**[BCH 322]**

**The effect of inhibitors on the rate of an enzyme catalyzed reaction**

**Method:**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| [S] mM | 0 | 0.5 | 1 | 2.5 | 5 | 10 | 25 | 50 |
| Without I | A | B | C | D | E | F | G | H |
| With I (adding 1 ml of I) | Ai | Bi | Ci | Di | Ei | Fi | Gi | Hi |

* **Prepare 16 tubes labeled as follows in which each tube will contain the following concentration:**

|  |  |
| --- | --- |
| Chemical | Volume (ml) |
| pH sodium acetate buffer | 0.5 |
| 0.1M MgCl2 | 0.5 |
| Corresponding p-nitrophenyl phosphate (pNPP) | 0.5 |
| Water | 5 |

* **To each of these tubes add :(Without Inhibitor)**

|  |  |
| --- | --- |
| Chemical | Volume (ml) |
| pH sodium acetate buffer | 0.5 |
| 0.1M MgCl2 | 0.5 |
| Corresponding p-nitrophenyl phosphate (pNPP) | 0.5 |
| Water | 4 |
| Inhibitor | 1 |

* **To each of these tubes add :(With Inhibitor)**
* **Place the tubes in a test tube rack situated in 37oC water bath and let stand for 5 min.**
* **Start the reaction by adding 0.5 ml enzyme and stop it by adding 0.5 ml KOH as in the following table:**

|  |  |  |
| --- | --- | --- |
| ***Tube*** | ***Start the reaction*** | ***Stop the reaction*** |
| ***A*** | ***0 min***  | ***0 min***  |
| ***B*** | ***0 min***  | ***5 min***  |
| ***C*** | ***2 min***  | ***7 min***  |
| ***D*** | ***4 min***  | ***9min***  |
| ***E*** | ***6 min***  | ***11 min***  |
| ***F*** | ***8 min***  | ***13 min***  |
| ***G*** | ***10 min***  | ***15 min***  |
| ***H*** | ***12 min*** | ***17 min*** |

* **Determine the absorbance at 405 nm for each sample, using the first tube (0 mM of S) as the blank.**

 ***Results:***

