LACTOSE INTOLERANCE:
Exploring Reaction Kinetics Governing Lactose Conversion of Dairy Products Within the Undergraduate Laboratory

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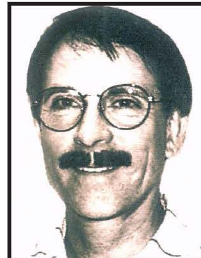
Lactose intolerance is a condition suffered by an estimated 50 million Americans. Certain ethnic and racial populations are more widely affected than others. As many as 75 percent of all African-American, Jewish, Native American, and Mexican-American adults, and 90 percent of Asian-American adults, are lactose intolerant.[1] Some populations in Africa are completely lactose intolerant, whereas some northern European populations are unaffected.[2] Why so? Genetic evidence indicates that lactose intolerance arose over 5,000 years ago and spread among populations due to positive selection. Correlations have revealed lactose intolerance is prevalent in geographical areas of extreme climates and where there have been persistent occurrences of communicable diseases affecting cattle.[3]

Lactose, or milk sugar, is a naturally occurring dissacharide found in dairy products and is normally converted to simpler sugars in the human digestion process. The lactase enzyme (β-Galactosidase) hydrolytically cleaves the lactose molecule to form monosaccharides, galactose, and glucose (see Figure 1). This enzyme is secreted by cells along the lining of the small intestine during normal digestion. In some people, this enzyme is absent or present in reduced concentrations. Interestingly, most other mammals completely stop producing lactase at weaning, and are thereafter intolerant of milk. If, during the human digestion process, lactose is not converted to these simpler sugars, people may suffer symptoms of bloating, nausea, cramps, and diarrhea. Hence the term lactose intolerance. Fortunately, it is generally not life-threatening and can be controlled through proper diet. Lactase-deficient people can also ingest commercial supplements such as Lactaid and Dairy Relief with their meal to aid in digestion of dairy products.

This undergraduate laboratory experiment demonstrates the effect of catalytic action (Dairy Relief) upon skim milk and the subsequent conversion of lactose to glucose. An inexpensive



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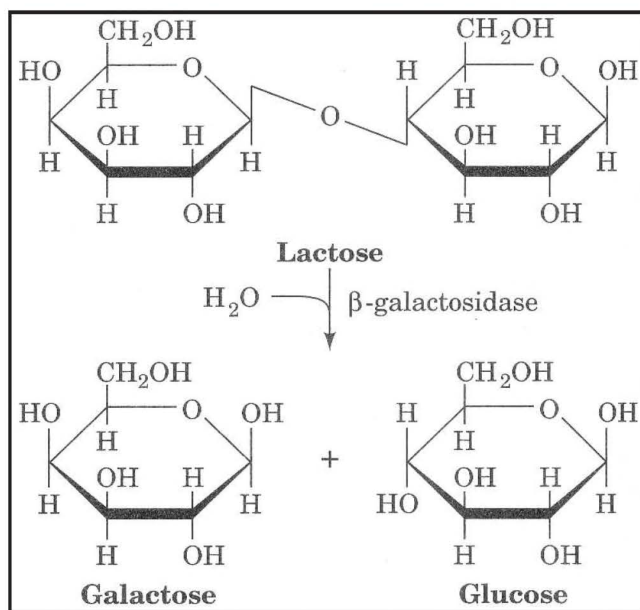


Figure 1. *Conversion of lactose to monosaccharides.*

blood glucose monitoring meter is used to record changes in glucose concentration over the course of the reaction. Two kinetic rate methods are offered to calculate reaction rates; these are the differential method and initial rate method. Effects of other reaction variables were studied, including pH, temperature, catalyst concentration, and catalyst accelerations/inhibition. A simple kinetic rate model is applied to demonstrate development of the Arrhenius expression. Finally, a more appropriate Michaelis-Menten model is applied to accurately model the complex enzyme kinetics.

CURRICULAR IMPACT

This experiment was primarily developed to be incorporated into a chemical engineering undergraduate unit operations laboratory (reactor design). Students could be required to develop reaction kinetics and use them to design/optimize a batch reactor to manufacture a lactose-free milk product based upon economic considerations. On the other hand, more fundamental portions, such as temperature and pH effects, could be assigned as part of an undergraduate semester project in a lecture class dealing with reactor design. Learning objectives might include determination of reaction rates, activation energy, optimum pH environment, catalyst deactivation temperature, Michaelis-Menten model parameters, and catalyst loading effects. The simplicity, portability, and quick variable response of this experiment also lend themselves well to K–12 audiences and other outreach activities. For these activities, it is recommended one omit the study of temperature effects (due to the inconvenience of transporting the control bath) and focus upon effects of pH and catalyst loading. The more advanced Michaelis-Menten model might be appropriate for demonstration within a chemical engineering graduate lecture class. This experiment also has broad appeal to other disciplines, including biomedical engineering and food science curriculums.

EQUIPMENT, SUPPLIES, AND OPERATING PROCEDURE

Chemicals and Supplies

- Individual instrument test strips for glucose monitoring, $0.60 ea.
- Skim milk, $0.605/L
- Household distilled white vinegar, 5% acidity, $2/L
- Household ammonia cleaning solution, dilute ammonium hydroxide, $0.87/L
- pH 4.5 - 10.0 pH test strips (Fisher), $0.09 ea
- Dairy Relief lactase tablet (9000 FCC units), $0.06 ea

Procedure

1. Milk is a complex food containing over 100,000 different molecular species. Skim milk contains about 87% water, 3.7 - 5.1% lactose, and 0.75% minerals. It has a density of 1.03 and a viscosity of 2.1 cP @ 20 °C.[4]
2. Fill the 1,000 ml glass-jacketed reactor with 490 ml of skim milk. Turn on the magnetic stirrer to a medium, reproducible setting (about 300 rpm). Set the temperature control bath to the desired temperature (between 12 and 38 °C) and wait for reactor temperature equilibration.
3. Initial studies can be conducted using the normal pH of skim milk, which is about 6.8. If runs will be made to examine effects of pH upon reaction rate, lower the pH of the milk with vinegar (about 10 ml for every pH unit) or raise the pH with ammonia cleaning solution (about 5 ml for every pH unit).
4. Calibrate the glucose meter according to the manufacturer's instructions. Once reactor conditions are at steady-state, measure the concentration of glucose in the skim milk (mg glucose/dL). Use a small bore pipette to deposit a single drop of sample mixture on a test strip. Each test requires about 60 - 75 seconds for total processing time.
5. Grind up two lactase enzyme tablets with a mortar and

TABLE 1
Equipment List

Equipment	Manufacturer	Cost
Blood glucose monitoring instrument	One Touch Basic (Johnson & Johnson, Lifescan Division)	$60
Standard laboratory temperature-control bath	NESLab RTE-100	$1,500
1000 ml glass-jacketed beaker	Ace Glass	$120
Magnetic stirrer with stirrer bar	Fisher	$200
—	TOTAL	**$1,880**

pestle. Wet the powder with 10 ml distilled water to dissolve the powder. When ready to begin a reaction run, add the catalyst/water mixture to the skim milk.

6. If sampling across long periods of time (differential method of analysis), sample the reaction mixture twice and average the results. If using the method of initial rates, sample reaction mixture consecutively over a 10- to 15-minute time period as there is insufficient time to repeat samples.

7. To start the reaction at a higher glucose concentration, dissolve 1.0 g reagent glucose into 20 ml of distilled water and add it to 480 ml of skim milk. This will raise the reading of the glucose monitoring meter by about 100 mg/dL. Also, reagent-grade lactose can be added to skim milk to study the effect of disappearance of reactant, though starting concentrations of lactose in our milk were not precisely known (reported to be 3.7 - 5.1%).

EFFECTS OF pH AND CATALYST CONCENTRATION

The conversion rate of lactose in milk with the use of a lactase catalyst is a function of several variables, including lactose substrate concentration, lactase catalyst concentration, temperature, pH, mixing rate, and ion concentration. The effect of catalyst concentration upon reaction rate is shown in Figure 2. As expected, reaction rate is accelerated with increases in catalyst concentration.

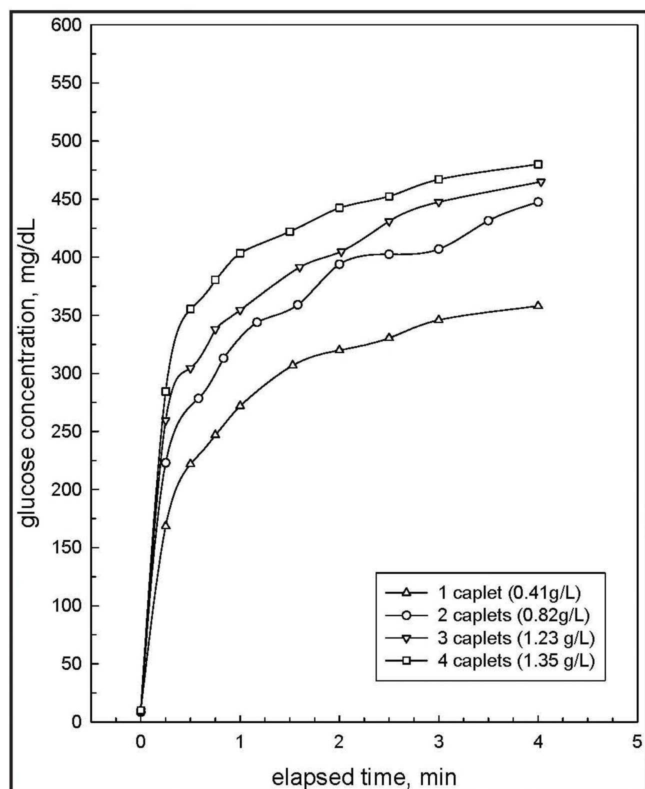


Figure 2. *Effect of catalyst concentration upon reaction rate (22 °C, 300 rpm, 500 ml skim milk).*

The activities of many enzymes vary with pH since the active sites of the enzyme contain significant acidic and basic groups. The influence of pH upon the reaction rate of lactose conversion to glucose is shown in Figure 3. A reduction in pH favors an increase in production rate until a pH of 4.5, where catalyst behavior becomes unstable. It has been reported that lactase in the Lactaid formulation is effective over the pH range of 3.5 - 8.5, with an optimum near 8.5.[5] Classically, initial rate of this reaction would be expected to vary with a bell-shaped distribution, with lower rates occurring at low and high pH and a high median rate at some intermediate pH.[6, 7] Optimum pH for crystalline lactase is reported to be 6.6 - 7.7 at 20 °C.[8] In this present investigation, however, reaction rates of lactase with milk appear to improve with decreasing pH—down to a pH of 5.0. Evidently, the Dairy Relief lactase formulation includes buffering agents to allow the lactase to operate in a more acidic environment, similar to that found within the stomach. At a pH of 4.5 (as measured by pH paper), the enzyme initially shows reduced efficiency. Finally after about 3/4 hour, it begins to behave erratically. Since the pH of stomach contents is commonly reported to be in the range of 2 - 4, this would seemingly be a problem for those people taking lactase to address lactose intolerance issues. The pH of the distal stomach contents, however, does not fall below a pH of 5 until some 60 - 75 minutes after the ingestion of a

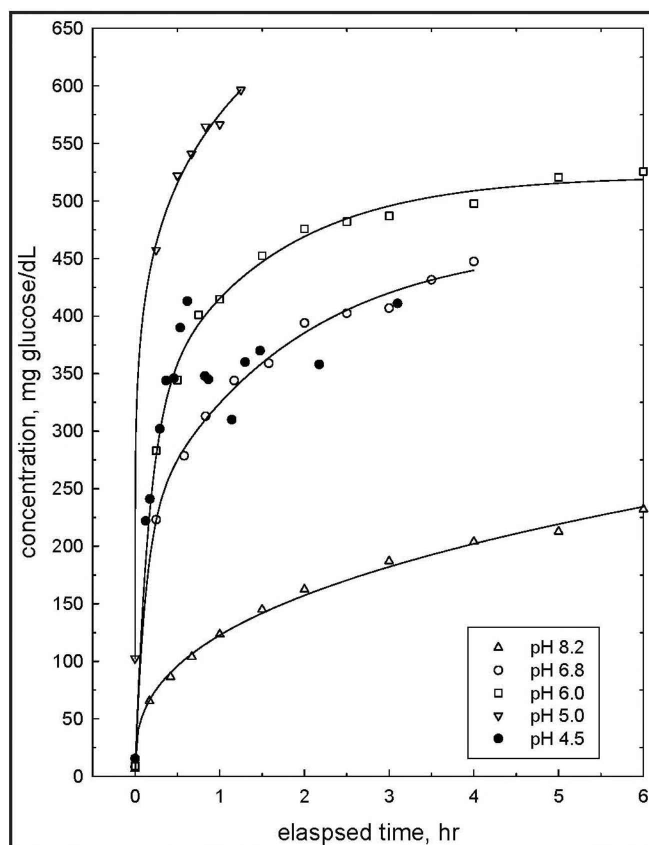


Figure 3. *Influence of pH upon reaction rate (500 ml milk, 2 tabs catalyst, 22 °C, 300 rpm).*

meal.[9] According to the pH 5 curve of Figure 3, 75 minutes would provide time for a conservative conversion of about 1/3 of the lactose contained in a glass of milk. The stomach does not mix at a rate of 300 rpm, however, but it does operate at the higher temperature of 37 °C (310K).

TEMPERATURE EFFECTS (METHOD OF INITIAL RATES)

The constructed pH plot (Figure 3) was based upon laboratory data collected over several hours. From this data, a differential method of analysis, such as graphical or numerical differentiation, could be used (though not performed here) to determine the reaction rate law for each set of experimental conditions. These longer data-collection time periods, however, are usually not available or practical in a typical undergraduate laboratory setting. Therefore, as an alternative, the method of initial rates can be used to investigate reaction kinetics. For example, Figure 4 illustrates the effect of temperature upon reaction rate. As an assignment, students were asked to generate data similar to Figure 4 and determine the rate constants and order of reaction for conversion of lactose to glucose across various temperatures. Their first approach (not the correct one) was to treat the reaction as first order, in the sense, lactose → products. A mole balance on a liquid phase batch reactor for the appearance of glucose can be written as:

$$\frac{dC_g}{dt} = r_g = kC_g^{\alpha} \qquad (1)$$

where r_g is the reaction rate for the appearance of glucose, k is the specific rate constant, α is the order of reaction, and C_g is the concentration of glucose. Upon integrating Eq. (1), the final result is:

$$t = \left(\frac{1}{k}\right)\frac{C_g^{(1-\alpha)} - C_{go}^{(1-\alpha)}}{(1-\alpha)} \qquad (2)$$

Using Excel Solver, a nonlinear regression was performed on Eq. (2) to solve for the specific rate constant and the order of reaction. The order of reaction was approximately first order (varied between 0.7 - 0.9 across the temperature range of 12 °C - 37 °C). Using the values of the specific rate constants as determined from the nonlinear regression, an Arrhenius plot was constructed and the activation energy for the reaction was graphically determined to be 39.1 kJ/mole. Assuming conversion of lactose to glucose to be a single, irreversible reaction is unjustifiably simple. As it turns out, however (see below discussion of Michaelis-Menten kinetics), the lumping together of all reactions and use of the Arrhenius equation is a fairly good approximation to an otherwise complex reactive

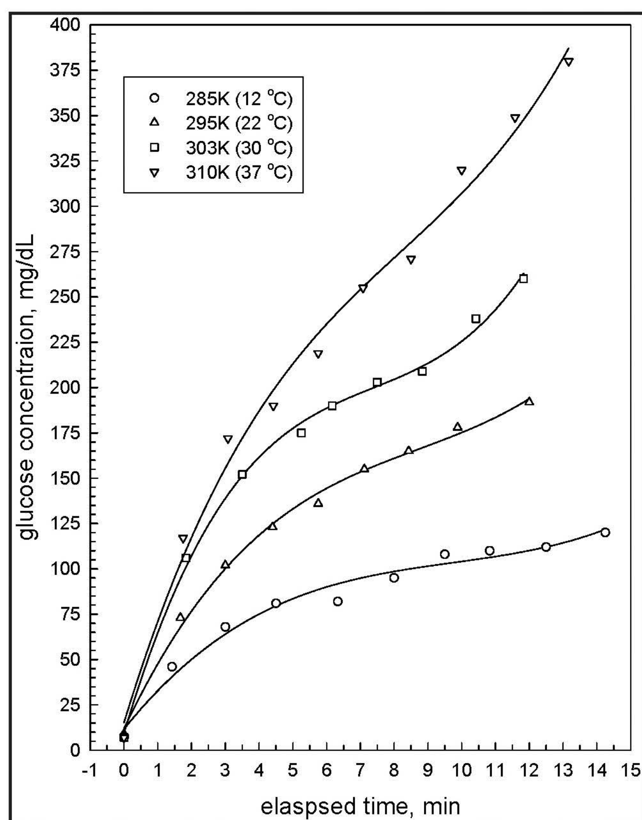


Figure 4. *Use of initial rate method to assess effect of temperature (500 ml milk, 2 tabs catalyst, 300 rpm).*

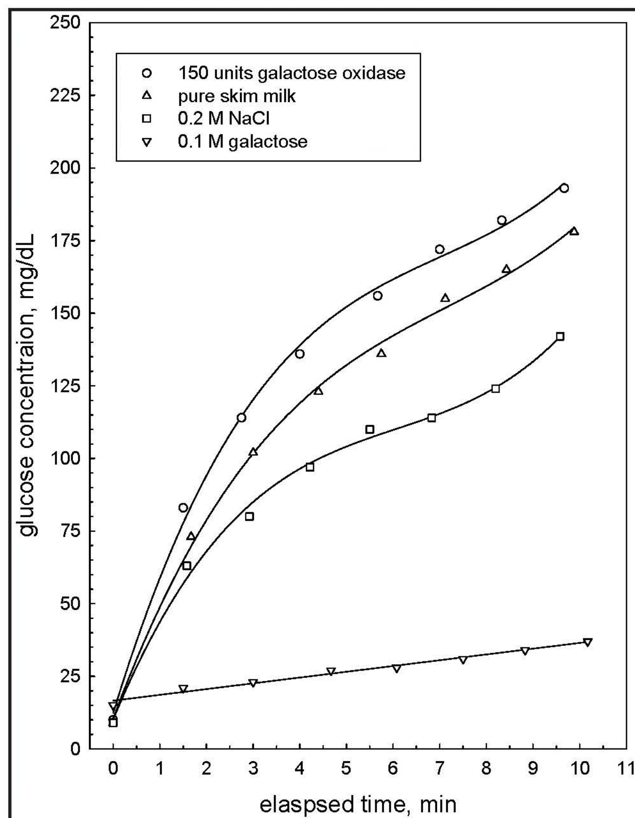


Figure 5. *Use of initial rate method to assess impact of enzyme inhibition (22 °C, 500 ml skim milk, 300 rpm, 2 tabs catalyst).*

Students were asked to generate data similar to Figure 4 and determine the rate constants and order of reaction for conversion of lactose to glucose across various temperatures. Their first approach (not the correct one) was to treat the reaction as first order, in the sense, lactose → products.

network (several reactions occurring with competing values of specific rate constants).

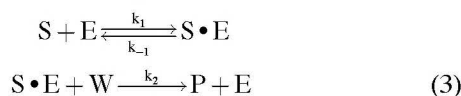
ENZYME INHIBITION

The surface of an enzyme contains many polar groups that are influenced by the surrounding ionic environment. It has been reported that sodium ions have a strong activating action on the lactase reaction.[8, 9] It is very likely a person might use significant quantities of table salt as they consume their hamburger along with a glass of milk. In this investigation, (see Figure 5, previous page) sodium chloride was not found to be an activating agent. At 0.2 M NaCl, the salt acted as a slight inhibiting agent. Using the initial rate method, the reaction rate with 0.2 M NaCl was 2.1×10^{-3} mol/L min. as compared to a rate of 2.4×10^{-3} mol/L min. with pure skim milk. At concentrations of 0.05 M NaCl and 0.05 M $MgSO_4$ (data not shown), there was no measurable change in the reaction rate. Other strong inhibitors of the lactase reaction are reported to be β- phenylthiogalactoside[10] and heavy metallic ions,[8] though these were not tested. Also, reported weak inhibitors of the reaction include some of the sugars, such as melibiose, galactose, glucose, and sucrose. According to Figure 5, galactose was shown to be a strong reaction inhibitor. When galactose was added to the reaction mixture (with no enzyme), the reversible reaction was slightly shifted to the right to produce higher concentrations of glucose—15 mg/dL vs. a routinely measured 9 mg/dL. This may indicate a reduction in normally available lactose in the initial reaction mixture of skim milk. Once the enzyme was added to the reaction mixture, the reaction proceeded at a much lower reaction rate (0.11×10^{-3} mol/L min vs. 2.4×10^{-3} mol/L min with pure skim milk).

Results with use of a reaction accelerating agent are also noted in Figure 5. Galactose oxidase converts any available galactose and drives the conversion of lactose into glucose at a slightly higher rate (2.9×10^{-3} mol/L min vs. 2.4×10^{-3} mol/L min with pure skim milk).

MICHAELIS-MENTEN KINETICS

The mechanism for conversion of lactose in milk to the products of galactose and glucose has been shown to follow the simple enzymatic reaction:



$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S \bullet E$$
$$S \bullet E + W \xrightarrow{k_2} P + E \qquad (3)$$

where S is the substrate, E is the enzyme, S • E is the substrate-enzyme complex, W is water, and P are the products. By writing simple rate laws and invoking the pseudo steady-state hypothesis, whereby the net rate of formation of the substrate-enzyme complex is assumed to be zero, the Michaelis-Menten equation can be expressed as:[11, 12]

$$-r_s = \frac{k_{cat} C_{Et} C_S}{C_S + K_M} \qquad (4)$$

where $-r_S$ is the net rate of disappearance of the substrate, $k_{cat} = k_2 C_W$, C_{Et} is the concentration of the total system enzyme concentration, C_S is the concentration of the substrate, and $K_M = (k_{cat} + k_{-1})/k_1$.

Interesting aside: Leonor Michaelis (1875-1945) was father of the permanent hair wave and Maud Menten (1879-1960) was one of the first women in Canada to earn a medical doctorate.[13]

The quantity k_{cat} is called the turnover number of the enzyme because it represents the maximum number of substrate molecules converted to products per active site per unit time, or the number of times the enzyme "turns over" per unit time. K_M, with units of mol/L, is called the Michaelis constant and measures the attraction of the enzyme for its substrate. K_M is often called the affinity constant. If we let $V_{max} = k_{cat} C_{Et}$, then the Michaelis-Menten equation takes the final familiar form of:

$$-r_S = \frac{V_{max} C_S}{K_M + C_S} \qquad (5)$$

where V_{max} is the maximum rate of reaction for a given total enzyme concentration, with units of mol/L sec.

According to the basic rate equation of enzyme kinetics as shown in Eq. (3), there are three rate constants. Each of these constants will obey the Arrhenius law and there will be three activation energies associated with each of the reactions. Sometimes, however, the Arrhenius law will follow a quite complicated enzyme process that involves several rate constants.[14] The Arrhenius law is reported to be obeyed for application of lactase to a variety of substrates over the range of 0 - 37 °C, with activation energies in the range of 12 - 20 kcal/mol.[15] Initially, the reaction rate increases with temperature and then begins to decrease at higher temperatures. At much higher temperatures, the complicated molecular structure of the enzyme begins to unfold and lose its catalytic properties. This is called thermal denaturation.[16] The point of thermal denaturation for crystalline lactase is reported to be

40 °C.[8] As a related example, Sizer[17] evaluated the thermal denaturation of the catalase-hydrogen peroxide system. According to Figure 6, the activity of this catalyst follows an Arrhenius relationship up to about 53 °C and then follows an inactivation curve at higher temperatures.

Investigators have found the conversion of lactose to glucose to follow the Michaelis-Menten relationship. This is true when $k_2 << k_{-1}$ and there is thermodynamic equilibrium between the initial free reactants and the enzyme-substrate complex.

Eq. (5) could be inverted and a Lineweaver-Burk plot constructed to graphically evaluate the slope of the plot as K_M/V_{max} and the intercept as $1/V_{max}$.[11, 18] This is not helpful, however, as the reaction rate for the disappearance of the reactant, $-r_s$, is not known.

A more useful method to evaluate the Michaelis parameters is to substitute Eq. (5) into Eq. (1) and integrate. Note, for every mole of reactant lactose disappearing, one mole of glucose is produced. For the appearance of glucose, the final result upon rearrangement is:

$$\frac{1}{t}\ln\frac{C_g}{C_{go}} = \frac{C_g - C_{go}}{K_M t} - \frac{V_{max}}{K_M} \qquad (6)$$

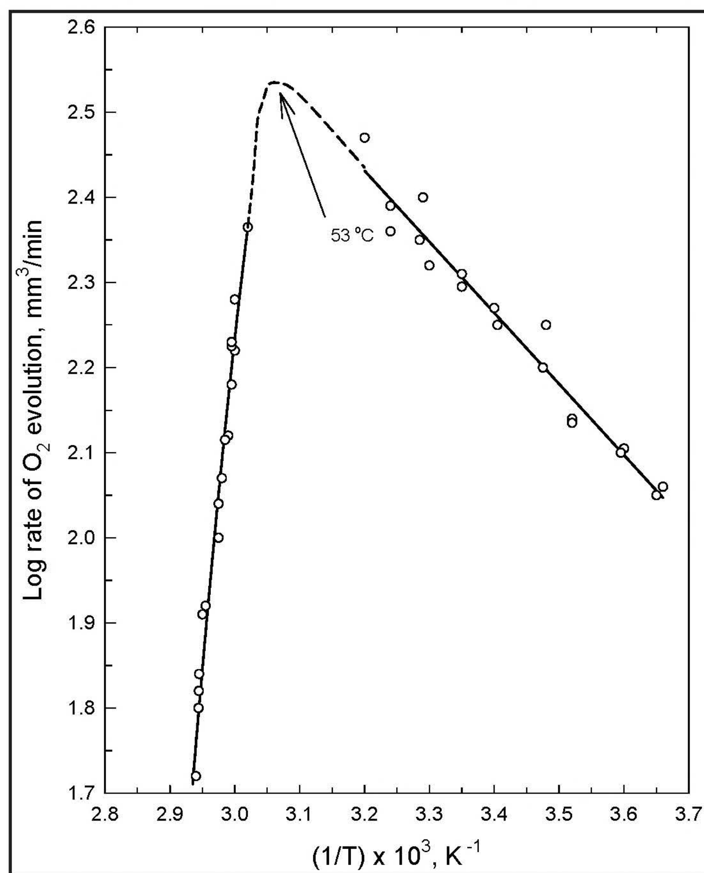


Figure 6. *Thermal denaturation of the catalase-hydrogen peroxide system (from Sizer, I.W., "Temperature Activation and Inactivation of the Crystalline Catalase-Hydrogen Peroxide System").[17]*

Using data from Figure 3 and plotting terms from Eq. (6), the slope of the line is $1/K_M$ and the intercept is $-V_{max}/K_M$ (see Figure 7, next page). From this plot, the Michaelis-Menten constant, K_M, assumes different values at different pH levels (also will vary with temperature), but V_{max} is only a function of enzyme concentration. According to Table 2, the enzyme is increasingly attracted to the substrate at lower pH (higher K_M), but V_{max} is relatively steady across the pH range (enzyme concentration was not varied).

The Michaelis-Menten constant, K_M, for lactose substrate is reported to be 10^{-3} moles/L and for maximal velocity, V_{max}, to be 3×10^{-3} μm/ml/min/std conc of enzyme[10] (9.0×10^{-4} mol/L sec for conditions of this investigation). Another investigator reports K_m and V_{max} for crystalline lactase acting on lactose substrate to be 3.85×10^{-3} mol/L and 1.9×10^{-5} mol/L sec, respectively, at 20 °C.[15]

GLUCOSE MONITORING ISSUES

In the interest of appealing to high school outreach programs and funding-challenged college undergraduate departments, an inexpensive means was sought to monitor the reaction kinetics associated with conversion of lactose to glucose in milk. The obvious choice to monitor the reaction would have been to adopt a standard glucose analyzer (Beckman, YSI, etc.) commonly found in research and hospital laboratories. This complete set-up, however, would cost over $10,000. Other methods considered were based upon refractive index, freeze point depression, use of a lactose probe, chromatography,[19] a colorimetric procedure,[20] a differential pH technique,[21] and various other wet chemistry methods.[22, 23] Finally, an inexpensive blood glucose monitoring meter, commonly used by diabetics to measure glucose levels in blood, was selected as a candidate for monitoring lactose conversion in milk.

Glucose monitoring meters are portable medical devices available from several manufacturers for use by diabetics as a quick, easy, and inexpensive means to monitor blood glucose levels. The meters are not recommended by the manufacturer for testing glucose in solutions other than blood. Currently, two different technologies that govern the operation of these meters are: (1) electrochemistry (amperometry), and (2) reflectance photometry. With reflectance photometry technology, the test strip for the

TABLE 2
Graphical Determination of Michaelis-Menten Parameters at Various pH (22 °C)

pH	K_M, mol/L	V_{max}, mol/L sec
8.2	1.35×10^{-3}	2.26×10^{-4}
6.8	3.46×10^{-3}	2.63×10^{-4}
6.2	4.39×10^{-3}	2.63×10^{-4}
5.0	12.5×10^{-3}	2.88×10^{-4}

meter contains a combination of glucose oxidase catalyst and a dye mixture. As a drop of blood is placed on the test strip, glucose and oxygen react in the presence of the catalyst to produce gluconic acid and hydrogen peroxide. Hydrogen peroxide, in turn, with mediation by peroxidase, oxidizes a dye impregnated in the test strip to produce a blue color. The meter senses the intensity of this blue color, which is proportional to the glucose concentration of the sample. The method is based upon Trinder's glucose oxidase method and the two coupled reactions are called Trinder's reaction.[24] Additional references[25, 26] can be consulted for more information about bioelectrochemical analytical methods.

The Bayer *Ascensia Contour* glucose meter (electrochemistry technology) was tested and found to be unsuitable for purposes of these experiments. Next, the Lifescan *One-Touch Basic* meter (reflectance photometry) was tested. Range of the meter is 0 - 600 mg glucose/dL (0 - 33.3 mmol/L). Normal range of glucose concentrations in blood for fasting humans is 70 - 110 mg/dL. Operation of the meter involves inserting a coded test strip within the meter and placing a small sample drop on the strip. After about 60 seconds, the meter provides a digital readout of glucose concentration. This meter was responsive when measuring glucose levels in skim milk across varied temperature and pH ranges, and was selected for use in this experimental investigation.

METER ACCURACY AND PRECISION

The blood glucose monitoring meter is designed to measure glucose concentrations in blood, but not necessarily glucose concentrations in milk. A typical meter measurement of raw skim milk is 9 -10 mg glucose/dL. The actual range of glucose in nonfat milk as measured by other sophisticated laboratory instruments has been reported to be 2.2 - 27.0 mg/dL.[27, 28] Even though our meter measurement was within this range, it is not known how accurate the meter is at the lower end of its range (0 - 600 mg/dL). In an effort to check the bias and accuracy of the glucose monitoring meter over its entire range, reagent-grade glucose was added to skim milk to make up various standard concentrations. According to Figure 8, there is significant deviation between actual concentration of glucose in skim milk and concentrations measured by the meter. Also, the meter was unable to reproduce concentrations of glucose in buffered deionized water solutions. When glucose was added to bovine blood, meter readings were accurate and linear. As noted above, the glucose monitoring meter measures glucose concentrations in blood based upon the result of two reactions with reagents contained on a standardized test strip (Trinder's reactions). Blood is a very complex mixture of numerous compounds, including various acid-base buffers to control its normal pH within the range of 7.3 - 7.4. It is well documented that Trinder's reactions are subject to bias by numerous interfering reagents, such as uric acid, cholesterol, hemoglobin, ascorbic acid, and maltose.[29, 30]

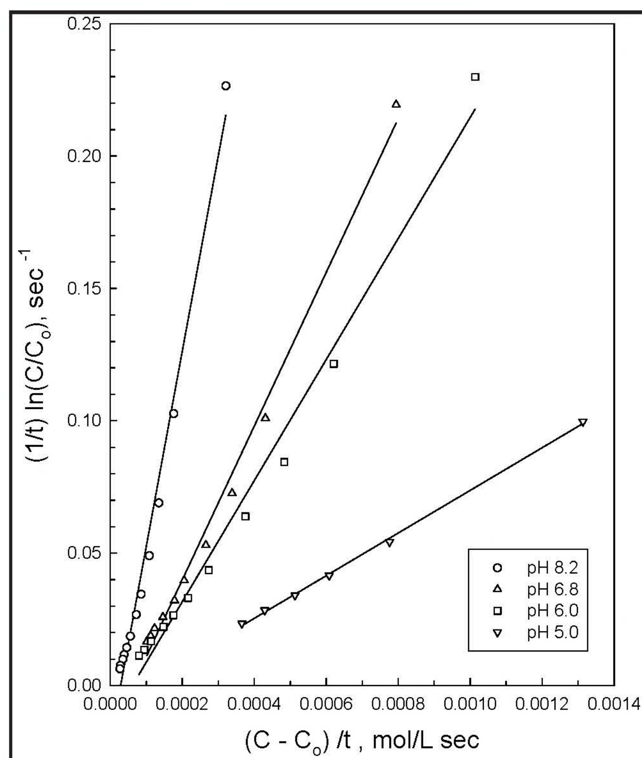


Figure 7. *Determination of Michaelis-Menten parameters for various pH (500 ml milk, 22 °C, 2 tabs catalyst, 300 rpm).*

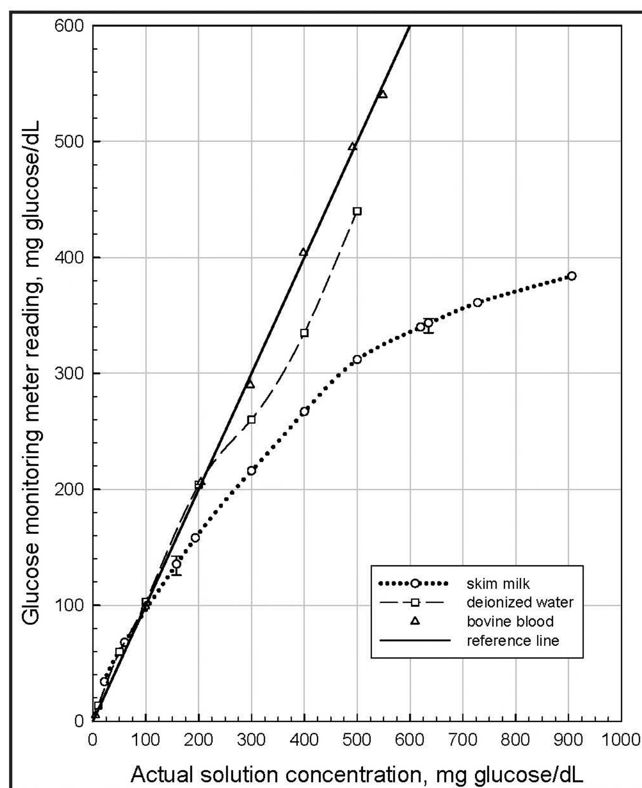


Figure 8. *Calibration of glucose monitoring meter (Lifescan One-Touch Basic Meter).*

Skim milk is a very complex mixture, containing many compounds that may interfere with use of Trinder's reactions to analytically quantify concentrations of glucose. It is believed matrix interferences between glucose and skim milk solutions contributed to nonlinearities in experimental meter measurements (see Figure 8). Unfortunately, these were unable to be resolved. Obvious interferences were investigated, including effects of pH (buffered solutions), solubility, viscosity, red color, and various ion concentrations. Glucose concentrations in milk could be linearized with the transform $y = -1.553e + 4 + 2022 \ Ln \ (x + 2184)$, where x is the actual glucose concentration and y is the meter reading. This approach would be acceptable if all other variables were to remain relatively steady, such as temperature and pH (which is the case with use of the meter to monitor glucose levels in human blood). The purpose of the use of this meter, however, is to evaluate the effects of changes in operating variables upon reaction rates in milk mixtures. Therefore, for use as a student exercise, these matrix interferences were ignored. Unfortunately, during the laboratory experiments, meter reading data does not correspond to true concentrations of glucose in milk mixtures.

The precision of the meter was found to be satisfactory for student testing. Error bars based upon results of 10 samples at various concentrations are shown in Figure 8 to demonstrate reproducibility. At the lower range, sample mean was 134.1 mg/dL with a sample standard deviation of 8.2. At the upper range, sample mean was 341.1 mg/dL with a sample standard deviation of 6.2. Typical precision can be roughly estimated to be about ± 10%. For laboratory runs occurring across long periods of time, students were required to test each sample two times and average the results. When using the initial rate method (occurring across about 10 minutes), there is insufficient time to repeat samples. Each test requires about 60 to 75 seconds to perform.

CONCLUSIONS

An inexpensive glucose monitoring meter has been shown as an effective tool for measuring relative rates of reaction for the conversion of lactose to glucose in skim milk. A number of variable effects can be studied using the meter, but absolute numbers associated with variable effects cannot be relied upon because the meter has proven not to be linear across ranges of glucose in skim milk. Trends can be studied, however, and the meter serves as a valuable teaching aid for use by undergraduates in the chemical engineering laboratory.

NOMENCLATURE

α reaction order, dimensionless
C_{Et} total system enzyme concentration, mol/volume
C_g glucose concentration, mg/dL
C_{go} initial glucose concentration, mg/dL
C_W water concentration, mol/volume
t time, sec
k specific rate constant, time $^{-1}$, if $\alpha = 1$
k_{cat} turnover number = $k_2 C_W$
r_g reaction rate for the appearance of glucose, mol/L min
$-r_S$ reaction rate for the disappearance of the substrate, mol/L min
V_{max} maximum rate of reaction for a given total enzyme concentration, mol/L sec
K_M Michaelis constant (affinity constant), mol/L

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