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Determination of Enzyme Activity

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Determination of Enzyme Activity

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Purpose: To show the enzyme activity of diastase upon starch by viscosity measurements.

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Taka-Diastase, 250 ml flask, corn starch, 50 ml beaker, ditilled water, burette clamp, stopwatch, Sam's viscometer, ringstand.

Procedure: The substrate is made by placing 10 g. of corn starch into 250 ml. of distilled water and then heating to 85 °C with continuous stirring. It is then cooled to room temperature.

Sam's viscometer can be easily made by using a 40 cm lengthof soft lime glass tubing, 6 mm O.D. This is then heated by holding its center in a Fisher burner flame. By pulling to a length of approximately 50 cm a small constriction is made in the center of the glass tube.

The viscometer is mounted on a ringstand and is held in place by a cork and a burette clamp. A small beaker is placed below the viscometer with 20 ml of starch suspension. To this is added 3 drops of liquid diastase. This is immediately stirred and a viscosity measurement taken. (Viscosity is determined in this case by sucking into the viscometer the starch-enzyme mixture from the beaker. The material is timed as it passes between two predetermined points on the viscometer.) Following one minute intervals the viscosity is checked until no further change in viscosity of the starch-enzyme mixture can be detected. Three trials are to be made and the average recorded.

Discussion: In this experiment viscosity is used to determine the activity of an enzyme upon its substrate. The enzyme in this case is liquid diastase and the substrate is starch suspension.

If one is familiar with the spot plate method for determining enzyme activity, he recalls that the enzyme activity is determined by the presence or absence of a blue-black color. Although the end point may be easily detected by this method the rate at which the reaction proceeds is harder to determine. It is my aim to show the rate of enzyme activity as well as the end point.

I think one may find this very well illustrated upon examination of the graph. One can see at a glance that immediately following the addition

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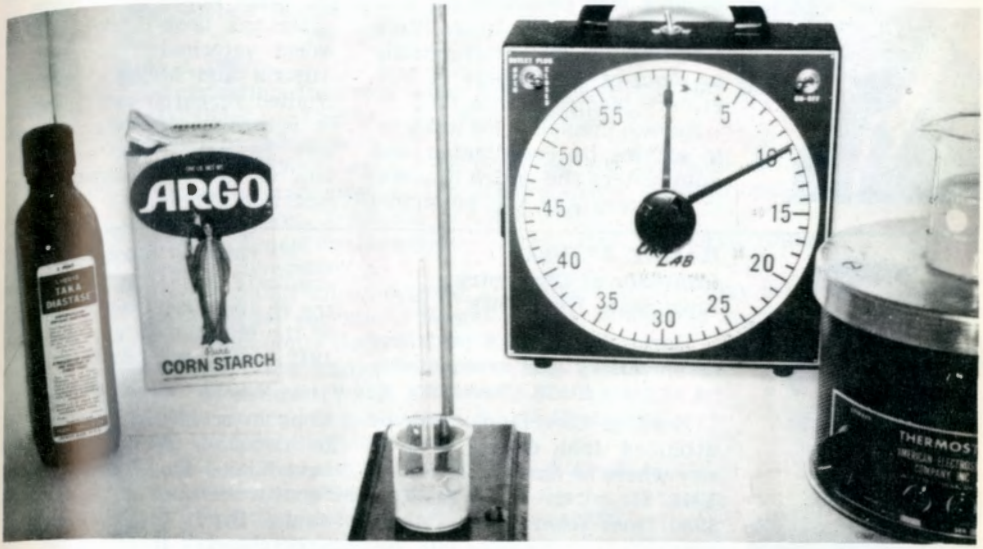
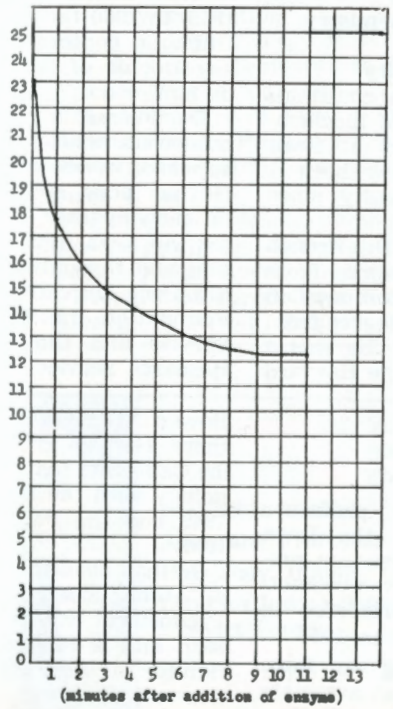
of the enzyme, the viscosity of the mixture changed from 23 seconds to 17.7 seconds after a period of one minute had elapsed. After a period of 9 minutes no enzyme activity can be noticed since the time for the mixture to pass out of the viscometer does not change.



Enzyme Activity by Viscosity



Graph: Showing enzyme activity when 3 drops of liquid diastase is added to 20 ml of starch suspension at 20° C.

DATA:

Time	Average viscosity
0	23
1	17.7
2	15.7
3	14.8
4	14
5	13.7
6	13.1
7	12.7
8	12.5
9	12.3
10	12.3
11	12.3