# Determination of Malachite Green in Aquaculture Water by Adsorptive Stripping Voltammetry

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**Abstract**

An adsorptive stripping voltammetric method for the determination of malachite green in fish farm water has been developed. Initial studies were made on the cyclic voltammetric behaviour of malachite green at a glassy carbon electrode in 0.1 M phosphate buffer over the pH range 2 to 10. The redox behaviour observed for malachite green was verified by both data analysis of malachite green its reduction product, leucomalachite green. Furthermore, leucomalachite green was found not to interfere with the determination of malachite green at pH 7.4, the optimum pH for malachite green determination. As a result, further studies were undertaken to exploit this as the basis of an adsorptive stripping voltammetric method for the trace determination of malachite green in fish farm water samples. The conditions necessary including voltammetric waveform, accumulation potential and time were investigated and optimised. Using differential pulse voltammetry the calibration plot was found to be linear from 0.2 µM to 1.2 µM of malachite green with a sensitivity of 0.8311 µA/µM. Using the method of multiple standard addition, fish farm water samples fortified with 0.5 µM and 0.75 µM malachite green were found to give mean percentage recoveries of 78.79 % and 87.20 % respectively (the coefficient of variation is 2.07 % and 1.45 %). Therefore, the performance data suggests that the method is reliable at the concentrations examined in this study and that a rapid, simple, economical and precise method of monitoring of malachite green in fish farm water and aquaculture applications is possible.

**Keywords**: Malachite green; Electrochemical behaviour; Reaction mechanism; Water sample

**Running head**: ELECTROCHEMICAL ANALYSIS OF MALACHITE GREEN

## INTRODUCTION

Malachite green ($C_{23}H_{25}N_2Cl$) is a member of the triphenylmethane class of dyes and is known to exhibit both antimicrobial and antiparasitic properties. It has been extensively used as a biocide in the aquaculture industry world-wide (Aiderman 1985). However, malachite green and its reduced form, leucomalachite green, have been shown to have toxic effects on the human immune system, reproductive system and to be a potential carcinogen (Srivastava et al. 2004; Meyer and Jorgenson 1983; Sudova et al. 2007; Gouranchat 2000). Though malachite green is not approved for use in aquaculture in territories such as Canada, the United States (Hernando et al. 2006), Europe (European Commission 2002) and Japan, its illegal use will probably continue due to its low cost, ready availability and effectiveness as a pesticide. In addition to its use in aquaculture, malachite green has been widely used as a dye in the textile industry, as well as a food additive and colouring agent (Culp and Beland 1996). A report prepared by the Water Research Centre for the Department of the Environment, Transport, and the Regions of the United Kingdom recommended an annual average environmental quality standard of 500 ng/L malachite green for the protection of freshwater aquatic life (Burchmore and Wilkinson 1993). Consequently, in order to preserve the environment and protect human health, it is very important to develop analytical methods for malachite green analysis.

A number of laboratory based analytical techniques have been developed for determination of malachite green, such as high performance liquid chromatography (HPLC) (Mitrowska et al. 2005; Bajc et al. 2007; Furusawa 2014; Swarbrick et al. 1997; Long et al. 2008; Bergwerff et al. 2003; Xie et al. 2013) and liquid chromatography-tandem mass spectrometry (Nebot et al. 2013; Lopez-Gutierrez et al. 2013; Hidayah et al. 2013). These methods can achieve high sensitivity and excellent accuracy. However, these rely on relatively expensive instruments which need to be operated by well-trained personnel. In

addition, the sample preparation using these methods can be complex and time-consuming.

Electrochemical sensors have been widely used for the determination of chemical contaminants in the environment and food industries (Privett et al. 2010) due to distinct advantages such as high sensitivity, good selectivity, rapid response, low cost and the possibility for use in the field. The electrochemical determination of malachite green at glassy carbon electrodes modified with carbon nanotube (MWNT) or colloidal gold-chitosan/MWNT has been reported (Yi et al. 2008; Liu et al. 2014). The addition of an anionic surfactant to the sample to enhance the signal has also been investigated (Huang et al. 2008). A modified carbon paste electrode based on a self-assembly layer of ethylenediamine and graphene oxide has been recently reported (Zhang et al. 2012).

To the best of our knowledge, the electrochemical properties of malachite green and the mechanisms underlying its voltammetric behaviour have not been studied in-depth. Therefore, to address this, the main objectives of this current work are to study the electrochemical properties of malachite green at an unmodified glassy carbon electrode and develop a convenient electrochemical method for its determination in water.

In the present investigation, the electrochemical behaviour of malachite green was investigated over a wide pH range by cyclic voltammetry. The effect of scan rate was examined at pH values between 2.0 and 10.0 and possible mechanisms for its voltammetric behaviour given. In addition, further cyclic voltammetric investigations were made on leucomalachite green, the reduction product of malachite green, to further elucidate the possible redox mechanism of malachite green. Studies were then made to explore the possibility of exploiting these findings to develop a method for its adsorptive stripping voltammetric determination in fish farm water samples at an unmodified glassy carbon electrode by optimizing the accumulation potential, accumulation time and electrochemical measurement waveform. This paper presents a detailed description of our studies.

## EXPERIMENTAL

### Apparatus and Chemicals

Cyclic voltammetry was performed using an Autolab potentiostat interfaced to a PC for data acquisition and instrument control via the Nova operating system (Autolab, The Netherlands). The voltammetric cell contained a graphite rod counter electrode, a silver/silver chloride reference electrode (Ag/AgCl, with 3 M KCl solution) (Chen Hua Instruments, Shanghai, China) and a 3 mm diameter glassy carbon electrode as the working electrode (Chen Hua Instruments, Shanghai, China). Cyclic voltammograms were initially recorded in plain solutions of 0.1 M phosphate buffer, then in the same solution containing malachite green. For initial studies a starting potential of 0.0 V with a switching potential of +1.0 V was employed. Differential pulse voltammetry was undertaken using a starting potential of 0.0 V and a final potential of +1.0 V; using a step height of 10 mV, pulse repetition time 0.2 s, pulse amplitude of 100 mV, and pulse duration of 50 ms.

All chemicals were supplied from Fisher Scientific (China), unless stated otherwise. Malachite green was obtained from Tanmo Quality Inspection Co., Ltd, Beijing, China. Leucomalachite green was obtained from Dr. Ehrensorfer, Germany and were both analytical grade. A 10 mM malachite green stock solution was prepared by dissolving the appropriate mass in ultrapure water. Working standards were then prepared by dilution of the primary stock solution with ultrapure water. Ultrapure water was obtained from a Milli-Q Academic System, (Millipore, USA). Solutions of disodium, trisodium, sodium o-phosphate and o-phosphoric acid (Sinopharm Chemical Reagent Co., Ltd, China) were made at a concentration of 0.2 M by dissolving the appropriate mass in ultrapure water. These were then titrated together to give the desired pH plus 0.9 % NaCl. An appropriate volume was then added directly to the voltammetric cell and diluted with sufficient ultrapure water to give an overall phosphate concentration of 0.1 M.

**Water Sample Collection and Pre-treatment**

The water sample was obtained from Badu River channel, Jumahe River Basin, Fangshan District, Beijing, 14th August, 2014 at a depth of 0.5 m. A 2.5 L glass hydrophore was used to collect the water which was transferred directly into sample bottles. Samples were refrigerated and sent to the laboratory as soon as possible for analysis. These were then filtered through a microporous membrane filter (water-system, 0.22 μm) prior to voltammetric analysis.

## RESULTS AND DISCUSSION

Figures 1 to 4 show the cyclic voltammetry obtained for a 1.0 mM solution of malachite green in 0.1 M pH phosphate buffer at pH values 2.0, 4.0, 7.4 and 10 at scan rates between 20 mV/s and 200 mV/s. Previous reports have shown that at pH 2.0 the electrochemical oxidation of malachite green leads to the formation of the oxidized form of $N,N,N,N$-tetramethylbenzidine ([1,1-biphenyl]-4, 4-diamine) characterised by quasi-reversible diffusion controlled behaviour (Ngamukot et al., 2006; Ma et al., 2008) $2e^-$, $2H^+$ redox couple (Galus and Adams., 1962). In the present study, the cyclic voltammograms obtained at pH 2.0 (Figure 1) show a similar behaviour, with a single oxidation peak on the initial positive going scan and single reduction peak on the return negative going scan. Plots of peak current ($i_p$) *vs.* the square root of scan rate ($v^{½}$) showed a linear dependence, showing the electrochemical process to be diffusion controlled. Consequently we conclude that, at this pH, malachite green undergoes similar redox behaviour as to that previously reported.

Figure 2 shows the cyclic voltammetric behaviour obtained at pH 4.0. At this pH, the oxidation seen at pH 2.0 is still observable, but the reduction peak seen on the return negative going scan is much broader and less well defined. Several other redox processes are also recorded concluded to result from the pH conditions being close to the pKa value of

malachite green. Such conditions in the results in two or more forms of malachite green being present producing the additional redox peaks recorded. However, at pH 7.4 (figure 3) and at values between 6.0 to 8.0 along with this same oxidation peak at +0.90 V an additional oxidation peak is seen (Ep = +0.5 V). Peak current values for this oxidation peak were found to be linearly related to scan rate ($v$) and further investigations of current function ($i_p/v^{½}$) versus the square root of scan rate ($v^{½}$) (Nicholson and Shain 1964; Wopschall and Sharin 1967) demonstrated this to be reactant adsorption in nature. On the return negative going scan a single reduction peak was seen at +0.4 V. In addition, there was very little response for leucomalachite green seen by cyclic voltammetry at pH 7.4, the pH at which we undertook the investigation of malachite green. Thus leucomalachite green would not interfere with the determination of malachite green.

It is believed that the oxidation peak seen at +0.5 V results from the oxidation of the carbinol form of malachite green. At pH values greater than 6.9 (Cuong et al. 2012) malachite green is chemically reduced to its carbinol form which can undergo a $2e^-$ oxidation to give malachite green and a hydroxyl ion. The carbinol form is more non-polar in nature and hence would explain the reactant adsorptive behaviour seen in our investigation. The more positive oxidation peak seen at +0.90 V is believed to result from oxidation of the amine nitrogen lone electron pair to form a radical species (eq.1). This phenomenon has also been reported and explained previously (Masui, Sayo and Tsuda 1968).

$$R_3 - N^{\bullet}_{\bullet} \quad \rightarrow \quad R_3 - N^{+}_{\bullet} \quad + \quad e^- \qquad \text{eq.1}$$

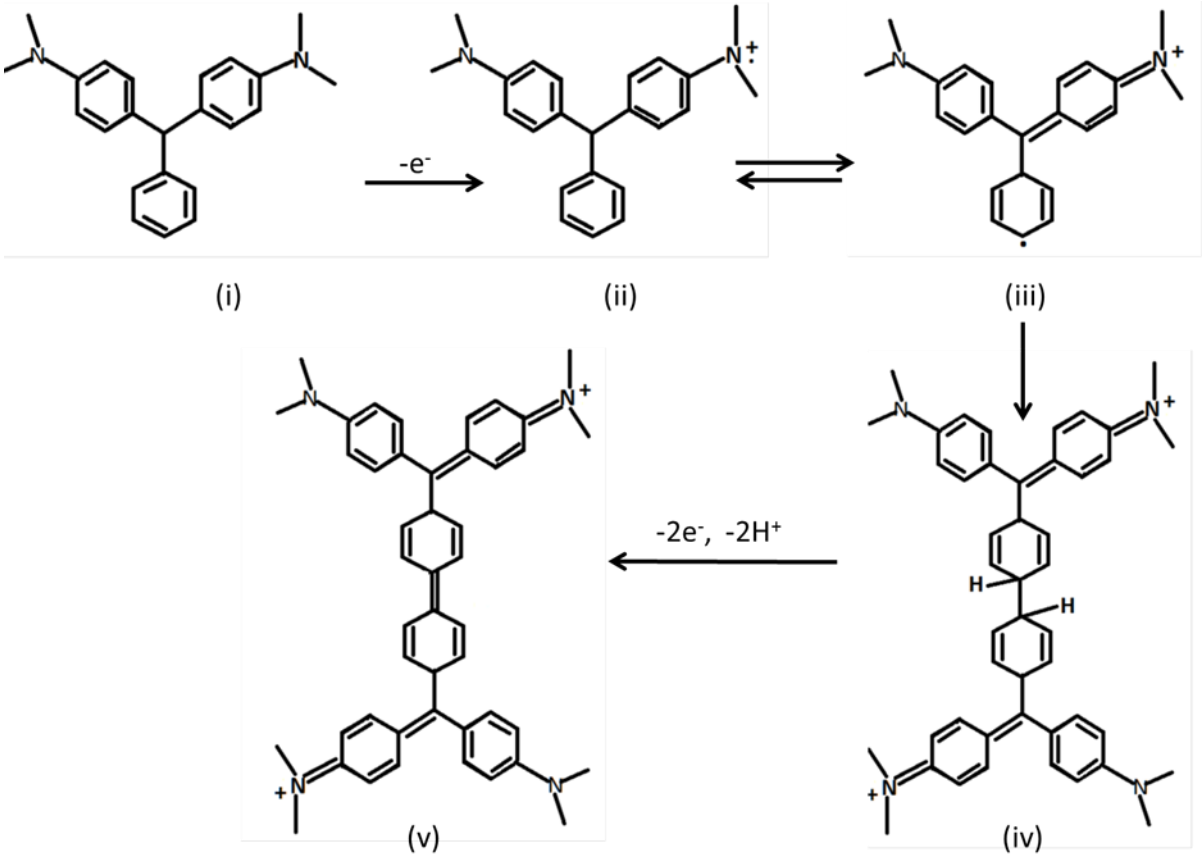
At pH 10 (figure 4) one oxidation peak was recorded over the potential range investigated concluded to result from the oxidation of the amine. The high pH and consequent high concentration of $OH^-$ makes the electrochemical oxidation reaction to be

unfavourable. In some scans at this pH, a further oxidation peak is observed. This was concluded to result from the instability of malachite green at this high pH.

To further investigate the possible underlying mechanism for the cyclic voltammogram of malachite green, the cyclic voltammetric behaviour of leucomalachite green was also investigated under the same conditions. The same oxidation peak for malachite green was also recorded for leucomalachite green (Figure 5). Moreover, the solution around the working electrode was seen to change from clear to green in colour when the potential was scanned from 0.0 V to +0.8 V at pH 2.0. It was concluded that this green colour resulted from electrochemical generated malachite green formed by the oxidation of leucomalachite green to malachite green. This phenomenon was not observed when the supporting electrolyte pH was increased. Further cyclic voltammetric investigations of leucomalachite green showed a second peak which became more obvious as the pH was increased to 4.0, however, the Ep peak potential remained constant (Figure 6). This may be due to the oxidation of the carbinol form of leucomalachite green; the results strongly supported the above assumptions. The voltammetric peaks of leucomalachite green decreased in magnitude with increasing pH, as it became increasing more difficult to electrochemically oxidize to malachite green under alkaline conditions.

Additional studies were made into the origin of the reduction peak recorded at +0.4 V for malachite green. A series of cyclic voltammetric investigations were undertaken using a switching potential of +0.6 V, before the beginning of the second oxidation process. Consequently, we concluded that this reduction process was a result of the species formed from the oxidation process at +0.5 V. A further two oxidation peaks were found when extending the switching potential to the more positive potential of +2.0 V, which was believed to result from the polymerisation of the malachite green (Lin et al. 1989; Raoof et al. 2013) at its amine group (Chen et al. 2007). The mechanism of the voltammetric dimerization

of aromatic amines has been investigated before (Hart, Smyth and Smyth 1981). Several different possible mechanisms for the polymerisation of malachite green have been previously given (Lin et al. 1989; Raoof et al. 2013). However, we believe that the tail-to-tail dimerization (Hart, Smyth and Smyth 1981, Honeychurch et al. 2004) shown in scheme 1 explains the voltammetric behaviour recorded in this study. Malachite green (i) is first electrochemically oxidised to a cation radical (eq.1) (ii). This electron radical (ii) can also form the resonance structure with (iii). The presence of (iii) which can lead to the electrophilic tail-to-tail dimerization of two of these radical species to give the dimer (iv). This can be converted to species (v) via a $2e^-$, $2H^+$ electrochemical oxidation.




**Scheme 1.**

The reactant adsorption of the malachite green at and around neutral pH values is analytically a very useful finding as it allows for the development of an adsorptive stripping

voltammetric assay for the determination of malachite green. Consequently, we chose to explore this further in the following sections.

## Adsorptive Stripping Voltammetry

### Effect of Accumulation Potential

Figure 7 shows the effect of accumulation potential on the resulting $i_p$ magnitude of both oxidation peaks using an accumulation time of 15 s. Peak current was found to increase from +0.6 V to a maximum value between +0.4 V and -0.2 V (*vs.* Ag/AgCl) which was then found to decline at potentials more negative than this. Consequently, further studies were carried out using an accumulation potential of 0.0 V (*vs.* Ag/AgCl). When using longer accumulation times or more negative accumulation potentials two oxidation peaks were recorded (Ep = +0.54 V, Ep = +0.63 V). The more positive peak is believed to result from the oxidation of a monolayer of malachite green on the glassy carbon electrode surface, whereas the more negative peak results from the oxidation of a multilayer of malachite green deposited on the glassy carbon electrode. This more negative peak was only seen at extended accumulation times, as it was only formed once the monolayer had been established (Honeychurch et al. 2000).

### Effect of Accumulation Time

Figure 8 shows the effect of increasing accumulation time at an applied potential of 0.0 V using a 2.0 µM malachite green solution. The first oxidation stripping peak (a) was found to increase with increased accumulation time and reached a maximum value at *ca.* 100 s. The second oxidation peak (b) was found to also increase with increasing accumulation time over the entry time frame studied.

### Effect of Electrochemical Measurement Waveform

In order to ascertain whether we could improve our detection limit we decided to examine the application of differential pulse voltammetry. In previous reports we have shown differential pulse voltammetry to greatly improve signal to noise ratio and consequently limit of detection (Honeychurch et al. 2000). Initially studies were performed under the optimised conditions, using a differential pulse voltammetry waveform described in the experimental section. The resulting oxidation peak of malachite green was greatly improved and additional smaller peaks were also observed (Figure 9). Consequently, differential pulse voltammetry was used for further investigations.

**Calibration Study**

A calibration study was carried out using differential pulse voltammetry in a 0.1 M pH 7.4 phosphate buffer over the concentration range 0.2 μM – 2 μM. The plot was linear up to 1.2 μM with a slope of 0.8311 μA/μM with an $R^2$ value of 0.990. The limit of detection based on a signal-to-noise ratio of 3 was 0.12 μM. Beyond 1.2 μM, the response was found to be quasi-linear up to at least 2 μM. These performance characteristics are able to meet the detection limits required for the determination of malachite green in environmental water samples (Burchmore and Wilkinson 1993). It should be mentioned that by simple extension of the relatively short accumulation times investigated in this study it would be possible to improve markedly on these performance characteristics. Table 1 gives a summary of previously reported methods for the determination of malachite green in various water samples. The spectrographic and chromatographic approaches require a separate concentration and clean-up step to obtain the detection limits quoted. In our method the concentration step and measurement step are undertaken in the same solution eliminating problems such as losses in sample preparation. Our method uses an unmodified carbon electrode which has the added advantage of being more stable compared to techniques which are based on modified electrodes.

**Interference Studies**

As mentioned above, cyclic voltammetric investigations showed that, under optimized conditions, equal molar concentrations of leucomalachite green did not interfere with the determination of malachite green. To further validate the performance of the method a number of possible interferences were studied. Thirteen metal and acidic ions previously reported to be common interferences were evaluated (Lin 2013; Sun et al. 2015) in the malachite green determination process (1 µM) and no interference was observed for ≥500-fold concentrations of: $K^+$, $Na^+$, $Ca^{2+}$, $Fe^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Cl^-$, $I^-$, $SO_4^{2-}$, $NO_3^-$, $CO_3^{2-}$, $PO_4^{3-}$and $CH_3COO^-$.

**Analytical Application**

The proposed analytical procedure was evaluated by carrying out malachite green determinations on a sample of fish farm water, before and after fortifying with known concentrations of malachite green. Samples were diluted one-to-one in 0.2 M pH 7.4 phosphate buffer, and the concentration of malachite green determined using the method of multiple standard additions. Table 2 shows the precision and recovery data that we obtained for replicate analysis of a single fish farm water sample fortified with 0.50 µM and 0.75 µM malachite green. This data demonstrates that the proposed method has promise for the determination of malachite green in such water samples. Further investigations of samples obtained from other environmental water sources showed similarly good recoveries and precision.

## CONCLUSIONS

We have investigated the redox behaviour of malachite green at a glassy carbon electrode and found that well-defined peaks could be obtained in 0.1 M pH 7.4 phosphate buffer using both cyclic voltammetry and differential pulse voltammetry. This is the first

report to investigate the voltammetric behaviour of malachite green over an extended pH range. It is also the first report to exploit the oxidation process seen at pH 7.4 at an unmodified glassy carbon electrode for the determination of malachite green in fish farm water samples. Unlike other previously reported methods, it was shown that no elaborate extraction or separation procedures were required as the method of multiple standard additions was shown to be both precise and accurate (table 2). It should be readily possible to improve the performance characteristics of the method by the application of longer accumulation times.

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TABLES

Table 1. Summary of various recently reported methods for the determination of malachite green

* LC-ESI-MS/MS is short for liquid chromatography-electrospray ion trap tandem mass spectrometry

| **Technique** | **Linear range** | **Detection limit** | **Sample** | **Comments** | **Reference** |
|---|---|---|---|---|---|
| Spectrophotometric | 50–250 µg/L | 2.2 µg/L | River water | Diffuse reflectance spectrophotometry 635 nm using sodium dodecylsulphate as a counter ion. | Robaina et al. (2011) |
| Spectrophotometric | 3.5 µg/L to 183 µg/L | 1.5 µg/L | Tap water | Cloud point extraction procedure using pH-sensitive hydrogel. Measurement at 617 nm. | Bahram et al. (2011) |
| Spectrophotometric | Malachite green, 9.9 µg/L to 800 µg/L; crystal violet 16 µg/L to 1000 µg/L | Malachite green 2.9 µg/L; crystal violet 4.8 µg/L | Pool and river water | Spectrophotometric determination of malachite green and crystal violet at 624 and 579 nm respectively following cloud point extraction. | An et al. (2010) |
| Spectrophotometric | 0.50 µg/L to 250 µg/L | 0.28 µg/L | Fish farm water | Malachite green and leucomalachite green isolated with maghemite nanoparticles modified with sodium dodecyl sulphate followed by magnetic | Afkhami et al. (2010) |

| | | | | | |
|---|---|---|---|---|---|
| | | | | separation. | |
| Spectrophotometric | 2.9 µg/L to 73 µg/L | 0.73 µg/L | Surface water | Method is based on the catalytic effect of silver nanoparticles on the oxidation of malachite green by hexacyanoferrate (III) in acetate-acetic acid medium measured at 610 nm. | Sahraei et al. (2013) |
| Spectrophotometric | | 3.2 µg/L | Water samples | Malachite green extracted with sodium dodecyl sulphate coated alumina solid phase. Ultraviolet (UV) detection at 617 nm. | Farhadi et al. (2010) |
| Spectrophotometric | 0.5 to 1.0 mg/L | -- | Drinking and river water samples | Malachite green pre-concentrated from 1 L of water with magnetic solid phase extraction using magnetic affinity adsorbent (magnetite with immobilized copper phthalocyanine dye). | Šafařka and Šafařiková (2002) |
| HPLC -UV | 0.20 to 100 µg/L | 0.01 µg/L | Yangtze River and pond waters | HPLC-Ultraviolet detection at 600 nm, following three-phase hollow fibre liquid phase microextraction | Zou et al. (2014) |

| | | | | | |
|---|---|---|---|---|---|
| HPLC diode array detection | 0.2 to 100 µg/L | 0.1 µg/L | Fish farm water | Dispersive liquid-liquid microextraction. | Maleki et al. (2012) |
| HPLC diode array detection | 0 to 200 µg/L | Seafood 0.05 µg/kg; seawater 0.1 µg/L | Seawater and seafood | Molecularly imprinted polymer solid phase extraction of malachite green followed by HPLC diode array detection. Seawater sample found to contain 1.30 µg/L. | Lian and Wang (2012) |
| HPLC diode array detection | 0.02–10, 0.5–100 and 0.2–100 µg/mL for MG, enrofloxacin and ciprofloxacin respectively. | 0.01, 0.07 and 0.10 µg/L for MG, enrofloxacin and ciprofloxacin respectively. | Fish farm water | Solid phase extraction of malachite green, enrofloxacin and ciprofloxacin | Sun et al. (2011) |
| HPLC | | | Fish and river water samples | Solid Phase extraction using a column with a monolithic molecularly imprinted epoxy resin-based polymeric stationary phase. | Wang et al. (2009) |
| LC-ESI-MS/MS | | 1.5 ng/L | Water samples from rural areas in North-western Spain | Solid phase extraction | Iglesias et al. (2014) |
| Micellar | 0.1 to 10 | 69.6 | Fish farm | Cloud point | Luo et al. |

| | | | | | |
|---|---|---|---|---|---|
| electrokinetic chromatography | µg/L | pg/mL | water | extraction followed by micellar electrokinetic chromatography | (2010) |
| Electrochemical detection | 18.4 µg/L to 82.2 mg/L | 2.2 µg/L | Water samples | Glassy carbon electrode modified with multi-wall carbon nanotubes. | Yi et al. (2008) |
| Electrochemical detection | 0.35 µg/L to 1.83 mg/L | 0.33 µg/L | Pond water | Glassy carbon electrode modified with multi-walled carbon nanotubes. Quantification cyclic voltammetry. | Liu et al. (2009) |
| Adsorptive stripping voltammetry | 43.79 µg/L to 437.9 µg/L | 43.79 µg/L | Fish farm water | Simple dilution of sample with phosphate buffer. Unmodified glassy carbon electrode | Present study |

Table 2. Recovery and precision data for malachite green obtained on fish water sample.

| *Sample | Original concentration(µM) | Added(µM) | Found(µM) | Recovery (%) |
|---|---|---|---|---|
| 1 | not detected | 0.50 | 0.406 | 81.24±0.36 |
| 2 | not detected | 0.50 | 0.387 | 77.42±0.20 |
| 3 | not detected | 0.50 | 0.401 | 80.22±0.18 |
| 4 | not detected | 0.50 | 0.389 | 77.86±0.32 |
| 5 | not detected | 0.50 | 0.386 | 77.20±0.27 |
| 1 | not detected | 0.75 | 0.66 | 89.20±0.28 |
| 2 | not detected | 0.75 | 0.663 | 88.80±0.32 |
| 3 | not detected | 0.75 | 0.658 | 87.87±0.40 |
| 4 | not detected | 0.75 | 0.654 | 85.99±0.26 |
| 5 | not detected | 0.75 | 0.659 | 87.33±0.20 |

FIGURE CAPTIONS

Figure 1. Cyclic voltammetric behaviour of 1.0 mM malachite green in 0.1 M phosphate buffer at pH 2.0 with different scan rate (a) 20, (b) 50, (c) 100, (d) 150, and (e) 200 millivolts per second.

Figure 2. Cyclic voltammetric behaviour of 1.0 mM malachite green in 0.1 M phosphate buffer at pH 4.0 with different scan rate (a) 20, (b) 50, (c) 100, (d) 150, and (e) 200 millivolts per second.

Figure 3. Cyclic voltammetric behaviour of 1.0 mM malachite green in 0.1 M phosphate buffer at pH 7.4 with different scan rate (a) 20, (b) 50, (c) 100, (d) 150, and (e) 200 millivolts per second.

Figure 4. Cyclic voltammetric behaviour of 1.0 mM malachite green in 0.1 M phosphate buffer at pH 10.0 with different scan rate (a) 20, (b) 50, (c) 100, (d) 150, and (e) 200 millivolts per second.

Figure 5. Cyclic voltammetric behaviour of malachite green and leucomalachite green (1.0 mM) in 0.1 M phosphate buffer at pH 2.0 (a)malachite green, (b) leucomalachite green.

Figure 6. Cyclic voltammetric behaviour of 1.0 mM leucomalachite green in 0.1 M phosphate buffer (a) pH 2.0, (b) pH 4.0.

Figure 7. Effect of accumulation potential for a 10 µM malachite green solution pH 7.4, 0.1 M phosphate buffer.

Figure 8. Effect of accumulation time for a 10 µM malachite green solution pH 7.4, 0.1 M phosphate buffer for peaks (a) Ep = +0.5 V, (b) Ep = +0.9 V.

Figure 9. Differential pulse voltammogram of 2 μM malachite green in 0.1 M phosphate buffer.

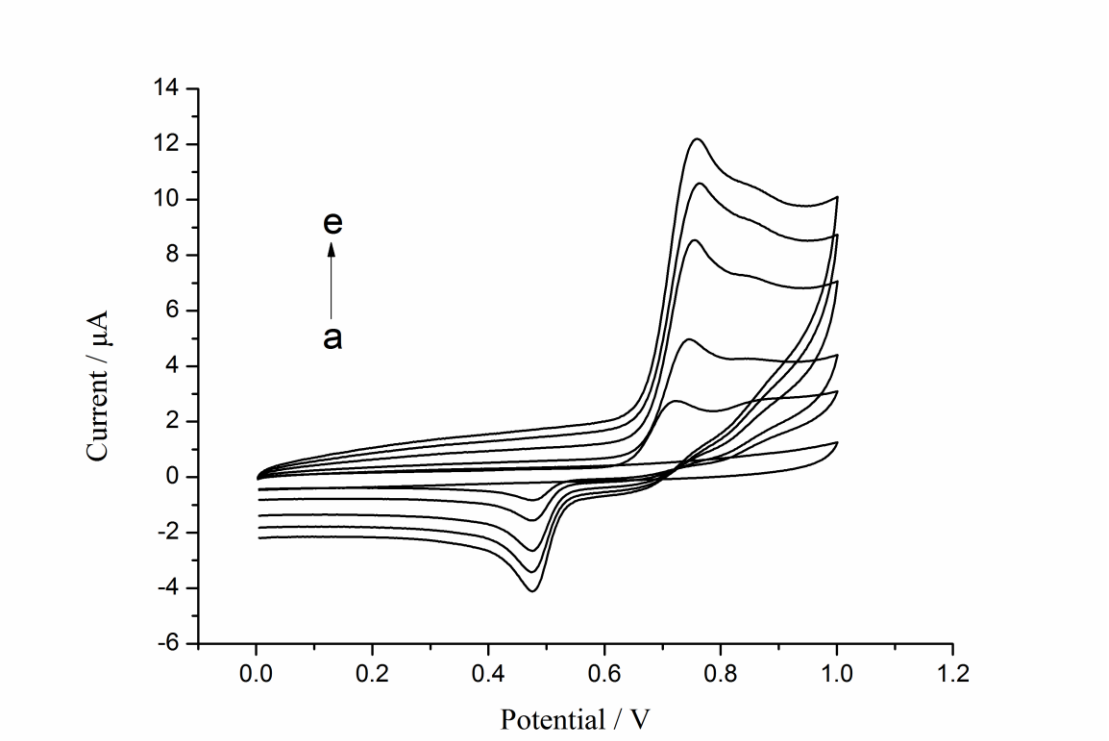


Figure 1. Cyclic voltammetric behaviour of 1.0 mM malachite green in 0.1 M phosphate buffer at pH 2.0 with different scan rate (a) 20, (b) 50, (c) 100, (d) 150, and (e) 200 millivolts per second. Voltammetric conditions: starting and end potential = 0.0 V; switching potential = +1.0 V.

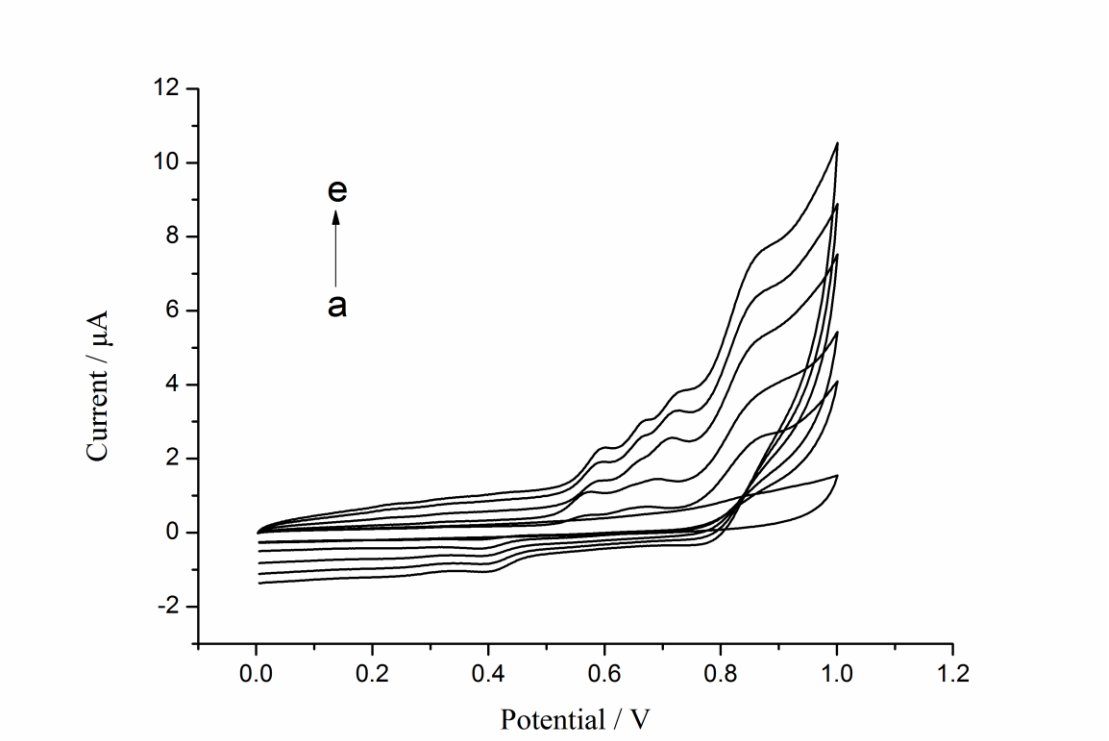


Figure 2. Cyclic voltammetric behaviour of 1.0 mM malachite green in 0.1 M phosphate buffer at pH 4.0 with different scan rate (a) 20, (b) 50, (c) 100, (d) 150, and (e) 200 millivolts per second. Voltammetric conditions: starting and end potential = 0.0 V; switching potential = +1.0 V.

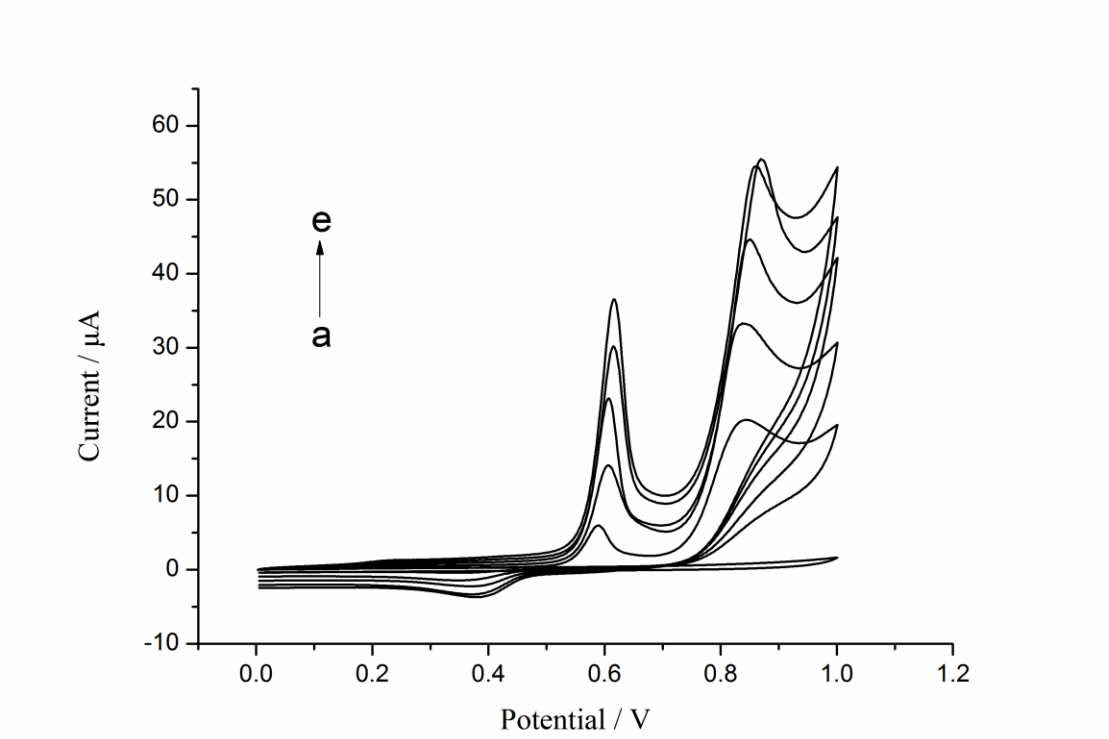


Figure 3. Cyclic voltammetric behaviour of 1.0 mM malachite green in 0.1 M phosphate buffer at pH 7.4 with different scan rate (a) 20, (b) 50, (c) 100, (d) 150, and (e) 200 millivolts per second. Voltammetric conditions: starting and end potential = 0.0 V; switching potential = +1.0 V.

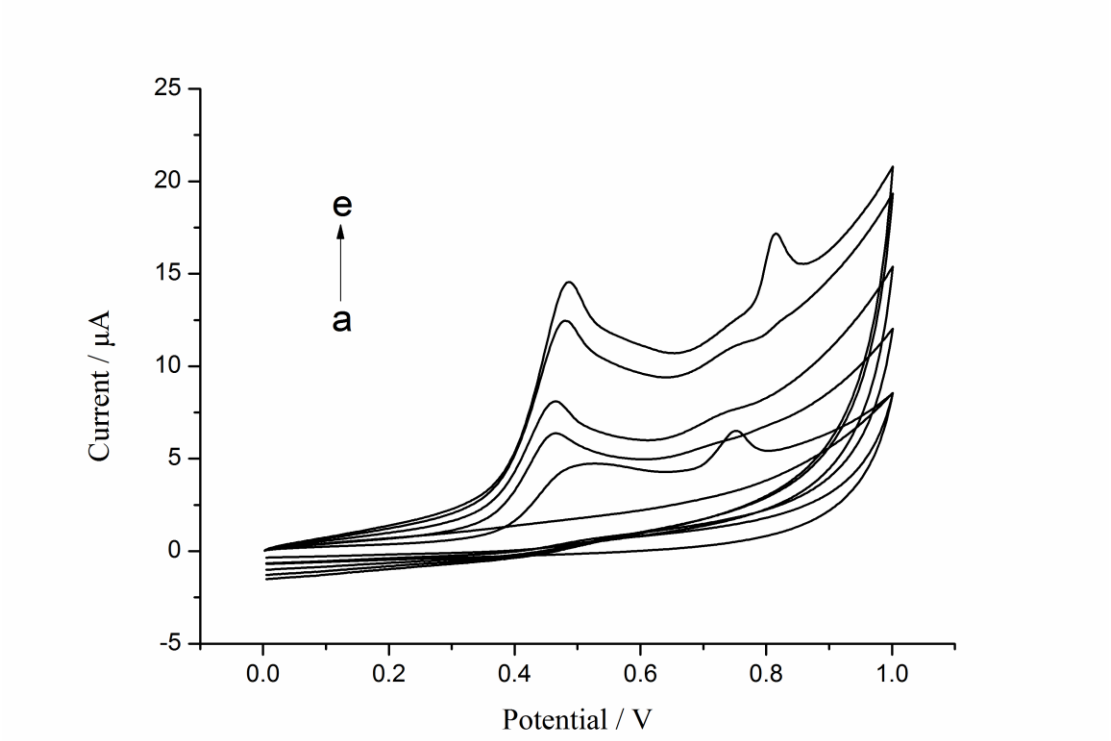


Figure 4. Cyclic voltammetric behaviour of 1.0 mM malachite green in 0.1 M phosphate buffer at pH 10.0 with different scan rate (a) 20, (b) 50, (c) 100, (d) 150, and (e) 200 millivolts per second. Voltammetric conditions: starting and end potential = 0.0 V; switching potential = +1.0 V.

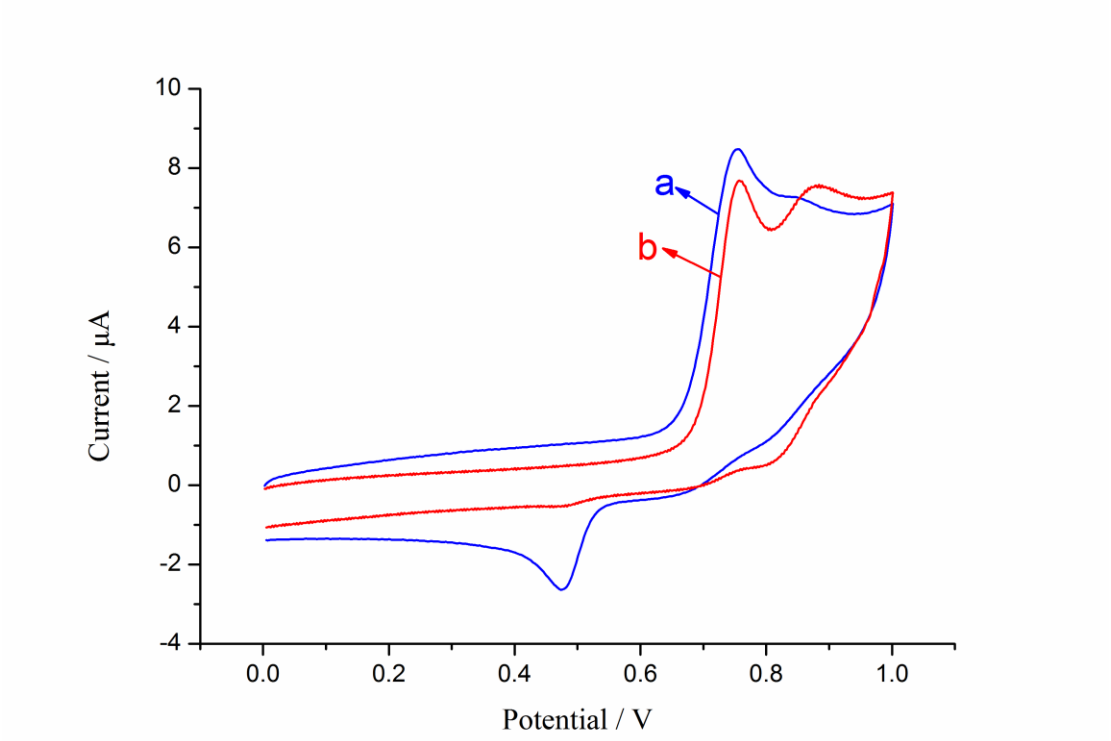


Figure 5. Cyclic voltammetric behaviour of malachite green and leucomalachite green (1.0 mM) in 0.1 M phosphate buffer at pH 7.4 (a)malachite green, (b) leucomalachite green.

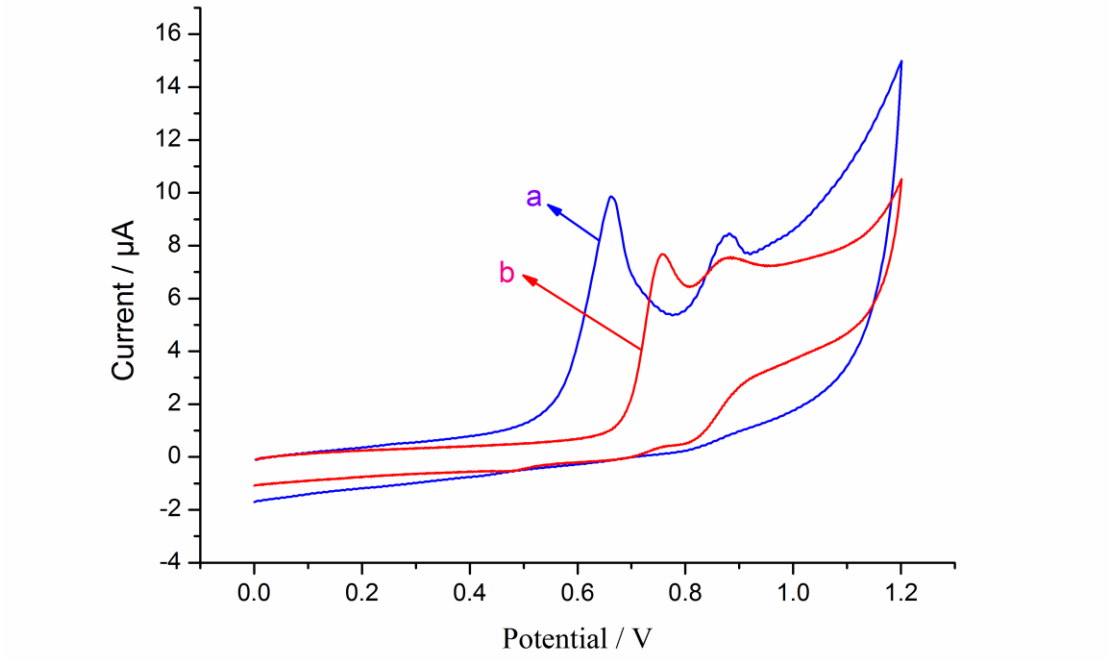


Figure 6. Cyclic voltammetric behaviour of 1.0 mM leucomalachite green in 0.1 M phosphate buffer (a) pH 2.0, (b) pH 4.0.

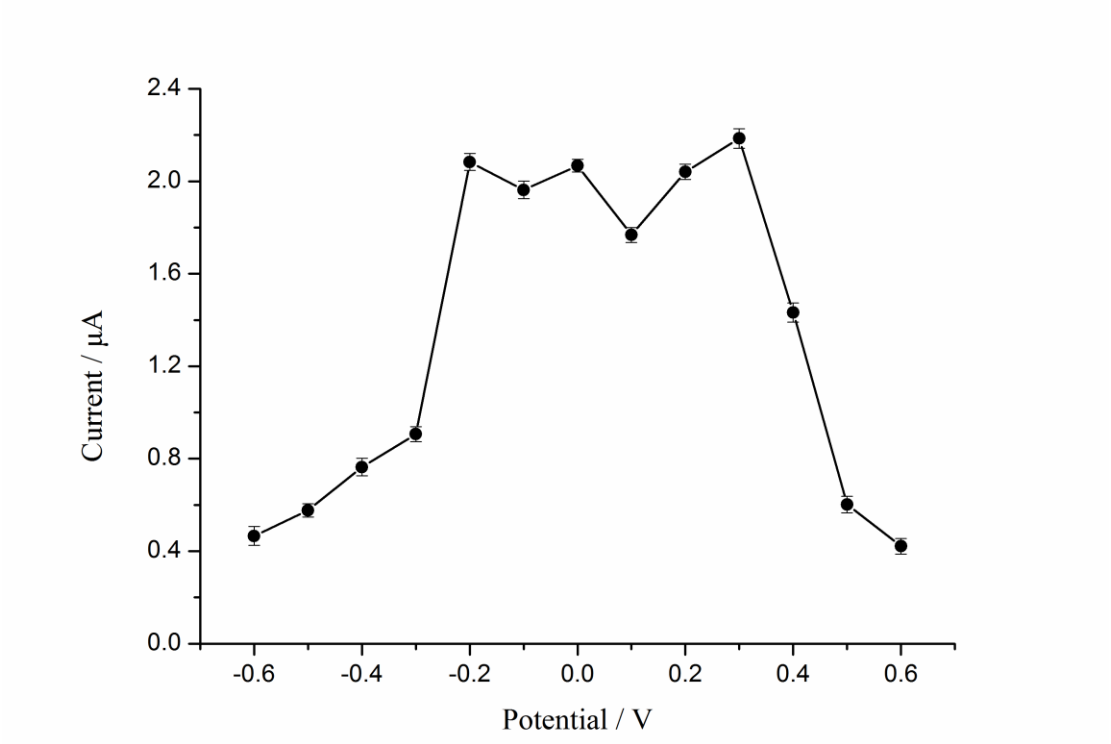


Figure 7. Effect of accumulation potential for a 10 µM malachite green solution pH 7.4, 0.1 M phosphate buffer.

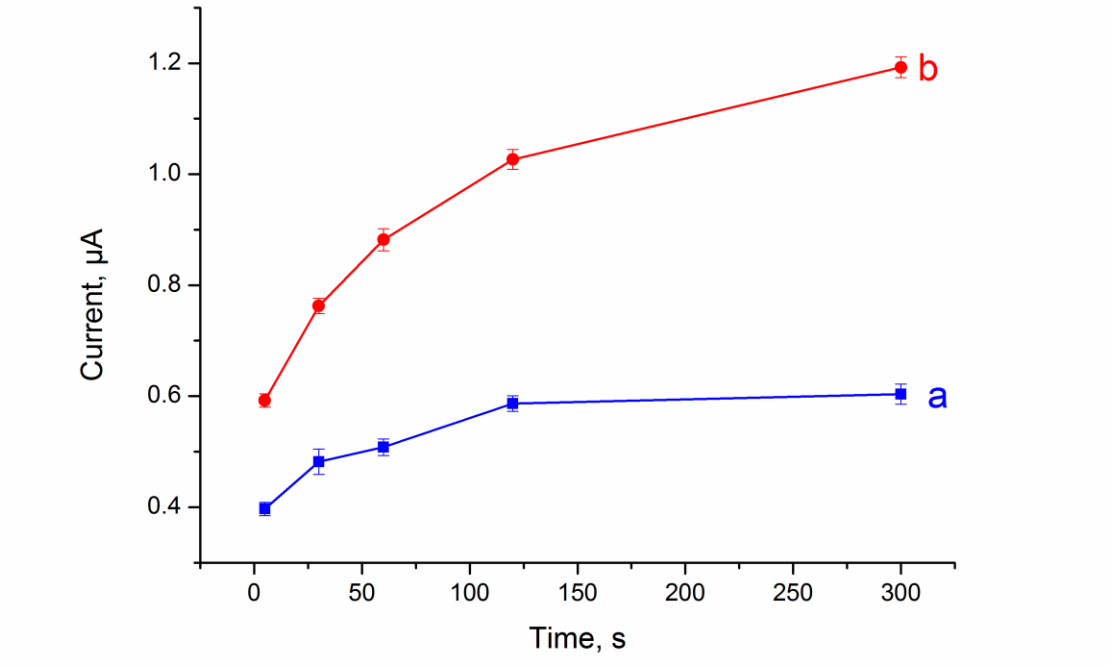


Figure 8. Effect of accumulation time for a 10 µM malachite green solution pH 7.4, 0.1 M phosphate buffer for peaks (a) Ep = +0.5 V, (b) Ep = +0.9 V.

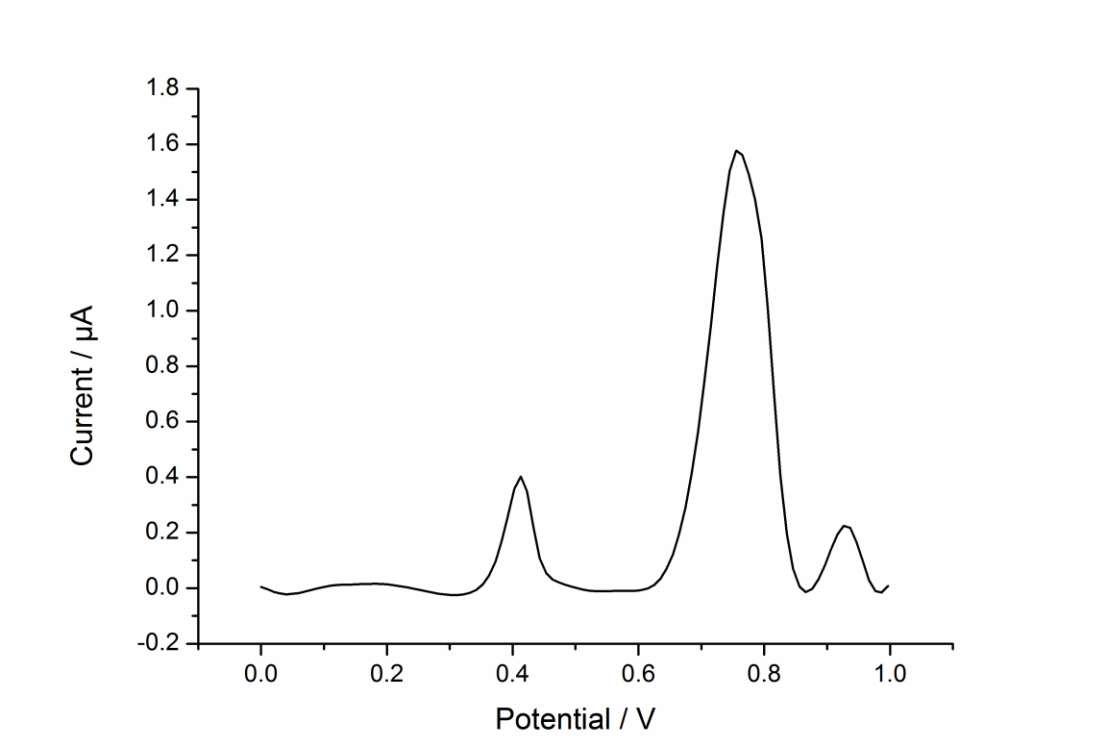


Figure 9. Differential pulse voltammogram of 2 µM malachite green in 0.1 M phosphate buffer.