation coefficients closest to one. The values for max Δ abs and

reaction rates for both of these estimates were substituted into the Michaelis—Menton equation and used to predict the overall change in absorbance. The observed and calculated total changes in absorbance are presented in Table 2, with percent deviations in parentheses.

Table 2

Observed and Predicted Values for Δ abs for Mixtures of BSA and Glucose*

Glucose I and BSA Observed			
	1 min	5 min	15 min
1:1	0.426	0.647	0.746
2:1	0.515	0.705	0.742
1:2	0.441	0.677	0.810
Glucose I and BSA Predicted with Lineweaver—Burk Estimates			
1:1	0.555 (18.4%)	0.746 (14.2%)	0.807 (12.7%)
2:1	0.696 (30.0%)	0.821 (15.2%)	0.863 (15.1%)
1:2	0.440 (0.2%)	0.672 (0.7%)	0.750 (7.7%)
Glucose I and BSA Predicted with Hanes—Woolf Estimates			
1:1	0.555 (18.4%)	0.750 (14.7%)	0.804 (7.5%)
2:1	0.749 (30.0%)	0.825 (15.2%)	0.860 (14.7%)
1:2	0.452 (2.5%)	0.676 (0.0%)	0.749 (7.8%)
Glucose II and BSA Observed			
1:1	0.558	0.772	0.852
2:1	0.756	0.758	0.808
1:2	0.516	0.725	0.867
Glucose II and BSA Predicted with Lineweaver—Burk Estimates			
1:1	0.645 (14.5%)	0.800 (2.0%)	0.852 (0.0%)
2:1	0.805 (6.3%)	0.902 (17.3%)	0.931 (14.1%)
1:2	0.485 (6.2%)	0.699 (3.7%)	0.773 (11.5%)
Glucose II and BSA Predicted with Hanes—Woolf Estimates			
1:1	0.616 (10.0%)	0.813 (5.2%)	0.873 (2.4%)
2:1	0.762 (1.0%)	0.921 (19.4%)	0.963 (17.5%)
1:2	0.471 (11.0%)	0.705 (2.8%)	0.783 (10.2%)

*Ratios are given as Glucose:BSA in terms of Joules/μl, total of 12 Joules/μl

The predicted values in most cases overestimated the total change in absorbance. Predicted and observed values for 1:2 Glucose:BSA for the first glucose standard are close with a deviation of under 8% in all cases. It is interesting to note that for both

glucose standards the Lineweaver—Burk and Hanes—Woolf estimates show similar errors for each prediction. This implies that the error that is present is consistent throughout the experiment.

The appropriate values were substituted into equation (5) and estimates of amount were calculated using the Lineweaver—Burk and Hanes—Woolf separately for both sets of mixtures. The results are summarized in Table 3 (next page).

Estimates of amount were made using two points, minutes one with five, and minutes one with fifteen. It is recommended that multiple points be used to calculate amount of material present and that these be averaged, as was done in Table 3. Minutes five and fifteen were not used to estimate amounts because differences in absorbance between the two times are not significant, especially when large amounts of glucose are present. Again, a small error is seen in the first glucose:BSA mixture for the 1:2 ratio. The errors are relatively consistent between the Lineweaver—Burk and Hanes—Woolf estimates.

Discussion

Clearly, the method described herein has some problems. Estimates for total change in absorbance have an error ranging from zero to 30%, while estimates for amount of specific compound range from 2.2% to 171%. This difference in magnitude of error indicates that absorbance measurements must be quite accurate at all time intervals to obtain quality estimates of individual compounds. There is consistency between the respective errors for the Lineweaver--Burk and Hanes--Woolf methods which implies that there is a procedural error is in the procedure itself, not the underlying theory. An examination of the method reveals numerous areas in which it may be improved.

Table 3
Observed and Estimated Values for Organic Content in Milligrams
Percent Errors in Parentheses

Glucose I and BSA: Lineweaver--Burk Estimates

	Expected		1 + 5		1 + 15		Average	
	BSA	Glucose	BSA	Glucose	BSA	Glucose	BSA	Glucose
1:1	0.333	0.385	0.475 (42.5%)	0.191 (50.5%)	0.527 (58.2%)	0.166 (57.0%)	0.501 (50.3%)	0.179 (53.6%)
1:2	0.167	0.578	0.346 (107%)	0.340 (41.1%)	0.294 (76.7%)	0.365 (36.9%)	0.320 (92.0%)	0.353 (39.0%)
2:1	0.500	0.193	0.511 (2.2%)	0.188 (0.23%)	0.628 (25.5%)	0.132 (31.3%)	0.572 (14.4%)	0.161 (16.7%)

Glucose I and BSA: Hanes--Woolf Estimates

	Expected		1 + 5		1 + 15		Average	
	BSA	Glucose	BSA	Glucose	BSA	Glucose	BSA	Glucose
1:1	0.333	0.385	0.448 (34.5%)	0.222 (42.5%)	0.465 (39.5%)	0.215 (44.2%)	0.457 (37.0%)	0.218 (43.3%)
1:2	0.167	0.578	0.354 (112%)	0.340 (41.1%)	0.294 (76.3%)	0.363 (37.1%)	0.324 (47.2%)	0.352 (39.1%)
2:1	0.500	0.193	0.474 (5.1%)	0.225 (17.0%)	0.678 (35.6%)	0.147 (23.7%)	0.578 (15.6%)	0.186 (3.3%)

Glucose II and BSA: Lineweaver--Burk Estimates

	Expected		1 + 5		1 + 15		Average	
	BSA	Glucose	BSA	Glucose	BSA	Glucose	BSA	Glucose
1:1	0.333	0.385	0.494 (48.3%)	0.253 (34.3%)	0.506 (51.8%)	0.249 (35.5%)	0.500 (50.2%)	0.251 (34.8%)
1:2	0.167	0.578	-0.0755 (145%)	0.634 (9.7%)	-0.0294 (118%)	0.593 (2.6%)	-0.0528 (132%)	0.613 (6.1%)
2:1	0.500	0.193	0.648 (29.6%)	0.223 (15.5%)	0.617 (23.4%)	0.171 (11.4%)	0.633 (26.7%)	0.198 (2.6%)

Glucose II and BSA: Hanes—Woolf Estimates

	Expected		1 + 5		1 + 15		Average	
	BSA	Glucose	BSA	Glucose	BSA	Glucose	BSA	Glucose
1:1	0.333	0.385	0.477 (43.0%)	0.305 (20.8%)	0.459 (37.8%)	0.268 (30.3%)	0.468 (40.5%)	0.287 (25.5%)
2:1	0.167	0.578	-0.264 (259%)	0.732 (26.7%)	-0.118 (171%)	0.687 (18.9%)	-0.191 (21.5%)	0.706 (22.2%)
1:2	0.500	0.193	0.356 (28.8%)	0.312 (61.7%)	0.465 (7.0%)	0.274 (42.0%)	0.411 (17.8%)	0.293 (51.8%)

First and foremost is the time differential. While this was accounted for in the second glucose standard, due to time constraints, the BSA standard retained this error. All mixtures using the second glucose standard were analyzed with time measurements beginning when dichromate was added. This means that the incubation times for the BSA standard differ from the incubation times of the BSA in the mixture by approximately six seconds. Comparison of the reaction rates of the glucose standards shows a significant difference. It is predicted that the estimate reaction rate for BSA will not show as significant a change, due to its larger value, but will be affected none-the-less.

Dichromate in a strongly acidic environment is an extremely powerful oxidizing agent. The heating block was used to accelerate the oxidation, and it was assumed that the ice-bath would stop the oxidation process. This assumption may be incorrect; oxidation may continue to occur while the samples sit in the ice-bath. Each sample cooled for about five minutes before being diluted and this would provide ample time for further oxidation, especially of glucose. This extra oxidation is not a problem when the dichromate test is used as it has traditionally been applied because complete oxidation is required. In this study, however, we seek to stop the oxidation at specific time intervals to observe how far the reaction has progressed. Due to the sensitivity of this assay, any subsequent oxidation may alter the results. The data collected to date indicates that this may be occurring. The second glucose standard (Figure 3) shows nearly complete oxidation after just ten seconds. While it is certainly possible that this is the case, allowing a partially oxidized glucose sample to sit for five minutes can cause further oxidation than seen in each time period. Therefore, it is recommended that dilution with 3.5 ml of distilled water take place first, followed by cooling in a colder environment such as dry ice

in ethanol. The dilution will weaken the acid, and coupled with the cooling, slow, and ideally stop, further oxidation.

Another potential problem is with the dichromate reagent itself. The assay, as it is currently performed, has a dichromate control absorbance over 2.0. This high absorbance measurement is near the extremes of detectability of the spectrophotometer, and thus more prone to systematic error. A solution to this problem is to add more water at the end of oxidation. This will reduce the absorbance measurements. This would also dilute the acid more and reduce subsequent oxidation after the desired time for oxidation has been reached.

In order to optimize the assay to perform to accurately predict amounts of organic material, the standards must have accurate max Δ abs and reaction rate estimates. This means a strong linear relationship must be developed for the linear transformations of the data. Only one of the methods, Hanes—Woolf, gave adequate correlations for both glucose and BSA. However, the accuracy of the predicted values decreases when the y-intercepts are close to zero. In both glucose and BSA, the y-intercept, used to determine max Δ abs, is statistically indistinguishable from zero, thus the estimate is extremely poor. The Lineweaver—Burk method, which does not have this limitation, consistently delivered the second highest correlations (Table 1), and for that reason has been used along with Hanes-Woolf. The correlation coefficients may be improved by the conditions outlined above.

There are also clues that there are problems with the data. The observed readings for total change in absorbance for the second glucose:BSA mixture at a 2:1 ratio (Table 2) show only a 0.002 difference in absorbance between one and five minutes. This is

unexpected, for all of the other data have shown that when protein is present a significant amount of oxidation takes place between minutes one and five. The error here is most likely random, but it demonstrates that numerous replicates must be included to minimize this error, and the utmost precision and accuracy is required.

Based on analysis of this data the following alterations in the procedure are recommended. First, all timing must begin upon addition of the dichromate reagent. The reaction should be terminated by the immediate addition of distilled water and placement in an ice bath. More than 3.5 ml of distilled water should be added to assure termination and reduce the absorbance of the control to within the limits of the spectrophotometer. It is recommended that between 4 and 4.5 ml of water be added. With these alterations, the accuracy of the assay should increase.

Despite the errors discovered in the procedure, this assay shows promise. Its high sensitivity necessitates that extreme care and precision be exercised in the production of standards and analysis of mixtures. Calculations predicting the total change in absorbance follow the same pattern as those found in the mixtures, increasing as more glucose is added in relation to BSA and vice-versa. In a few cases, the amount of glucose and BSA has been predicted to within 5 percent of the actual amount present. However, this may be fortuitous. Even if error cannot be reduced to below ten percent the assay may have use. If a consistent amount of error is present the assay can account for it and our goal will have been reached.

Further exploration into this assay and its underlying theory must be performed. Improved methodology and precision can lead us to a functioning assay to distinguish the amount of protein and carbohydrates in unknown samples. Once this has been

accomplished, a third term, to account for lipids can be incorporated and a fully functioning assay for determining the amount of carbohydrates, proteins and lipids in biological systems will be developed.

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Appendix 1

A Brief Description of the Lineweaver—Burk Approximation Method

Four methods were used to estimate max Δ abs and reaction rates for glucose and BSA. All four of these rearranged the Michaelis—Menton equation to a form that has a linear relationship between the variables. The slope and intercepts of these can be found and used to calculate max Δ abs and reaction rate. One of these, the Lineweaver—Burk method, will be explained in some detail below.

Beginning with the Michaelis-Menton equation used in this project, equation (3):

$$\Delta \text{ abs} = \frac{(\text{time})*(\text{max } \Delta \text{ abs})}{(\text{reaction rate} + \text{time})} \qquad (3)$$

The reciprocal is taken:

$$\frac{1}{\Delta \text{ abs}} = \frac{(\text{reaction rate} + \text{time})}{(\text{time})*(\text{max } \Delta \text{ abs})} \qquad (6)$$

The terms in the numerator are then separated:

$$\frac{1}{\Delta \text{ abs}} = \frac{(\text{reaction rate})}{(\text{time})*(\text{max } \Delta \text{ abs})} + \frac{(\text{time})}{(\text{time})*(\text{max } \Delta \text{ abs})} \qquad (7)$$

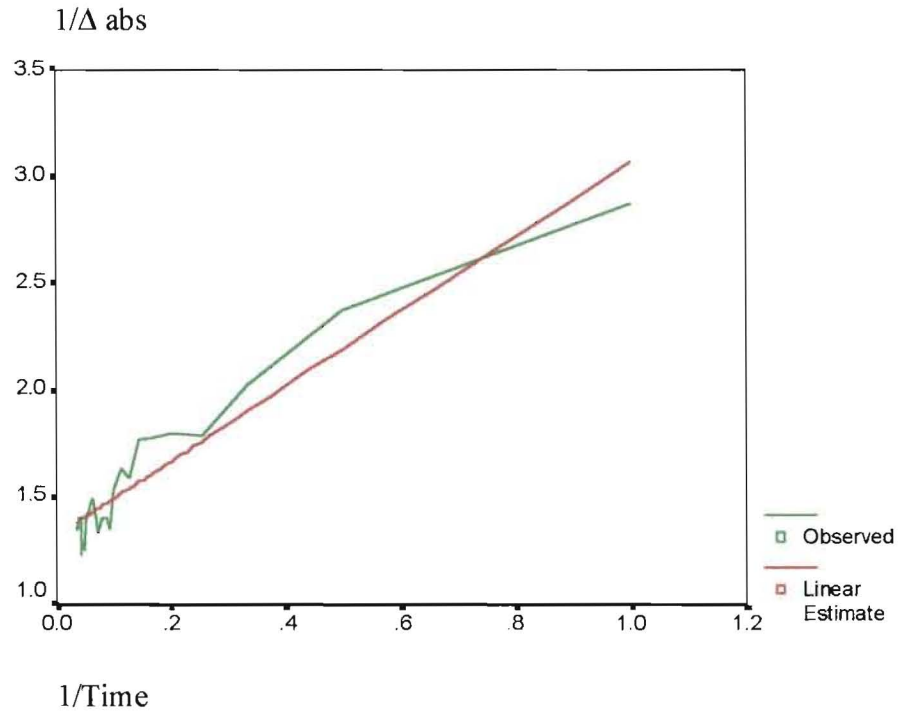
Separating out the first term and simplifying the second yields:

$$\frac{1}{\Delta \text{ abs}} = \frac{(\text{reaction rate})}{(\text{max } \Delta \text{ abs})} * \frac{1}{(\text{time})} + \frac{1}{(\text{max } \Delta \text{ abs})} \qquad (8)$$

A plot can be generated of (1/ max Δ abs) vs. (1/time). These values can be calculated from the raw data. The equation then takes on the form y = mx + b, with the slope being equal to (reaction rate/ max Δ abs) and the y-intercept being equal to (1/ max Δ abs). A typical Lineweaver—Burk plot is shown below in Figure 4:

Figure 4

Lineweaver Burk Plot of BSA Data



SPSS for Windows was used to perform the linear regression and the results of all of the linear estimations are displayed in Table 1. The slope and y-intercept are used to easily calculate max Δ abs and reaction rates.

The other methods use a similar method, but use different manipulations of the data. A thorough discussion of this can be found in *Biochemical Calculations*.

Figure 1
BSA Standard

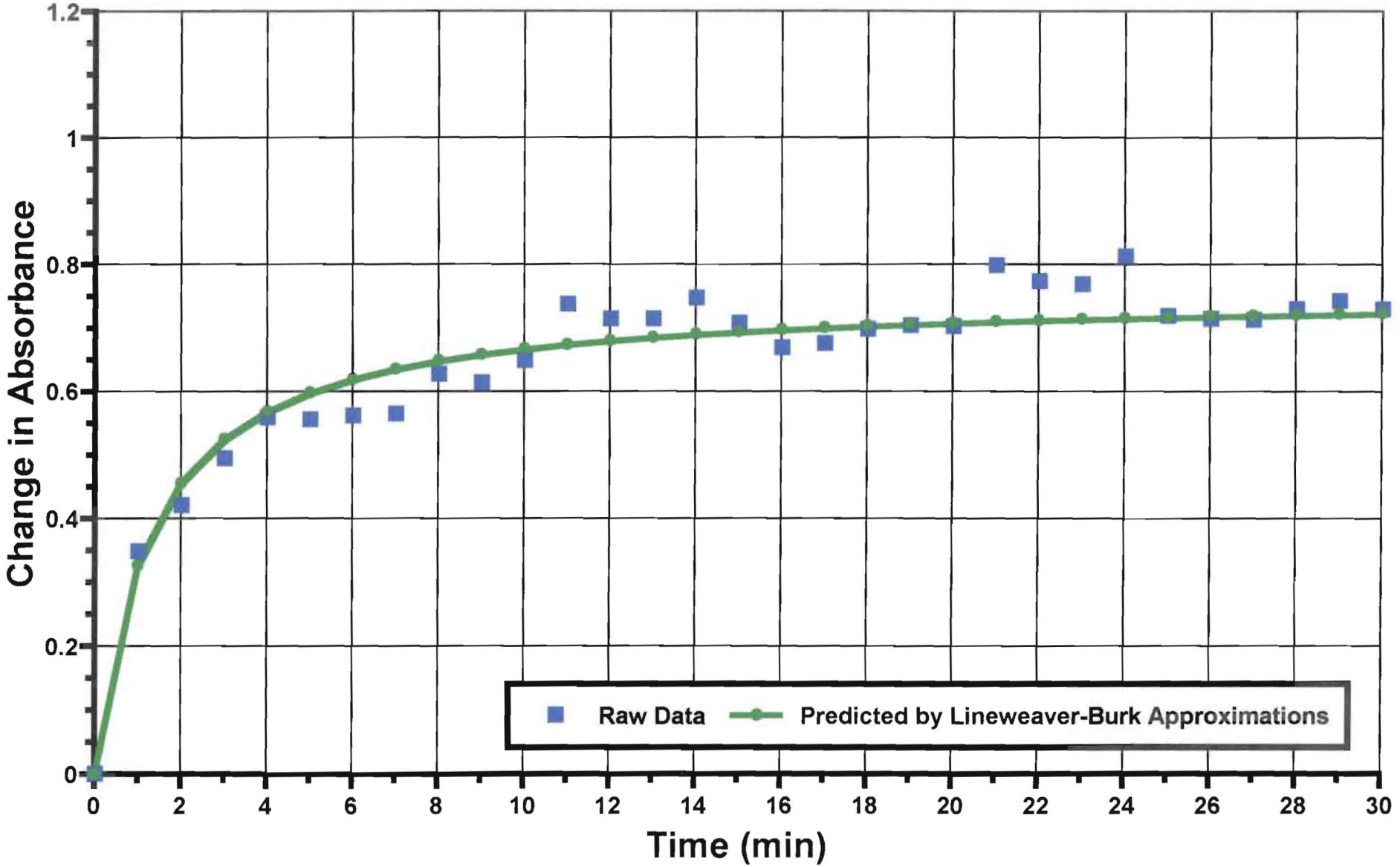


Figure 2
First Glucose Standard

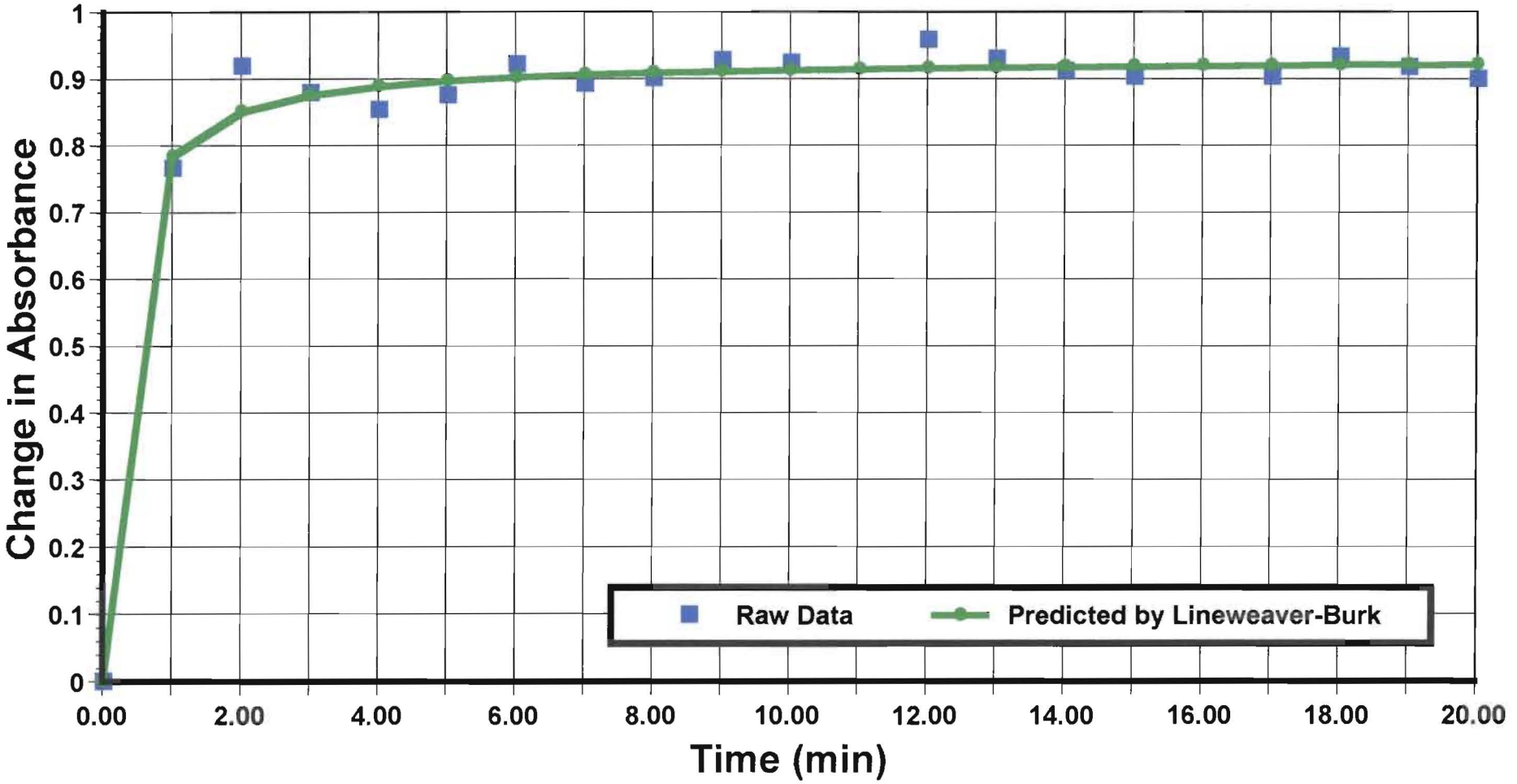


Figure 3
Second Glucose Standard