Microchemical Journal 144 (2019) 56–63

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

A new method for spectrophotometric determination of carbaryl based on rubber tree bark peroxidase enzymatic reaction

Sam-ang Supharoek[a,b], Kraingkrai Ponhong[c,*], Watsaka Siriangkhawut[c], Kate Grudpan[d]

[a] Department of Sciences and Liberal Arts, Mahidol University, Amnat Charoen Campus, Amnat Charoen 37000, Thailand
[b] Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University, Rama 6 Road, Ratchathewi, Bangkok 10400, Thailand
[c] Creative Chemistry and Innovation Research Unit, Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahasarakham University, Maha Sarakham 44150, Thailand
[d] Department of Chemistry, Faculty of Science and Center of Excellence for Innovation in Analytical Science and Technology, Chiang Mai University, Chiang Mai 50200, Thailand

ARTICLE INFO

Keywords:
Carbaryl
Enzyme catalytic reaction
Peroxidase
Rubber tree bark
UV–Visible spectrophotometry
DLLME

ABSTRACT

A method for the determination of carbaryl in vegetable samples was developed employing peroxidase enzyme extracts from newly excised rubber tree bark strips as a catalyst. The solid phase extraction method QuEChERS was utilized for sample preparation followed by dispersive liquid-liquid microextraction (DLLME) to increase the concentration of the analyte before analysis by UV–Visible spectrophotometry. Parameters influencing this enzymatic development method and efficiency of DLLME were carefully studied. Under selected conditions, absorbance was in proportion to carbaryl concentration in the range of 0.1–3.0 mg L^{-1} with linear regression (r^2) of 0.9999. Limit of detection (LOD) and limit of quantitation (LOQ) were 0.06 mg L^{-1} and 0.25 mg L^{-1}, respectively. Relative standard deviation (RSD) values for the samples were 2.51–4.06% (n = 7). Good recoveries of carbaryl were obtained in the range of 83–118%. The proposed method proved rapid, reliable and selective for determination of carbaryl in vegetable samples.

1. Introduction

Carbaryl is an insecticide in the carbamate family, widely used to eradicate agricultural pests for increasing crop yields. Unfortunately, carbaryl is toxic to humans; small concentrations can remain in food products and also contaminate and pollute the environment. Humans absorb carbaryl via skin contact, inhalation and ingestion. This causes inhibition of the key enzyme acetylcholinesterase (AChE) that controls neurotransmitters and headaches, memory loss, proximal muscle weakness and anorexia can result [1]. Therefore, sensitive and selective analytical methods are required to determine concentrations of carbaryl residues in food products.

Chromatographic separation, both as high performance liquid chromatography (HPLC) and gas chromatography (GC) using various detection methods are popular choices for quantification of carbaryl [2–8]. These techniques can simultaneously determine multi-analytes at the same injection but they require highly skilled operators. Moreover, the instrumentation is expensive and not portable compared with UV–Visible spectrophotometry.

Simple and rapid analysis of carbaryl using spectrophotometric methods have been developed [9–16] with many advantages in terms of high sensitivity, medium to high selectivity, high precision and high accuracy. Moreover, spectrophotometry was employed for flow-based analysis detection to increase sample throughput using an automated approach [16–20].

Selective and sensitive carbaryl detection methods utilizing acetylcholinesterase [21–24] and choline oxidase and/or immunoassay [3,4,25] have also been successfully developed. These methods are highly efficient for the determination of carbaryl but they need well-trained operators to perform the complicated and tedious operational steps. Therefore, an inexpensive, simple, portable, and highly sensitive analytical technique is urgently required for the detection of pesticides residues.

To the best of our knowledge, there are no publications concerning the exploitation of peroxidase enzyme extracts from excised rubber tree bark strips for quantification of carbaryl. Here, a spectrophotometric method based on peroxidase enzyme catalytic reaction was developed for detection of carbaryl. The major advantages of using enzymes in analysis are to react specifically with individual components of a mixture, reduces the time needed for an analysis, require the small amount

* Corresponding author.
E-mail address: kraingkrai.p@msu.ac.th (K. Ponhong).

https://doi.org/10.1016/j.microc.2018.08.048
Received 8 June 2018; Received in revised form 24 August 2018; Accepted 24 August 2018
Available online 28 August 2018
0026-265X/

of substrate (sample) for analysis, and employ under the mild conditions [26]. The advantages of utilizing non-purified enzyme are that cells provide a natural environment for the enzymes, preventing conformational changes in the protein structure that would lead to loss of activity in non-conventional medium, and are able to efficiently regenerate co-factors [27]. Also, with the minimum process in obtaining the enzyme for utilizing the enzyme for the analysis would offer various benefits in green analytical chemistry approaches. In this work, non-purified peroxidase extracts from a rubber tree bark strip were utilized, as rubber tree bark is waste after rubber tapping process, it is a rich source of enzyme peroxidase. Rubber tree bark peroxidase is aimed as alternative for the commercially available ones which are expensive isolation and purification horseradish enzyme (HRP) to develop enzymatic reaction for carbaryl assay. Moreover, dispersive liquid-liquid microextraction (DLLME) was employed to pre-concentrate the analyte before spectrophotometric analysis step.

2. Experimental

2.1. Chemical and reagents

All chemicals and reagents used were analytical and/or HPLC grade. Deionized water purified by Milli-Q, Millipore apparatus was used to prepare solutions. Standard carbaryl, 4-aminoantipyrine, 1-dodecanol and lyophilized powder horseradish peroxidase were purchased from Sigma-Aldrich (Sigma-Aldrich, USA). Sodium hydroxide, monosodium dihydrogen phosphate, disodium hydrogen phosphate and sodium chloride were obtained from Fisher Chemical (Fisher Chemical, Australia). Hydrogen peroxide (H_2O_2) 30%, acetonitrile, ethanol, methanol and 1-octanol were acquired from Merck (Merck, Germany). Acetone and dichloromethane were bought from QRëC (QRëC, New Zealand). Magnesium sulfate anhydrous 96% and charcoal activated powder were obtained from Panreac (Panreac, Germany). Chloroform was purchased from VWR Chemical (VWR Chemical, Ecuador).

Stock standard solution of 1000 mg L^{-1} carbaryl was prepared by dissolving 0.01 g of carbaryl in 10 mL of 96% ethanol. Working solution of carbaryl was obtained by diluting the appropriate volume of stock standard 1000 mg L^{-1} carbaryl in 50% ethanol.

4-Aminoantipyrine solution (1000 mg L^{-1}) was made by weighing 0.01 g of 4-aminoantipyrine and dissolving in 100 mL of water.

Daily stock solution of H_2O_2 (100 mmol L^{-1}) was generated by dilution of 30% H_2O_2 in 10 mL of deionized water. Working solutions of H_2O_2 were prepared by diluting the appropriate volumes of the stock H_2O_2 in water.

Sodium hydroxide solution (1.0 mol L^{-1}) was prepared by dissolving 2 g of sodium hydroxide in 50 mL of deionized water and then diluting to working solutions.

Enzyme extract solution as 50 mmol L^{-1} of phosphate buffer pH 6 was prepared by dissolving of 3.67 g monosodium dihydrogen phosphate and 0.2 g disodium hydrogen phosphate in water. The solution was then adjusted to pH 6 with HCl or sodium hydroxide and the final volume was made up to 500 mL with water.

2.2. Instruments and apparatus

A Cary 60 Agilent UV–Vis spectrophotometer (Agilent Technologies, Australia) was utilized to measure absorbance of the reaction at wavelength of 504 nm. A benchtop centrifuge model 1040 series from Labquit (Labquit, England) was used to eliminate particle remains in the extract solutions, yielding a clear supernatant. A pH meter model 713 from Metrohm (Metrohm, Switzerland) was employed to check pH of the buffer. Reaction temperature was controlled by a water bath (Julabo, Eco Temp TW12, Germany). A vortex mixer was used to increase mass transfer of QuEChERS (Quick Easy Cheap Effective Rugged Safe) and DLLME steps. A cooking blender model EBR 2601 from Electrolux (Electrolux, Thailand) was utilized to homogenize the vegetables.

2.3. Extraction of peroxidase enzyme from excised rubber tree bark strips

Excised rubber tree bark strips were obtained from Roi Et Province, Thailand. The strips were collected immediately after rubber tapping and placed in a cool box. In the laboratory, the dry rubber latex remaining on the bark was removed and weighed into 130 g batches for extraction of the enzyme. Before extraction, the rubber tree bark was washed with deionized water 3 times. The cool enzyme extract solution (100 mL) was added and the rubber tree bark was homogenized together with phosphate buffer in a blender for 5 min. The suspension solution was filtered through four layers of cotton gauze and centrifuged at 14000 rpm and 4 °C for 30 min to remove turbidity. The supernatant was then filtered through filter paper (Whatman No.1) into amber-colored bottles and stored at −18 °C until required for enzyme activity analysis.

2.4. Peroxidase enzyme activity test

Peroxidase enzyme has been previously determined in rubber tree bark [28]; however, peroxidase activity was assessed to ensure the presence of the enzyme in the extract solution. Enzyme activity was evaluated by mixing a crude extract solution (10 μL) with specific peroxidase enzyme substrate as ABTS ready to use (ABTS solution, Roche, Germany). Absorbance at 420 nm was immediately monitored for 1 min and the initial slope of the enzymatic reaction was calculated. Enzyme activity of 1 U was defined as the amount of enzyme required to generate 0.001 absorbance of product per 1 min under the described conditions.

2.5. Optimization of the conditions for determination of carbaryl using peroxidase catalytic reaction

Quantification of carbaryl is based on the reaction between 1-naphthol as a hydrolysis product of carbaryl under alkaline condition with H_2O_2 and 4-aminoantipyrine in the presence of peroxidase enzyme extracts from rubber tree bark. Effect of various influencing parameters including pH, concentration of sodium hydroxide, concentration of H_2O_2, concentration of 4-aminoantipyrine, reaction time and volume of crude enzyme extracts were carefully investigated and optimized using a univariate experimental method throughout. The effect of pH was optimized between 3 and 7. Concentrations of sodium hydroxide for carbaryl hydrolysis were investigated in the range of 1–20 mmol L^{-1}. Hydrolysis time was performed for 1 to 15 min and H_2O_2 concentrations in the range of 0.05–0.50 mmol L^{-1} were studied. Concentrations of 4-aminoantipyrine (50–500 mg L^{-1}) were examined, while volumes of crude enzyme extract were varied from 10 to 200 μL. Reaction time of peroxidase was varied between 1 and 30 min.

2.6. DLLME optimization for carbaryl assay

An environmentally friendly extraction method also known as DLLME was employed to increase the concentration of the enzyme catalytic product before the spectrophotometrically determination step. Various parameters affecting the DLLME extraction method were studied including type and volume of extraction solvent. Variety and volume of disperser solvent were examined. Salt addition was investigated by varying sodium chloride concentration between 0 and 2% w/v. Increasing mass transfer was performed by vortex solution for 10 to 180 s with centrifugation time in the range of 1 to 10 min to complete phase separation between the organic and aqueous phases.

2.7. Vegetable sample preparation

QuEChERS [29,30] was utilized to prepare vegetable samples to determine carbaryl content. Briefly, a vegetable sample was cut into small pieces and then mixed homogeneously using a cooking blender. A