June 2, 1970 D. N. KRAMER ETAL 3,515,644

REVERSED ENZYMATIC DETECTION METHOD FOR ANTICHOLINESTERASES

Filed April 10, 1967 2 Sheets-Sheet 1

Fig. 1 HYDROLYSIS OF 3',5' DICHLORO INDOPHENYL ACETATE BY ALBUMIN AT pH 8.0

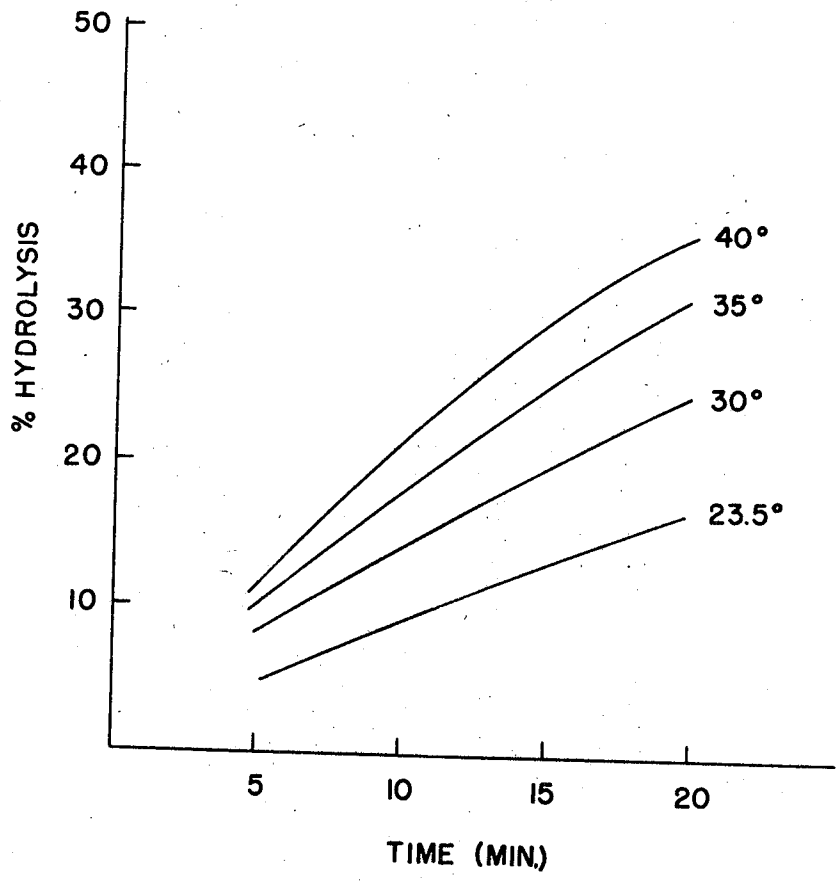

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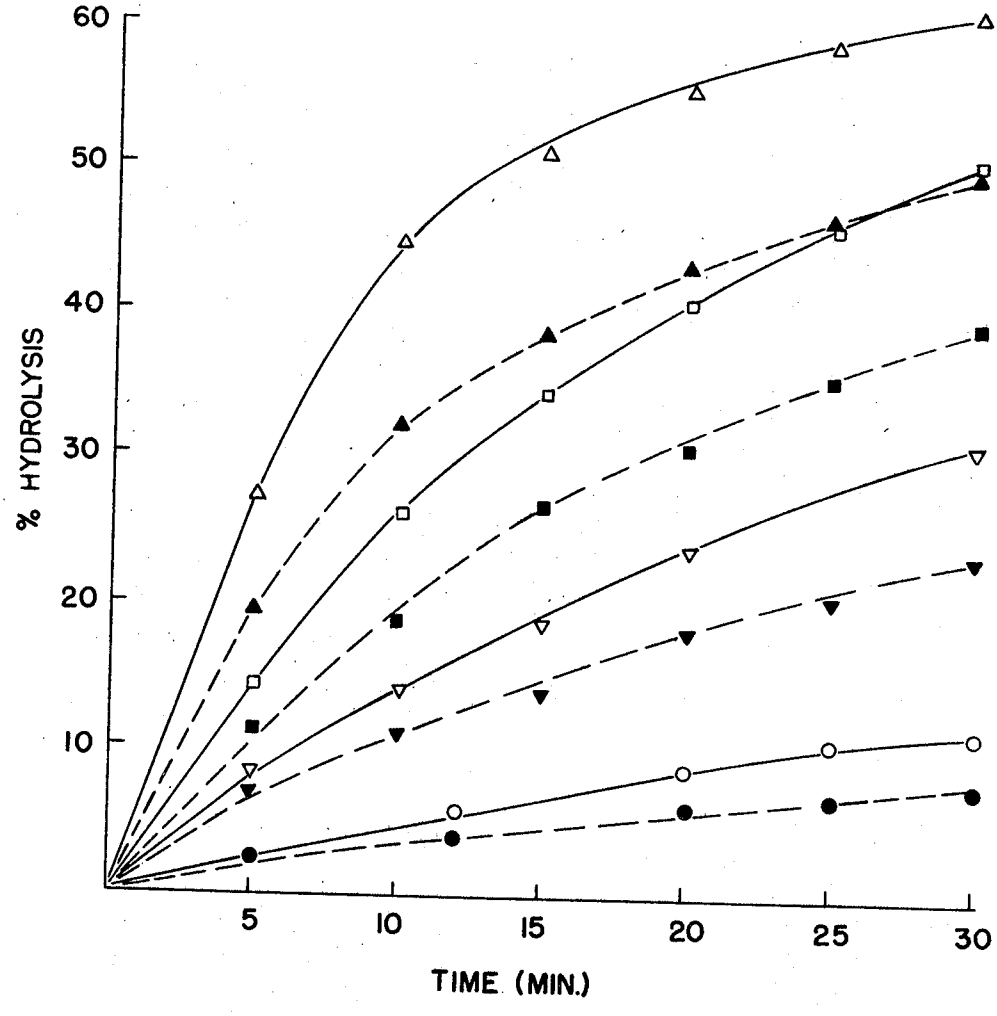
Fig. 2

EFFECT OF TOXIC AGENT ON HYDROLYSIS RATE OF 3',5' DICHLOROINDOPHENYL ACETATE BY ALBUMIN AT 23° C



TOXIC AGENT ABSENT

pH
9.0
8.5
8.0
7.4

TOXIC AGENT PRESENT

pH
9.0
8.5
8.0
7.4

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REVERSED ENZYMATIC DETECTION METHOD FOR ANTICHOLINESTERASES

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ABSTRACT OF THE DISCLOSURE

New enzyme detection method for toxic vapors and aerosols of chemical agents, for example G and V, or any anticholinesterases utilizing a dual substrate and enzyme-catalyst system.

SPECIFICATION

This invention is directed to the detection of toxic vapors and aerosols of anticholinesterase chemical agents, for example G and V agents, by a new dual substrate and enzyme-catalyst method.

In the drawing:

FIG. 1 shows the percent hydrolysis of dichloroindophenyl acetate by albumin with temperature variation.

FIG. 2 shows the percent hydrolysis of dichloroindophenyl acetate by albumin with pH variation.

Numerous prior art methods have been reported for the quantitative and qualitative determination of toxic vapors and aerosols by means of enzyme reaction. An example of such prior art method is the publication of the instant inventors on pp. 251–253 of vol. 30, No. 2, February 1958 of Analytical Chemistry.

The example as well as other prior art had the inherent drawback in the method of giving a negative color response in the enzyme detection system; that is, appearance of color indicated the absence of toxic vapor or aerosol.

It is the object of this invention to determine a system based on an enzyme-substrate reaction which would yield a positive color response in the presence of toxic vapor or aerosol; that is, appearance of color indicated the presence of toxic vapor or aerosol.

A further object was to develop a system based on the enzyme-substrate reaction which would permit a continuously operable one-batch system.

Such a system to meet the above objectives was evolved after the discovery that crystalline bovine plasma albumin effected rapid hydrolysis of a number of substituted indophenyl esters, and that a dual substate and dual enzyme-albumin rather than single system could be utilized.

The preferred system of the invention consisted of a substrate of 0.1 N acetylthiocholine iodide in distilled water, horse serum cholinesterase with an activity of 2.18 μM acetyl choline hydrolyzed/mg./min./ml. in 0.1 N tris buffer added to said acetylthiocholine, 2×10^{-3} M 3',5' dichloroindophenyl acetate in dioxane, and 5% by weight of crystalline bovine plasma albumin in pH 8.0 0.01 N tris buffer added to said dichloroindophenyl acetate. The albumin was heated for five minutes at 55° C. to inactivate aliesterase. The albumin-dichloroindophenyl acetate solution was then mixed with the acetylthiocholine-cholinesterase solution to constitute the detection system to be used to monitor for the presence of G and V agents. While the preferred system was buffered to pH 8.0, it was found that a range of pH 7.4 to pH 9.0 was operable. Also, while 3',5' dichloroindophenyl acetate substrate is preferred, substrates in Table I can be substituted for the preferred substrate.

TABLE I

Compound:	Rate of hydrolysis
Indophenyl acetate	Slow
2,6-dimethoxyindophenyl acetate	Slow
2,6-dibromo-3'-methoxyindophenyl acetate	Slow
2,6-dimethoxy-3',5'-dichloroindophenyl acetate	Fast
2,6,3'-tribromoindophenyl acetate	Fast

In the system of the invention the crystalline bovine plasma albumin hydrolyzes the indophenyl ester to the free indophenol without affecting the acetylthiocholine. On the other hand, the cholinesterase hydrolyzes the acetylthiocholine to produce thiocholine. The thiocholine, thus produced, reduces the blue indophenol to a colorless substance and the solution remains colorless as long as the reactants are unaffected, i.e., if the cholinesterase is uninhibited. In the presence of toxic vapors and aerosols, such as organophosphorous compounds such as G and V agents, the essentially colorless system becomes increasingly bluer with time. The increasing blueness is evidenced by Table II.

TABLE II

Hydrolysis in the presence of toxic agent such as G agent

Time (min.):	Absorbance
3	0.080
5	0.107
10	0.183
20	0.247

In the above described manner a continuous enzyme detecting system was made possible. The hydrolysis of dichloroindophenyl acetate by albumin is pH and temperature dependent, as shown by FIG. 1 and FIG. 2 in the drawing, and is of considerable magnitude under all conditions.

While a preferred system has been described herein, we wish our invention to be limited solely by the scope of the appended claims.

We claim:

1. A continuous organophosphorous compound toxic agent detecting system comprising an acetylthiocholine substrate adapted to be hydrolyzed by cholinesterase, albumin adapted to hydrolyze indophenyl esters, indophenyl ester substrate adapted to be hydrolyzed by the albumin to produce free indophenol, and cholinesterase adapted to hydrolyze the acetylthiocholine to produce thiocholine; said system being controlled within the pH range of 7.4 to 9.0 and said indophenyl ester being an acetate.

2. The system of claim 1 wherein the organophosphorous compound is selected from the group consisting of G and V agents.

3. The system of claim 1 wherein the acetylthiocholine is an 0.1 N solution of the iodide in distilled water.

4. The system of claim 1 wherein the cholinesterase is horse serum cholinesterase.

5. The system of claim 1 wherein the albumin is crystalline bovine plasma albumin.

6. The system of claim 1 wherein the acetate is selected from the group consisting of 2,6 dimethoxy; 2,6 dibromo 3 methoxy; 2,6 dimethoxy-3',5' dichloro; 3',5' dichloro; and 2,6,3' tribromo.

7. The system of claim 1 wherein the indophenyl ester substrate is 3',5'-dichloroindophenyl acetate.

8. A method of detecting organophosphorous toxic agents selected from the group consisting of G, V, and anticholinesterases comprising the steps of preparing an 0.1 N solution of acetylthiocholine iodide in distilled

water; adding enzyme with an activity of 2.18 μM acetyl choline hydrolyzed/mg./min./ml. of horse serum cholinesterase in a pH 7.4 to 9.0 range buffer to said acetylthiocholine iodide; heating 5% by weight of crystalline bovine plasma albumin in pH 7.4 to 9.0 range buffer at 55° C. for 5 minutes to inactivate aliesterase; preparing a 2×10^{-3} M solution of 3',5'-dichloroindophenyl acetate in dioxane; adding the albumin to the dichloroindophenyl acetate solution; mixing the dichloroindophenyl acetate-albumin solution with the acetylthiocholine-cholinesterase solution; monitoring for the presence of said toxic agent with the system of mixed solutions.

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