PHOTOMETRIC DETERMINATION OF CATALASE ACTIVITY

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Catalase activity may be measured quantitatively by the method of von Euler and Josephson (1) by allowing the enzyme solution to react with hydrogen peroxide for varying periods of time and measuring the excess peroxide remaining by titration with potassium permanganate. The similarity of this titration procedure to the assay of ascorbic acid by titration with 2,6-dichlorophenolindophenol suggested to the authors the possibility of measuring catalase activity photometrically (in a manner similar to the photometric estimation of vitamin C) by addition of an excess quantity of potassium permanganate and subsequent photometric measurement of the color. This procedure has been found to be practical and rapid. It gives reproducible results and, by photometric means, obviates the human error in reading the end-point of the potassium permanganate. As both hydrogen peroxide and potassium permanganate in the present procedure are used in the same concentrations as in the conventional von Euler and Josephson method, compounds that inhibit catalase such as HCN should have no different effect in this procedure from that in the conventional one.

Apparatus and Reagents—The photometric determination of catalase activity may be carried out with any standard colorimeter or spectrophotometer.¹

The reagents required for this measurement are as follows.

Hydrogen peroxide (approximately 0.01 N) in phosphate buffer at pH 6.8. Use 5.67 ml. of 3 per cent hydrogen peroxide per liter.

Potassium permanganate (approximately 0.005 N); 0.158 gm., made to 1 liter with distilled water.

A solution of diluted catalase enzyme. For our study, a 1:2500 catalase-Sarett² solution was used.

Sulfuric acid (5 N); 142 ml. of concentrated sulfuric acid (sp. gr. 1.84) made to 1 liter with distilled water.

¹ A Coleman spectrophotometer, a Cenco “photelometer,” or an Evelyn colorimeter will be found satisfactory for this purpose.

Preparation of Standard Curve

In a volumetric flask, 10 ml. of hydrogen peroxide (3 per cent by volume) are diluted to 100 ml. with distilled water and mixed. Then into consecutive 100 ml. volumetric flasks are pipetted 6, 5, 4, 3, 2, and 1 ml., respectively, of the 0.3 per cent hydrogen peroxide solution. The contents of the flasks are made to volume with phosphate buffer (0.0067 M) and mixed. From each flask, a 5 ml. amount is pipetted into six reaction test-tubes containing 2 ml. of 5 N sulfuric acid. The photometer is set to transmit maximally at 515 m\(\mu\) and is adjusted to 100 per cent transmission with a reference tube which contains 2 ml. of 5 N sulfuric acid plus 15 ml. of distilled water.

Into one of the six reaction tubes, 10 ml. of the 0.005 N permanganate solution are blown. The contents are quickly mixed, and the transmission is read at once. This is repeated for all six tubes.

A seventh tube is prepared with 2 ml. of sulfuric acid, 5 ml. of water, and 10 ml. of permanganate. The transmission of this mixture is read and serves as the “blank.”

Table I lists the contents of each of the tubes and presents a typical set of readings obtained with the use of a Coleman Universal spectrophotometer.

The optical densities which correspond to the transmissions, expressed in per cent, are calculated and can be plotted with reference to the rela-

<table>
<thead>
<tr>
<th>Contents of tube, 17 ml.</th>
<th>Tube 1</th>
<th>Tube 2</th>
<th>Tube 3</th>
<th>Tube 4</th>
<th>Tube 5</th>
<th>Tube 6</th>
<th>Blank</th>
<th>Reference tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ml. 0.3% H(_2)O(_2) per 100 ml.</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>% H(_2)O(_2)</td>
<td>0.018</td>
<td>0.015</td>
<td>0.012</td>
<td>0.009</td>
<td>0.006</td>
<td>0.003</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>H(_2)O(_2), ml.</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Water, &quot;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>K(_2)MnO(_4),&quot;</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>H(_2)SO(_4), &quot;</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Transmission, %</td>
<td>90(^a)</td>
<td>83(^b)</td>
<td>75(^c)</td>
<td>71(^d)</td>
<td>66(^e)</td>
<td>61(^f)</td>
<td>57(^g)</td>
<td>100(^h)</td>
</tr>
<tr>
<td>Optical density</td>
<td>0.0458</td>
<td>0.0796</td>
<td>0.1206</td>
<td>0.1478</td>
<td>0.1805</td>
<td>0.2147</td>
<td>0.2422</td>
<td>0.0000</td>
</tr>
<tr>
<td>(D_0\text{ml} - D_x)</td>
<td>0.1964</td>
<td>0.1626</td>
<td>0.1216</td>
<td>0.0935</td>
<td>0.0617</td>
<td>0.0275</td>
<td>0.0275</td>
<td>0.0275</td>
</tr>
<tr>
<td>Concentration</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.0327</td>
<td>0.0325</td>
<td>0.0304</td>
<td>0.0312</td>
<td>0.0309</td>
<td>0.0275</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average slope 0.0309
tive concentrations of hydrogen peroxide in the test-tubes to obtain the "standard curve." As the relationship between optical density and percentage of concentration is linear in this case, it is actually unnecessary to plot the standard curve. All that is necessary is to calculate the amount of decrease in optical density below that of the blank for each increase of 1 per cent in relative concentration. This is done by subtraction of the value for the optical density of each concentration from that of the blank, and division of the result by the relative concentration. The resulting quotients or "slopes" are averaged to obtain the mean slope of the standard curve.

**Measurement of Catalase Activity**

The necessary dilutions of a preparation of catalase are made and placed in an ice-water bath. In this work, a 1:2500 solution of catalase-Sarett was used. To 50 ml. of 0.01 N hydrogen peroxide containing 0.007 M phosphate buffer at a pH of 6.8 (which has been allowed to sit in a beaker of ice water for 5 minutes) add 1 ml. of properly diluted enzyme. Mix, and immediately pipette 5 ml. of this solution into a reaction tube containing 2 ml. of the 5 N sulfuric acid. Mix, and add 10 ml. of the permanganate solution. Mix, and read in the colorimeter against the reference tube with a 515 m\(\mu\) filter. Repeat again after 3, 6, 9, and 12 minutes. Then calculate the \(K_0\) value and the \(Kat. f.\) value (2).

A blank reading is made with a mixture containing 2 ml. of acid, 5 ml. of distilled water, and 10 ml. of the permanganate solution.

The volume of permanganate remaining after the reaction with hydrogen peroxide is calculated according to the following formula:

\[
\frac{D_{bl.} - D_s}{\text{Slope}} = \text{ml. permanganate used}
\]

where \(D_{bl.}\) is the optical density of the blank reading, \(D_s\) is the optical density of the sample reading, and "slope" is the average slope of the standard curve calculated as described above.

**Comparison of Measurements of Catalase Activity by Photometric Method and by Conventional Permanganate Titration Method**

For comparative purposes the activity of a sample of "catalase-Sarett" was determined by the photometric method described above and by the conventional permanganate titration method. The results are summarized in Table II. As can be seen from the data, the \(Kat. f.\) values are in good agreement.
Photometric Determination of Catalase

Precautions

Several precautions should be emphasized. The time between pipetting of the permanganate solution into the tube and the photometric reading should be not more than 1 minute. After a period of from 1 to 1½ minutes, a colloidal suspension forms in the tubes, which is especially pronounced if the quantity of hydrogen peroxide remaining is significant.

Table II

Comparison of Measurements of Catalase Activity by Photometric and Permanganate Titration Methods

<table>
<thead>
<tr>
<th>Reaction time</th>
<th>Photometric method</th>
<th>Titration method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transmission</td>
<td>Optical density</td>
</tr>
<tr>
<td>min.</td>
<td>% per cent</td>
<td>ml.</td>
</tr>
<tr>
<td>0</td>
<td>86¹</td>
<td>0.064</td>
</tr>
<tr>
<td>3</td>
<td>81₀</td>
<td>0.092</td>
</tr>
<tr>
<td>6</td>
<td>77¹</td>
<td>0.112</td>
</tr>
<tr>
<td>9</td>
<td>74¹</td>
<td>0.129</td>
</tr>
<tr>
<td>12</td>
<td>70³</td>
<td>0.150</td>
</tr>
<tr>
<td>Blank</td>
<td>56²</td>
<td>0.248</td>
</tr>
</tbody>
</table>

Kat. f. ‡

\[
\frac{0.0245 \times 2500^§}{0.0271^§} = 2260
\]

\[
\frac{0.078 \times 1000^§}{0.0271^§} = 2880
\]

Slope \( \Delta t \) = \( \frac{\Delta t}{\Delta t} \)

\( \tau \) Kₜ = \( \frac{1}{T} \log_{10} \frac{a}{a - x} \), where Kₜ is the reaction rate, T time, a initial KMnO₄ concentration, and a - x is the KMnO₄ remaining. The Kₜ value for T = 0 is obtained by extrapolation.

\‡ \( \text{Kat. f.} = \frac{K_5 (0 \text{ time})}{\text{gm. enzyme (dry weight)}} \). See Sumner and Somers (2).

§ Enzyme dilution used was 1:2500 in the photometric method and 1:1000 in the titration method; the enzyme content was 0.0271 gm. (dry weight) per ml. in both instances.

One standard curve, if properly obtained with good duplicate checks and small deviations in slopes between the individual points, suffices for the particular instrument, wave-length, and permanganate pipette. A 10 ml., graduated pipette with a wide tip to facilitate rapid flowing is recommended.

Increasing or decreasing the permanganate concentration or increasing or decreasing the peroxide concentration has no effect on the slope of the curve, which is constant. Hence for such a determination, one has only
to ascertain the blank reading of permanganate, acid, and water and the reading of the unknown solution containing the reaction products of catalase activity.

SUMMARY

A technique is described for measuring catalase activity photometrically with the use of a Coleman spectrophotometer or any colorimeter.

A properly diluted enzyme solution is allowed to react with hydrogen peroxide for a specified period of time. The reaction is then stopped by use of a sulfuric acid solution. Potassium permanganate in excess is next added to the mixture and allowed to react with the peroxide not decomposed by the catalase. Within 1 minute after addition of the permanganate, the excess in potassium permanganate is determined photometrically.

BIBLIOGRAPHY
