DETECTION OF PESTICIDE IN ORGANIC SOLVENT BY CHOLINESTERASE INHIBITION TO AVOID HEAVY METALS INTERFERENCE

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Pesticides are among the most important environmental pollutants because of their high toxicity and their significant presence in the environment. Among the many methods reported for pesticide detection, chromatographic methods such as HPLC (High Performance Liquid Chromatography) and GC (Gas Chromatography) are used as reference methods. Despite their high sensitivities, these techniques are expensive, time consuming and required highly qualified personnel. Thus enzymatic methods have been adopted as an alternative to classical methods (GC, HPLC) for faster and simpler detection of some environmental pollutants.

The use of cholinesterase enzymes for inhibition-based determination of pollutants has shown great promise for environmental screening analysis [1,2]. The measurement of enzymatic activity can be accomplished by spectrophotometric method [3,4]. However, several problems have to be solved in order to make this kinetic approach effective.

A major problem relates to the effect of the organic solvents normally used for pesticide extraction on Acetylcholinesterase activity as many of these solvents are known to inhibit the AchE reaction [5]. At the same time, liquid extraction with organic solvents are the procedures usually adopted for pesticide determination. A second major problem involves the interference of heavy metals with the enzymatic determination of pesticides based on their inhibition of Acetylcholinesterase activity.

The need of an extraction method to avoid the presence of heavy metals during the measurement of pesticides based on Acetylcholinesterase (AchE) inhibition has been demonstrated by studying the reaction between thiocholine and heavy metals. Mercury (Hg²⁺) and Copper (Cu²⁺), usually present in contaminated samples, together with Silver (Ag⁺) have shown a strong affinity towards thiocholine.

We present the preliminary results obtained by using two different phases, one organic and the other aqueous, in which pesticide and enzyme are respectively solubilised. Firstly the concentration of the substrate acetylthiocholine (1 mM), of enzyme (7 mU/ml) and the reaction time (20 min) were optimised in aqueous solution. The organic phase was then added and the effect of various solvents on the enzyme activity was evaluated after 10 minutes of mixing. It was found that using hexane, the enzyme retained almost 100 % of activity and it was chosen for pesticide assays.

Hexane was spiked with different concentration of pesticides and then added to the enzyme aqueous phase. The pesticide are able to inhibit the enzyme at the interface between the two solutions. The degree of inhibition obtained with increasing amounts of pesticide was evaluated. A 50% inhibition has been observed by using paraoxon solution of $9 \times 10^{-7}$ M and carbofuran solution of $1 \times 10^{-7}$ M.


