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# Malachite Green and Leuco-Malachite Green Detection in Fish using Modified Enzyme Biosensor

Nurul Hidayah, A. P[a]*., Faridah, S.[a], Nur Azura, M. S.[a,] Gayah, A. R.[a], Othman, M.[b], and Fatimah, A. B[c].

[a]*Agri-Nanotechnology Program, Biotechnology and Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor D.E., Malaysia*
[b]*Fishery Research Institute Malaysia, FRI Batu Maung, Bayan Lepas, 11960 Batu Maung, Pulau Pinang, Malaysia*
[c]*Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia*

**Abstract**

A modified enzyme-based biosensor was developed for amperometric measurement of total Malachite Green (sum of MG and LMG) in tilapia fish. The 4 $UmL^{-1}$ butyrylcholinesterase enzyme (BuChE) was used and immobilized onto the surface of carbon paste electrode during the electro-polymerization of polypyrrole (PPy). It is a simple, rapid and sensitive technique used for determination of total MG using 0.1 M phosphate buffer pH 8.0. In this study, butyrylthiocholine iodide (0.3 Mm BTCi) was used as a substrate which produced the thiocholine due to the enzymatic hydrolysis. Then, it was oxidized at 0.4 V against silver-silver chloride electrode for 100 s. The modified enzyme biosensor provided a high sensitivity and linear concentration range from 0.25 to 10 ppb with a limit of detection (LOD) at 0.25 ppb. The results showed the linear standard curve of total MG standard solution (based on the current output in microampere) with the equation of $y = - 0.9113x + 10.84$, $R^2 = 0.9445$.


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Keywords: Malachite Green; Leuco-Malachite Green; Biosensor; Butyrylcholinesterase; Polypyrrole

## 1. Introduction

Malachite Green (MG) is a cheaper anti-fungal and anti-bacterial, which is extensively used in aquaculture. However, the presence in water possesses a potential hazard to aquatic environment and human health due to its toxicity (Mitrowska and Posyniak, 2004). Recently, the total MG was determined using LC-MS/MS method which is expensive and time consuming (FAO/WHO Expert Committee, 2008; Mitrowska et al., 2007).

* Corresponding author. Tel.: +6-03-8953-6550; fax: +6-03-8953-6606.
*E-mail address:* nurulhidayah@mardi.gov.my


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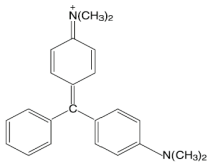
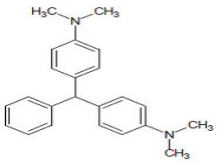
Therefore, a rapid, simple and sensitive method is needed for the detection of total MG (MG and LMG). Currently, a modified enzyme biosensor (based on the inhibition of the butyrylcholinesterase enzyme (BuChE)) has gain great attention (Andreescu and Marty, 2006; Çokuğraş, 2003) where the enzyme was immobilized together with the conducting polymer solution by the electro-deposition method (produces a uniform layer) for more stable electrode with the higher enzyme recoveries. It can be ensured the continuous operation of enzymatic processes with the multiple reuses of enzymes and rapid termination of reactions. It also can extend the linear range for total MG detection (Krajewska, 2004). All the optimization and analysis of total MG was carried out using the amperometric technique. The chemical structures of MG (a) and LMG (b) have showed in the Fig. 1 (Liu *et al.,* 2009; Sudová *et al.,* 2007; Srivastava *et al,* 2004) meanwhile the Fig. 1 (c) has showed the biosensor approaches that used in this study.



a) Malachite Green (MG)



b) Leuco-Malachite Green (LMG)


c) Screen Printed Electrode (SPE)

**Figure 1.** The chemical structures of MG (a) and LMG (b) (Liu *et al.,* 2009; Sudová *et al.,* 2007; Srivastava *et al,* 2004) (c) Screen Printed Electrode (Carbon)

## 2. Material and Methods

All chemicals were obtained from the commercial sources and used without further purification. Butyrylcholinesterase (E.C.3.1.1.8, 221 U/mg, equine serum sources), butyrylthiocholine iodide, Malachite Green oxalate (free zinc), Leuco-Malachite Green and pyrrole were purchased from Sigma-Aldrich chemicals (Malaysia), disodium hydrogen phosphate dihydrate, natrium dihydrogen phosphate monohydrate, potassium chloride and acetonitrile were purchased from Merck. This experiment was carried out using a potentiostat machine model AUT128N, PGSTST128N (Eco-Chemie, The Netherlands), connected to a personal computer and analysis was done using NOVA 1.7 software. A modern three in one screen printed carbon electrode that was purchased from Science Technology (Malaysia) Sdn. Bhd. was used as a transducer.

### 2.1 Methods

#### 2.1.1 Amperometric Analysis

Amperometric analysis was carried out by scanning the set potential (a range of 0 to 1.2 V for 120 s) of total MG standard solution at different concentration, where the fix concentration of BTCi substrate (0.3 mM). The measurement readings are available in microampere (current output) and the percentage of enzyme inhibition by total MG (MG and LMG) was obtained by doing some calculation. Then, the calibration curve will be plotted.

#### 2.1.2 Specificity Study

The cross-reactivity study was carried out between the MG, LMG, Pararosanaline, Methylene Blue and Nile Blue A (triphenylmethane dye) for total MG specificity. This amperometric measurement was done at 0.4 V for 120 s.