

TP4 In vitro model of cytotoxicity/protein precipitation effect.

1/ Principle

The protein denaturation test is interesting both as a test simulating one of the basic mechanisms of cytotoxicity and as an alternative to the use of animals in research. The principle of protein denaturation dosing is very simple. When a chemical is incubated with a protein (BSA), an increase in opacity occurs as a result of protein precipitation. Opacity is directly proportional to the amount of precipitated protein, which is measured spectrophotometrically at 405 nm. The ability of a chemical to precipitate a protein is considered a measure of its cytotoxicity.

2/ Protocol

0.9 ml of 5% BSA (prepared in 0.9% NaCl) are added to 0.1 ml of the substance or 10mM HCl or 10mM acetic acid as reference denaturing agents. The volume is completed to 6ml with NaCl 0.9%. The mixture is incubated for 1 hour at 37°C followed by reading the OD at 405 nm. A control containing methanol instead of the test substance is prepared under the same conditions. A blank corresponding to each test series is also prepared under the same conditions. The percentage of protein denaturation is calculated according to the following reaction:

$$\% \text{ of Ovalbumin denaturation} = \left(\frac{\text{Optical density of the sample} - \text{Optical density of the control}}{\text{Optical density of the control}} \right) \times 100.$$

In this practical work we aim to test the denaturing effect of the proteins of the crude extract of the leaves of the Nerium Oleander plant at the concentrations 1000ug/ml and 500µg/ml

Questions

- 1/ Indicate the pharmacological interest of cytotoxic substances.
- 2/ Discuss the advantages and limitations of this in vitro model of protein denaturation.
- 3/ Indicate the composition of the control tubes and the blank tubes used Indicate the purpose of each of these tubes
- 4/ Explain why HCl and acetic acid are used as reference molecules in this test.
- 5/ Present the results obtained on a histogram and run a statistical analysis using the student test
- 6/ Interpret and discuss the obtained results.