# Inversion of Sucrose[1]

Purpose: The rate of reaction between sucrose and water catalyzed by hydrogen ion is followed by measuring the angle of rotation of polarized light passing through the solution. Non-linear curve fitting is used to extract the rate constants. Hydrochloric and monochloroacetic acid will be used as the catalysts and the acids will be compared.

Introduction

The angle of rotation of polarized light passing through the solution is measured with a polarimeter. The reaction is

$$C_{12}H_{22}O_{11} + H_2O + H^+ \longrightarrow C_{16}H_{12}O_6 + C_6H_{12}O_6 + H^+$$

Sucrose   Glucose Fructose
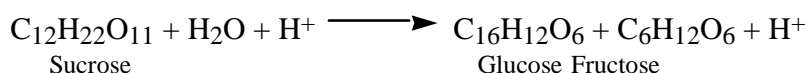
Sucrose is dextrorotatory, but the resulting mixture of glucose and fructose is slightly levorotatory, because the levorotatory fructose has a greater molar rotation than the dextrorotatory glucose. As the sucrose is used up and the glucose-fructose mixture is formed, the angle of rotation to the right (as the observer looks into the polarimeter tube) becomes less and less, and finally the light is rotated to the left. The rotation is determined at the beginning (α_o) and at the end of the reaction (α_∞), and the algebraic difference between these two readings is a measure of the original concentration of the sucrose.

The reaction proceeds too slowly to be measured in pure water, but it is catalyzed by hydrogen ions. The water is in such large excess that its concentration does not change appreciably, and the reaction follows the equation for a first-order reaction, even though two different kinds of molecules are involved in the reaction.

This same reaction in biological systems is catalyzed by an enzyme called invertase. The hydrolysis of sucrose into single sugars is necessary before the sugars can be metabolized. In humans, the invertase enzyme is found in saliva.

Theory

The rate law for the inversion of sucrose is in the form:

$$\frac{d[\text{sucrose}]}{dt} = k\,[\text{sucrose}]^m\,[H_2O]^n\,[H^+]^p \tag{1}$$

The concentration of water in this experiment is large, 55M, and essentially constant. We will also use a large excess of acid to maintain a constant hydrogen ion concentration. We can then rewrite Eq. 1 to isolate the dependence on the concentration of sucrose:

$$-\frac{d[\text{sucrose}]}{dt} = k_{eff}\,[\text{sucrose}]^m \tag{2}$$

with $k_{eff} = k\,[H_2O]^n\,[H^+]^p$ (3)

We will assume that the reaction is first order in sucrose, m=1, which we will verify by curve fitting to a first-order integrated rate law. In the equations that follow we will set [sucrose] = c.

It is assumed that the reaction goes to completion so that practically no sucrose remains at "infinite" time. The integrated form of the first-order-reaction differential equation is then:

$$c = c_o\, e^{-k_{eff}\, t} \tag{4}$$

where c_o is the concentration of sucrose at the beginning of the reaction. Taking logarithms,

$$\ln \frac{c}{c_o} = -kt \tag{5}$$

Thus the slope of a plot of ln c/c_o versus t is -k. However, in this experiment we don't measure the concentration of sucrose.

Instead of measuring concentration directly, in this experiment the optical rotation, α, is measured. Optical rotation is linear function of the concentration for each optically active molecule in solution:

$$\alpha_i = A_i\, c_i \tag{6}$$

for A_i a constant dependent on the molecule i. For a solution of sucrose, glucose, and fructose:

$$\alpha = A_{sucrose}\, c_{sucrose} + A_{glucose}\, c_{glucose} + A_{fructose}\, c_{fructose} \tag{7}$$

At the end of the reaction, no sucrose remains and:

$$\alpha_\infty = A_{glucose}\, c_{glucose,\infty} + A_{fructose}\, c_{fructose,\infty} \tag{8}$$

$$\text{From the stoichiometry,}\quad c_{glucose,\infty} = c_{fructose,\infty} = c_{sucrose,o} \tag{9}$$

where $c_{sucrose,o}$ is the initial concentration of sucrose. Substituting Eq. 9 in to Eq. 8 gives:

$$\alpha_\infty = (A_{glucose} + A_{fructose})\, c_{sucrose,o} \tag{10}$$

Likewise at the beginning of the reaction:

$$\alpha_o = A_{sucrose}\, c_{sucrose,o} \tag{11}$$

Subtracting Eq. 10 from Eq. 11 gives:

$$\alpha_o - \alpha_\infty = (A_{sucrose} - A_{glucose} - A_{fructose})\, c_{sucrose,o} \tag{12}$$

During the course of the reaction at time t:

$$\alpha = A_{sucrose}\, c_{sucrose} + A_{glucose}\, c_{glucose} + A_{fructose}\, c_{fructose} \tag{13}$$

However, from the 1:1 stoichiometry and Eq. 9 we have

$$c_{glucose} = c_{fructose} = c_{sucrose,o} - c_{sucrose} \tag{14}$$

which when substituted into Eq. 13 and then Eq. 10 is subtracted gives:

$$\alpha - \alpha_\infty = A_{sucrose}\, c_{sucrose} - A_{glucose}\, c_{sucrose} - A_{fructose}\, c_{sucrose} \tag{15}$$

or grouping terms:

$$\alpha - \alpha_\infty = (A_{sucrose} - A_{glucose} - A_{fructose})\, c_{sucrose} \tag{16}$$

Dividing Eq. 16 by Eq. 12 then gives the desired concentration ratio:

$$\frac{\alpha - \alpha_\infty}{\alpha_o - \alpha_\infty} = \frac{c_{sucrose}}{c_{sucrose,o}} \tag{17}$$

Finally substituting Eq. 17 into Eq. 4 gives:

$$(\alpha - \alpha_\infty) = (\alpha_0 - \alpha_\infty)\, e^{-k_{eff}\, t} \tag{18}$$

or $\alpha = (\alpha_0 - \alpha_\infty)\, e^{-k_{eff}\, t} - \alpha_\infty \tag{19}$

Substituting Eq. 17 into Eq. 5 gives

$$\ln \frac{\alpha - \alpha_\infty}{\alpha_0 - \alpha_\infty} = -kt \tag{20}$$

or $\ln(\alpha - \alpha_\infty) = -kt + \ln(\alpha_0 - \alpha_\infty) \tag{21}$

The slope is the same as it would be if actual concentrations had been plotted.

   Summary: In kinetics experiments any property, such as optical rotation, that is a linear function of concentration can be used in place of the concentration for curve fitting.

Apparatus. Polarimeter (described in the Appendix); sodium-vapor lamp; thermostat and circulating pump; two water-jacketed polarimeter tubes; two #3 rubber stoppers; pure sucrose; 100 ml of 4 M hydrochloric acid; 100 ml of 4 M monochloroacetic acid; three 25-ml pipettes.

Procedure. Twenty grams of pure cane sugar (sucrose) is dissolved in water (filtered, if necessary, to give a clear solution) and diluted to 100 ml. The sucrose solution, the 4 M hydrochloric acid solution, and the 4 M monochloroacetic acid are placed in the 25° thermostat and allowed to stand a few minutes to come to temperature.

   Two jacketed polarimeter tubes are connected in series with the circulating water from a thermostat at 25° . A zero reading is taken.

   Twenty-five milliliters of the sucrose solution is thoroughly mixed with 25 ml of the 4 M monochloroacetic acid solution, and small portions of the solution are used to rinse out one polarimeter tube. The tube is then filled and stoppered. The second polarimeter tube is filled in a similar manner after rinsing, using 25 ml of the sugar solution and 25 ml of the 4 M hydrochloric acid solution. The tubes are filled rapidly, and readings are taken as soon as possible after mixing. The first reading is taken on the hydrochloric acid solution and recorded, and subsequent measurements are recorded about every 10 min for the first hour or so. As the reaction slows down, the observations may be taken less frequently. The observations should extend over a period of 3 hr or more. The reaction goes much more slowly with the monochloroacetic acid, and the readings are taken less frequently. Monochloroacetic acid readings are taken at convenient intervals of time when the polarimeter is not needed for the hydrochloric acid solution.

Cleanup: At the end of the day, please empty the cells, rinse with soap, and rinse the cells at least three times. Remove the end caps and cell windows. Wash the cell windows and the exterior of the cells. Leave the windows on a ChemWipe to dry. Don't use paper towels on the cell windows. Wash all your glassware with hot soapy water and rinse. Wash the base of the refractometer and the cell holder with soapy water and dry with paper towels. Leave all the glassware at the bench for the next group.

 Caution: Monochloroacetic acid is corrosive. Please, immediately clean up any spills with <u>soap</u> and water.

Calculations: The Problem with Linear Curve Fitting of Exponential Processes

Recasting the exponential form of Eq. 4 or 19 to give a linear equation has two important and well-known consequences. The first problem concerns the effect of experimental error. The raw data in kinetics experiments usually has a constant error for every data point. That is, the uncertainty in the early measurements is the same as in later measurements when the reaction is almost complete. When the ln function is taken, something striking happens. Early data points are little effected by the errors, but data points toward the end of the reaction have large deviations caused by the basic nature of logs. Therefore, when you do a linear curve fit, the error in the data points at the end of the kinetics run can have an inordinate effect on the fit value of the rate constant. This effect is shown in Figure 1. The standard deviation in the raw data is the same for every measurement, but the transformed data shows much more scatter at the end of the reaction than the early data points. Because of this effect of error, it is always advisable to use non-linear curve fitting to the exponential form of the integrated rate law. Non-linear curve fitting is just as easy as linear curve fitting and uses the same basic philosophy. Many computer programs are available to do the calculations.
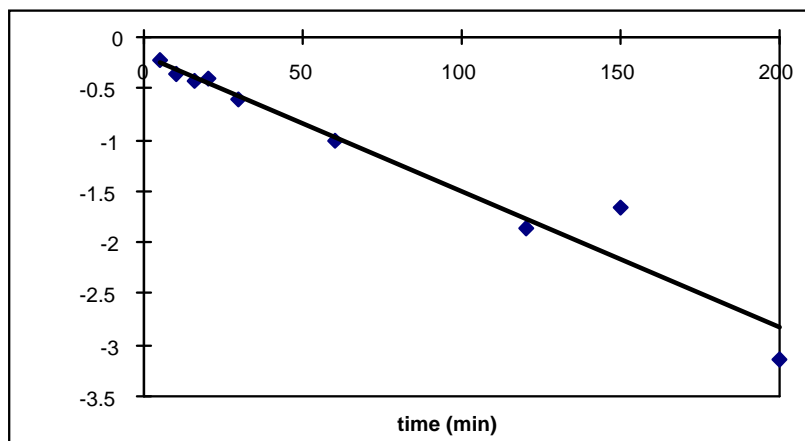

Figure 1. The effect of a constant experimental error in a natural log plot.

The second problem with the linear form of the integrated rate law is the effect of the value of α_∞, the infinite time value in Eqs. 19 or 20. Small errors in the determination of α_∞ cause significant curvature in a straight line plot of the data, Figure 2.
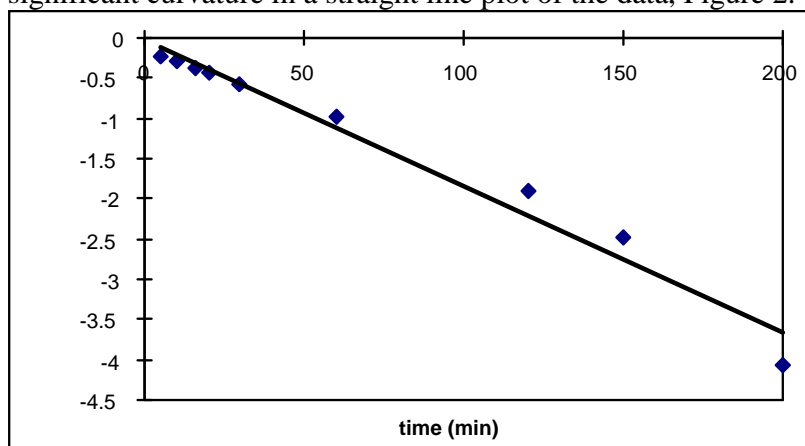

Figure 2. Curvature caused by a 1.5° error in the value of α_∞ in a natural log plot.

Many methods have been devised to get around this problem. For example, you could treat α_∞ as a variable parameter in Eq. 21 and do a three parameter fit to the data: α_∞, k, and the intercept. In so doing you would need to do a non-linear fit to the data, so it is best to reject the linear form of the integrated rate law, and fit Eq. 19 directly as discussed above. Eq. 19 has three fit parameters, $\alpha_0 - \alpha_\infty$, k, and α_∞. The next section discusses how to do this fit using the "Nonlinear Least Squares Curve Fitting" applet on the course Web site.[2]

Sucrose Kinetics Example Calculation Using Non-Linear Cyrve Fitting

Based on Eq. 19, we want to fit an equation of the form from:

$$y = a\, e^{-bt} + c \tag{22}$$

The data for this problem is given in Figure 3, so you can cut and paste into the applet as a trial run. Enter rough guesses for the fit coefficients, a, b, and c. Estimates for the rate constant can be easily obtained from a preliminary linear plot using Eq. 21, or simply by trial and error. For Figure 3, the first data point was used as a guess for a and the last data point as a guess for c. If your initial guesses don't give convergence, you can scroll down in the Results window and note the "Fit values from the initial guesses." This section just calculates the fit values without changing your guesses for the fit parameters, so you can judge how close your initial guesses are. You can vary your guesses until these initial fit values more closely resemble the input data. The final results are shown in Figure 4.
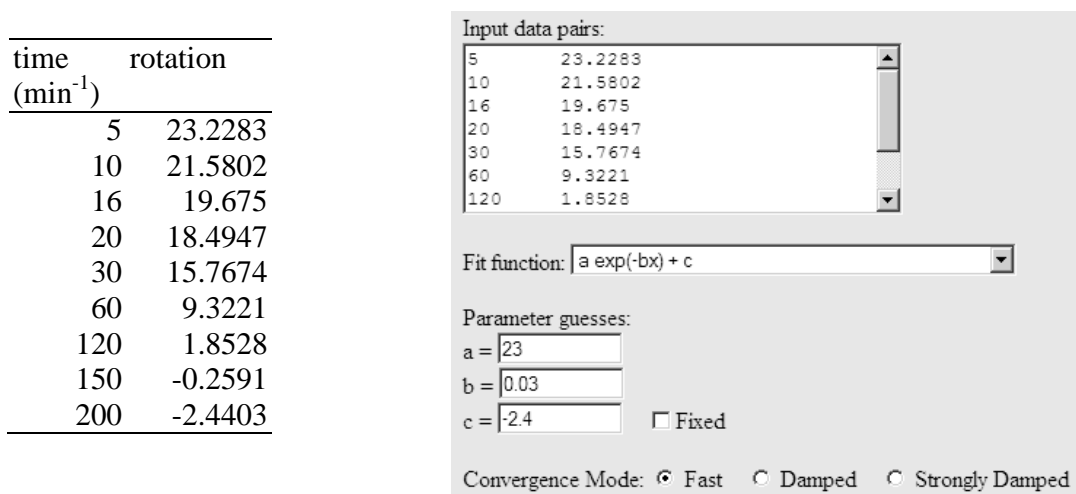
time	rotation
(min^{-1})	
5	23.2283
10	21.5802
16	19.675
20	18.4947
30	15.7674
60	9.3221
120	1.8528
150	-0.2591
200	-2.4403

Figure 3. Example data and initial input into the Nonlinear-Curve Fitting" applet.[2]

The rate constant is given by $b = 0.012335 \pm 0.000033$ min^{-1}, with small uncertainty. Non-linear curve fitting procedures give statistically valid estimates for the uncertainties of the fit parameters and an estimate of the correlation coefficient between the fit parameters. For our example, a correlation coefficient of 0 between the fit parameters a and b means that the error in a has no effect on the error in b. For a correlation coefficient of ±1, the errors in a and b are completely correlated. For high correlation coefficients, a small change in the value of one of the data points, caused by experimental error, will give a large change in the fit values of both a and b. Such a complete correlation means that neither parameter estimate is valid. For our example,

Figure 4, the correlation coefficient between the fit parameters a and b is -0.7007. Such a correlation coefficient is acceptable, especially for exponential curve fitting. Correlation coefficients larger than 0.95 suggest that one of the fit parameters needs to be specified as a fixed parameter, which is independently determined either directly from the data or from another experiment. The correlation coefficient between b and c in our example is 0.9252. This large fit parameter correlation coefficient tells us the final results for b and c are strongly related. A small change in the value of one of the data points, caused by experimental error, will give a large change in the fit values of both b and c. Our example's correlation is large, but still acceptable. However, this large correlation suggests that if you can determine the c value accurately from very long time measurements, then it would be better to use c as a fixed parameter and just fit a and b. The large fit parameter correlation also suggests that you should repeat the experiment to try to obtain data with greater precision.

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===================   Results   ========================
a= 30.036 +- 0.0241
b= 0.0123352 +- 0.000033
c= -4.986 +- 0.029
_____ Output Data _____
      x                 y                  y(fit)     residual
     5.0          23.2283        23.25342    -0.02512
    10.0          21.5802        21.56434     0.01586
    16.0          19.675         19.67027     0.00473
    20.0          18.4947        18.48323     0.01147
    30.0          15.7674        15.75967     0.00773
    60.0           9.3221         9.34293    -0.02083
   120.0           1.8528         1.84974     0.00306
   150.0          -0.2591        -0.26459     0.00549
   200.0          -2.4403        -2.43791    -0.00239
-----------------------------------------------------------
sum of squared residuals= 0.001575
stand. dev. y values= 0.0162
correlation between a & b= -0.7007
correlation between b & c= 0.9252
correlation between a & c= -0.8846
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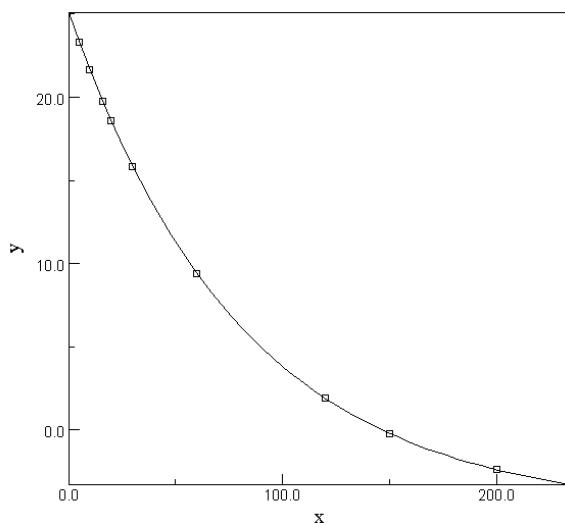Figure 4. Final results for the example data set.

Figure 5. Final curve fit for the example data set.

The r^2 correlation coefficient that is commonly reported for linear least squares curve fitting and the fit parameter correlation coefficients should not be confused. The r^2 correlation coefficient should be close to 1 for a good fit, but the between fit parameter correlation coefficients should be small for a valid fit.

Conclusions

Report your rate constants and uncertainties for the two acids. Include your plots. Discuss the validity of the curve fits based on the fit parameter uncertainties and the fit parameter correlation coefficients. Relate your results to the acid strength of hydrochloric acid and monochloroacetic acid. Use your data for the two acids and the K_a for monochloroacetic acid to calculate the order of the reaction with respect to $[H^+]$. Explain the relative acid strengths of monochloroacetic acid and hydrochloric acid on the basis of molecular structure.

Precise measurements of the rate of inversion of sucrose are available.[3,4] The inversion of sucrose is historically important, because it was the first quantitative kinetics study.

References
1. This write up is loosely adapted from F. Daniels, J. W. Williams, P. Bender, R. A. Alberty, C. D. Cornwell, J. E. Harriman, *Experimental Physical Chemistry*, *7th Ed*, McGraw-Hill, New York, NY, 1970, Experiment 24.
2. Nonlinear Least Squares Curve Fitting:
    http://www.colby.edu/chemistry/PChem/scripts/lsfitpl.html
3. S. W. Pennycuick, *J. Am. Chem. Soc.,* **1926**, *48*, 6.
4. G. Scatchard, *J. Am. Chem. Soc.,* **1926,** *48*, 2259.

Appendix: Polarimeters[1]

   The rotation of plane-polarized light may be determined by the use of a polarimeter. Electromagnetic radiation consists of sinusoidally varying electric and magnetic fields, the directions of which lie in mutually perpendicular planes. If the electric component is restricted to a single plane (as illustrated in Fig. A1), the light is said to be plane-polarized. In ordinary light the electric component has all possible orientations, and none is preferred. This is because the individual atoms and molecules that are radiating act independently. Such unpolarized light may also be considered to consist of two plane-polarized waves which are at right angles to each other and have a completely random phase relationship; i.e., at any instant the phase difference between the two waves is equally likely to have any value between 0 and 2π.
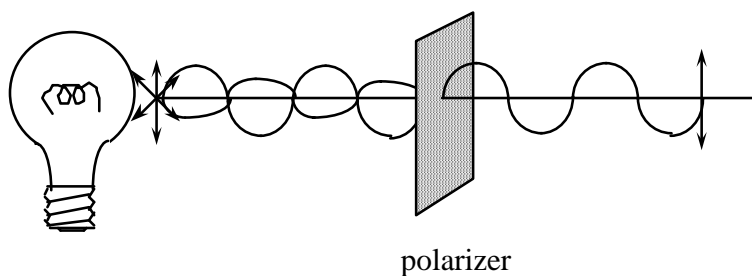

polarizer

Figure A1. A polarizer selects light waves that oscillate in a chosen direction.

Polarizers select waves that oscillate in a particular direction. Simple polarizers can be made by stretching a piece of clear adhesive tape to orient the polymer molecules. High quality polarizers are made from anisotropic crystals. In optically *isotropic* crystals the index of refraction is independent of the direction of propagation of the light through the crystal. In *anisotropic* crystals, such as calcite ($CaCO_3$), an incident beam of unpolarized light is split into two beams in the crystal. It is found that these two beams are plane-polarized, their planes of vibration being at right angles. The velocities of propagation of these two components through the crystalline medium are different because of the difference in the index of refraction of the medium for the two differently polarized rays. This makes possible the elimination of one component, so that plane-polarized light is obtained. A Nicol prism is constructed by cutting a calcite prism in half along a suitable diagonal plane and cementing the sections together with Canada balsam. At the calcite-Canada balsam interface, one component is totally reflected to the side, where it is absorbed by a black coating applied to the prism. The other component, for which the refractive index of the balsam and the calcite are almost equal, is freely transmitted.

To get a better understanding of the rotation of the plane of polarization of light by certain solutions and crystals, it is useful to consider circularly polarized light. If plane-polarized light (Figure A2a) is allowed to pass into a suitably cut slab of calcite, the light will be separated into two waves that are plane-polarized at right angles to each other and have equal amplitudes, but are propagated in the same direction through the crystal. The waves travel through the crystal at different speeds, and so there will be a phase difference between them when they emerge from the crystal. If the thickness of the crystal is chosen so that this angle is 90º, the emerging light is
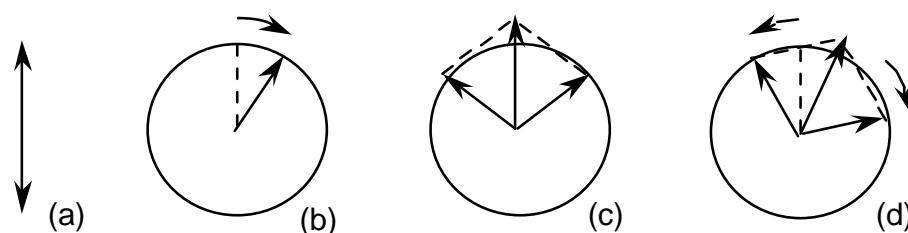

Figure A2. (a) Electric vector of plane-polarized light moving in a direction perpendicular to the page; (b) circularly polarized light in which the electric vector rotates around the direction of propagation; (c) plane-polarized light is the resultant of two vectors representing circularly polarized light with opposite senses of rotation; (d) rotation of the plane of polarization of plane-polarized light is caused by one circularly polarized wave propagating through the medium at a higher velocity than the other.

circularly polarized and the ground crystal is referred to as a *quarter-wave plate*. Such a combination of two plane-polarized waves can be represented by a vector rotating about the direction of propagation as shown in Fig. A2b. Plane-polarized light may be considered to be made up of two oppositely rotating circularly polarized beams which are in phase. As shown in Fig. A2c, the resultant of the addition of these two vectors remains in a single plane.

The optical rotation by a gas, liquid, or solid may be considered to arise from a difference in the velocity of clockwise and counterclockwise circularly polarized light. As shown in Fig. A2d, the resultant of the addition of the two vectors representing circularly polarized light is rotated through and angle if one component travels more rapidly than the other.

   Molecules that can be distinguished from their mirror images are able to rotate the plane of polarized light when it passes through them. The presence in the molecule of an asymmetric carbon atom (one for which all four attached groups are different) leads to such a structure and is the most common, but not the only, cause of optical activity.

   In the study of proteins it is found that there is present an intrinsic optical activity, resulting from the contributions of the individual amino acids, whatever the overall conformation of the protein; it derives from the asymmetric carbon atoms. In the cases of those proteins with α-helical content, however, there is a second and additional contribution to the optical rotation which originates in the asymmetry of the α helix itself. From observations of the magnitude of this second contribution it has become possible to make an estimate of the fraction of the polypeptide which is in the helical conformation.

   The magnitude of the optical rotation is measured with a source of monochromatic light and a polarimeter, which consists primarily of two Nicol prisms, between which the optically active substance is placed. When the second Nicol, known as the *analyzer*, is placed at right angles to the first, no light can pass through if the cell contains an optically inactive substance. When a
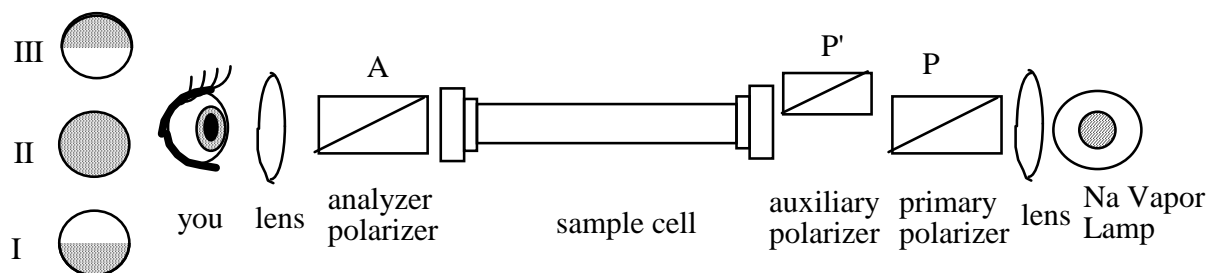

Figure A3. Polarimeter.

substance that is capable of rotating the plane of polarized light is inserted between the Nicols, light can again be seen through the analyzer. The angle through which the analyzer must be turned to darken the field again is represented by α. If the analyzer is turned clockwise (as seen by the observer) to restore darkness, the substance is said to be dextrorotatory. If darkness is restored when the analyzer is turned counterclockwise, it is levorotatory.

   Because of the error inherent in locating the point of minimum intensity, it is better to employ a scheme in which the eye is required only to compare one field with another field of nearly the same intensity, as is done in half-shadow polarimeters.

   The principle of the Laurent half-shadow polarimeter is illustrated in Figure A3. Behind the large polarizing Nicol prism, P, is placed an auxiliary Nicol, P', whose plane of polarization has been adjusted to make an angle θ with that of the principal polarizing prism P. The angle of the analyzer A, is indicated by the instrument scale. When the analyzer is turned so that it is at smaller angles than the main polarizing Nicol, the field, as viewed through the magnifying eyepiece, is dark on one side and light on the other, as shown at I. When the analyzer is turned through the small angle θ to cross with the smaller Nicol, the sides are reversed in intensity, as shown at III. When the analyzing Nicol is turned back through half of this small angle, it gives a uniform field as shown at II. This proper setting, II, is readily found, and the corresponding reading of the scale is recorded.

   The magnitude of the optical rotation is affected by the concentration of the solution, the length of the path of the light in the solution, the wavelength of the light, the temperature, and the nature

of the solvent. The specific rotation $[\alpha]^t_\lambda$ for a given wavelength λ at a given temperature t is defined by the relation

$$[\alpha]^t_\lambda = \frac{\alpha}{\mathit{l}c} = \frac{\alpha}{\mathit{l}pd}$$

where α = observed angle of rotation

l = length of light path, decimeters

c = concentration of solute, grams solute per milliliter solution

p = concentration of solute, grams solute per gram solution

d = density of solution, $g\ ml^{-1}$

   The specific rotation depends upon the wavelength of the light, and this dependence is called *optical rotatory dispersion.* The dispersion of optical rotation is closely related to light absorption; in the vicinity of absorption bands, the optical rotation usually increases rapidly and then decreases through zero to give an opposite rotation as the wavelength is changed. This is referred to as the *Cotton effect*. These changes are more sensitive than most properties to changes in molecular conformation. This tool has recently been developed into a very powerful one for studying molecular structures of complicated bio-molecules.

1. Excerpted with a few changes from F. Daniels, J. W. Williams, P. Bender, R. A. Alberty, C. D. Cornwell, J. E. Harriman, *Experimental Physical Chemistry , 7th Ed.* McGraw-Hill, New York, NY, 1970, Exp. 37, pp 236-239.