# CARACTERIZACIÓN DE LA β-GLUCOSIDASA

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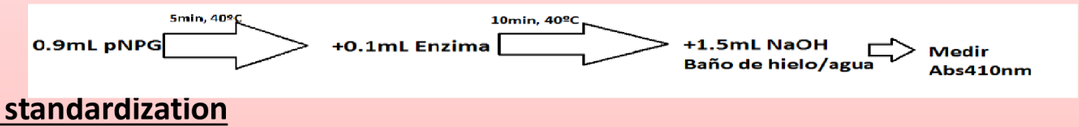
## INTRODUCCIÓN

β-glucosidasE (EC 3.2.1.21) is an enzyme with hydrolase activity and, under certain conditions, transferase (or transglycosidase) activity. This group of enzymes catalyzes mainly the hydrolisis of O- o S-glycosidic bonds between two carbohydrates or one carbohydrate and an aglicona (compound of a different nature). These enzymes are found throughout nature, from bacteria to humans, where they perform different functions (1). In the case of humans, β-glucosidase is directly related to Gaucher's disease (2). The three-dimensional structure of β-glucosidases is known thanks to techniques such as X-ray crystallography (3). The object of the study is to propose a kinetic mechanism for the enzyme, in the aim of looking for an industrial use if possible.

## MATERIALS AND METHODS

The enzyme used was isolated from sweet almonds' emulsin (*Prunus dulcis*), from FLUKA house. All the assys which have been carried out follow the following protocol:.
The activity of the enzyme was quantified using a chromogenic substrate: pNPG .




**Assay standardization**

- Construction of the pNP standard line: the enzyme's activity is directly proportional to the concentration of this compound (it is one of the products).
- To check that the assay is carried out at the optimum time and enzyme concentration.
- To calculate the approximate value of Km (Km *), based on tests carried out using a theoretical Km

**Kinetic characterization**

The values of the kinetic parameters were obtained from the results of the test (it was done in duplicate).

**Temperature effect on catalysis**

These studies are intended to verify that the test is carried out under optimal temperature conditions.

**Reversible inhibition studies** This study allows us to propose a kinetic mechanism, since the type of inhibition of each of the substrates helps determine the order of realease of the products. Assays with different concentrations of β-gluconolactone and β-glucose were carried out in this study.

## RESULTS

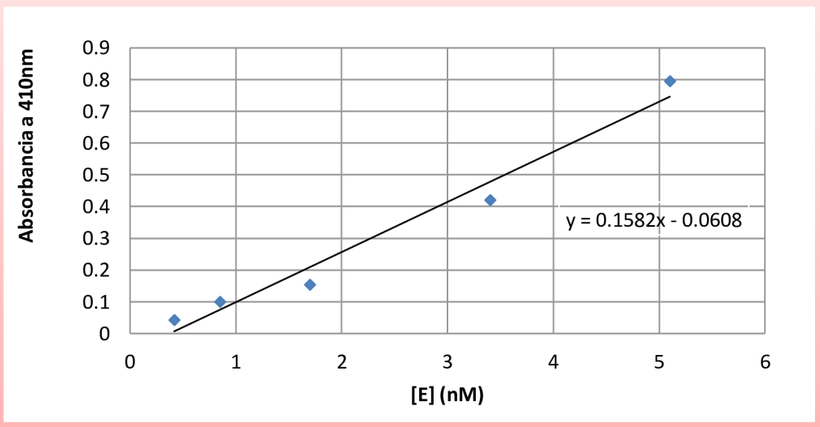



*Figure 1: graph to determine the optimal enzyme concentration.*

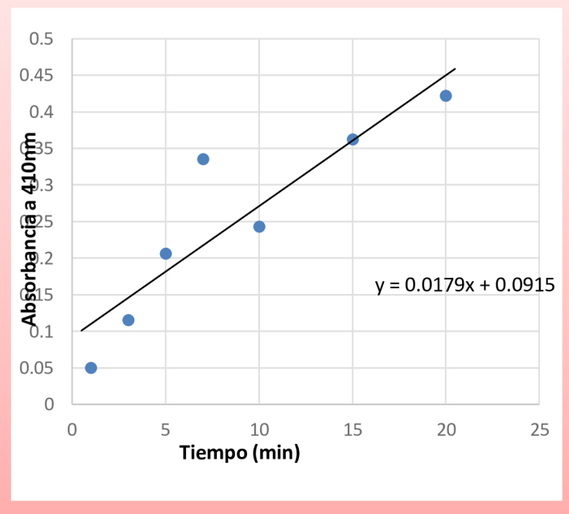



*Figure 2: plot to check the linearity of velocity with the time.*

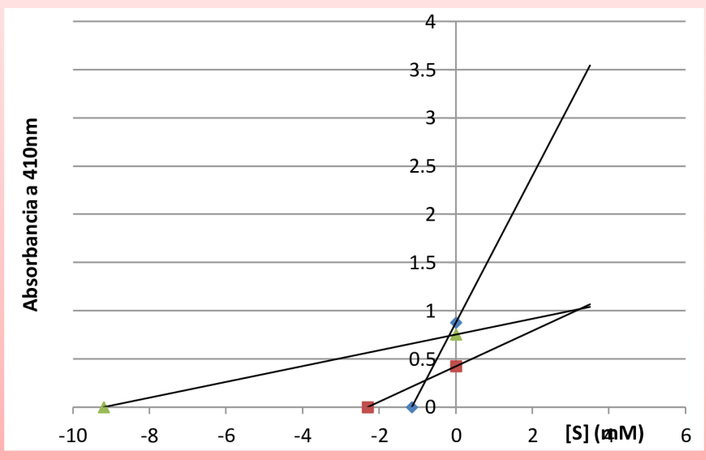



*Figure 3: Cornish-Bowden representation to determine the approximate value of Km (Km *)*

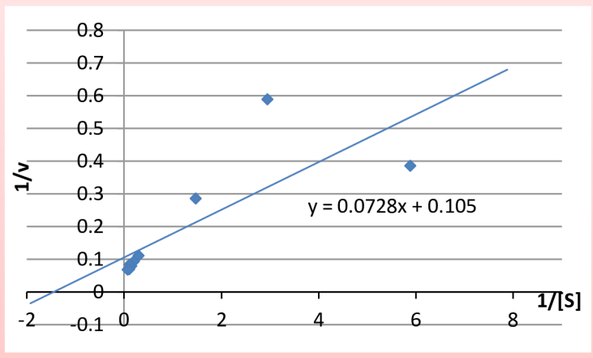



*Figure 5: Lineweaver-Burk plot*

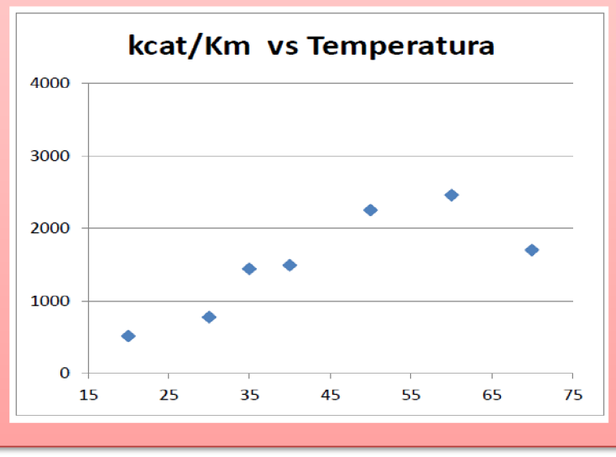



*Figure 6: catalytic efficiency at different temperatures.*

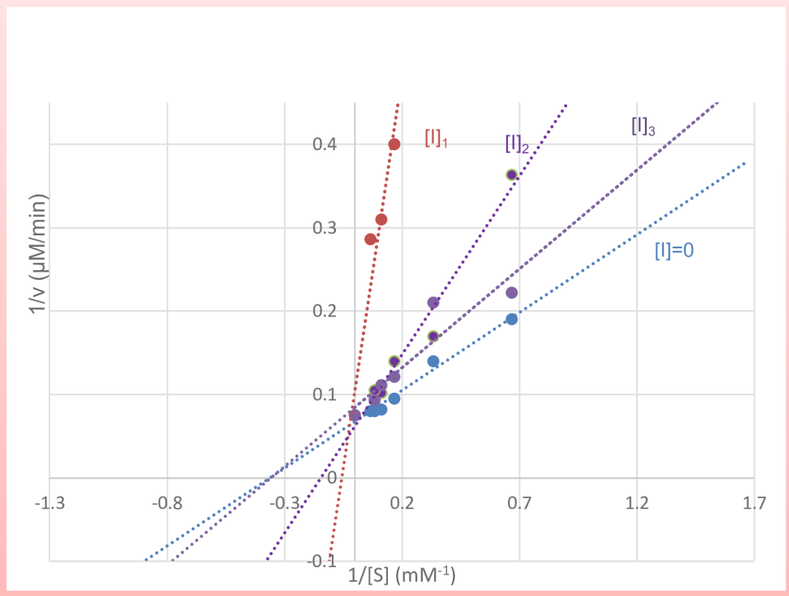



*Figure 8: Lineweaver-Burk plot at different concentrations of β-gluconolactone: same Vmax, different Km.* ***Competitive inhibitor***

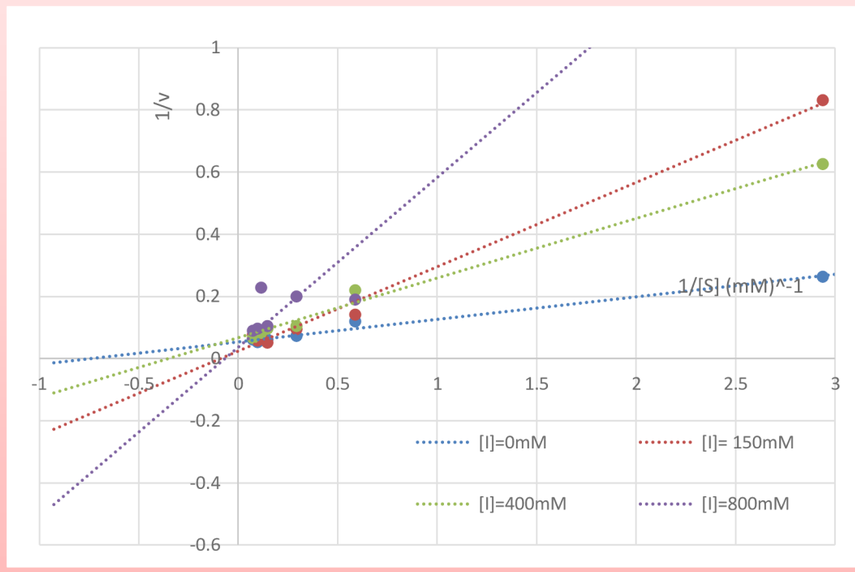



*Figure 7: Lineweaver-Burk plot at different concentrations of D-glucoSE: same Vmax, different Km.* ***Competitive inhibitor***

## CONCLUSIONS:

The β-glucosidase in this experiment has a Km of 2,776mM and its maximum velocity is 18,53µM/min. Taking into account that β-D-glucose works as a competitive inhibitor (Kis=200mM), an organized sequential mechanism may be proposed for the catalytic mechanism of the enzyme.

β-gluconolactone also shows a strong (Kis=0,06)competitive inhibitor behavior. Besides, its structure isn't similar to D-glucose's (the principal substrate). Then, the only available option is that its structure resembles the stationary state, which would explain the results in Kis and the structural differences.
This study could be completed with an additional experiment which could show that pNP is the first product to leave the enzyme. The object of the designed experiment would be to check that the pNP is a mixed inhibitor in the hydrolysis reaction of pNPG by the β-glucosidase. In this type of inhibitors, both of the secondary representations show variation in their slope (non of the parameters stay constant).

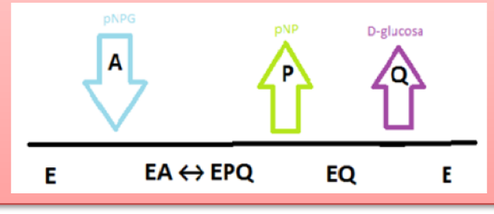



*Figura 9: catalitic mechanism proposed*

**REFERENCES**

1. Bhatia, Y.; Mishra, S.; Bisaria, V. S., *Microbial beta-glucosidases: Cloning, properties, and applications*. Critical Reviews in Biotechnology 2002, **22**, 375-407.
2. Grabowski, G. A.; Gatt, S.; Horowitz, M., *Acid beta-glucosidase: enzymology and molecular biology of Gaucher disease*. Critical Reviews in Biochemistry and Molecular Biology 1990, **25**, 385-414.
3. Cairns, J.R.K.; Esen, A., *β-glucosidases*. Cellular and Molecular Life Sciences 2010, **67**, 3389– 3405.