

AN APPROACH TO ESTIMATE THE CLOTTING TIME OF MEASURING THE ACTIVITY OF PEPSIN

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INTRODUCTION

Pepsin is aspartic proteases. Pepsin is most active in acidic environments between 37°C and 42°C [1,2]. Accordingly, its primary site of synthesis and activity is the stomach (pH 1.5 to 2). Pepsin exhibits maximal activity at pH 2.0 and is inactive at pH 6.5 and above, however pepsin is not fully denatured or irreversibly inactivated until pH 8.0. Therefore pepsin in solution of up to pH 8.0 can be reactivated upon re-acidification [3].

MATERIALS AND METHOD

To prepare substrate solution of milk powder, 12 g dry milk powder was added in 94 ml of CaCl₂ son after that it was stirred magnetically for 20-30 min and makeup 100 ml with 0.01M CaCl₂. Than it was distributed in 10-ml portions in separate test tubes and keep them at 30±0.2 °C for at least 10 min, but not more than 1 h. To prepare the enzyme dilutions, introduce 2 ml and 4 ml respectively of 1.25 M sodium acetate buffer pH 5.7 was added in 50 ml or 100 ml flask, add distilled water was added to two-thirds of the volume of the flask and pipet accurately measured aliquots of the enzyme sample (dissolve, if powdered), and the reference enzyme, dilute to volume. To obtain the desired dilutions (about 1:5,000 strength, arrived at by diluting original sample solution between 25 and 200 fold), distribute 1-ml aliquots of each dilute enzyme solution in test tubes at (18x250 mm) and incubate at 30±0.2 °C. To start the reaction, substrate solution contained in one test tube was poured into that containing the dilute enzyme and starts concomitantly a stop watch and mix the test solution twice by rapid inversion of the test tube and keep in a slanted position in the water bath kept at 30±0.2 °C. Rotate the test tube slowly by hand until the first clots are observed whereupon the watch is stopped and the time is recorded to the nearest 0.1 sec [1,2].

Determination of clotting time

Plot the clotting times obtained with enzyme dilutions being assayed and that with the reference standard against the dilution factors employed. Straight lines are obtained for clotting times between 50 and 300 sec.

RESULT

Clotting reaction: clotting time of enzyme –substrate reaction which have 40 mg/ml to 120 mg/ml conc of enzyme decreased with increased concentration of enzyme, showed decreased clotting time. (Table:1) Minimum clotting time was observed at 120 mg/ml concentration of enzyme.(Figure:1)

Table:1 clotting time on various concentration of enzyme

Pepsin.Concentration (mg/ml)	Initial clotting time (min)	Final clotting time (min)
120	1:20	2:17
100	2:20	2:52
80	3:00	3:54
60	4:40	5:24
40	6:00	7:24

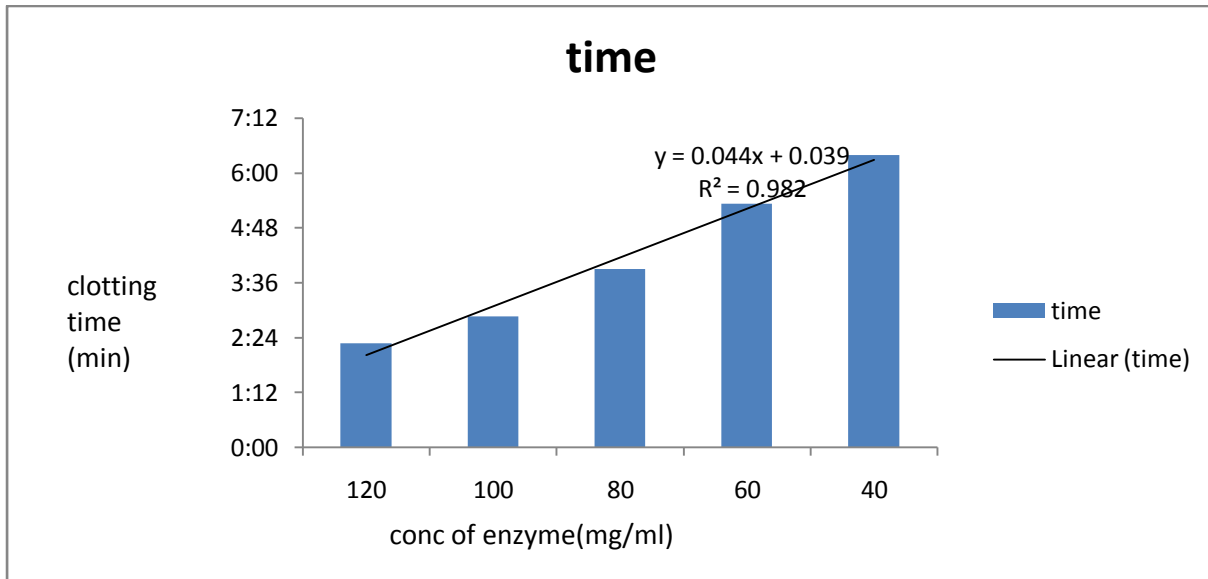


Figure:1 Effect of enzyme concentration on clotting time

CONCLUSION

Clotting time of enzyme substrate reaction is decreased with increase concentration of enzyme indicate the active form of enzyme present in it, and other soluble form does not give the clot.

REFERENCES

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3. M. Bergmann, W.F. Ross, J. Biol. Chem., 114,1936,717.

