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Enzymeless Flow Injection Analysis of 2,4,6-Trichlorophenol Based on Preoxidation by Ammonium Cerium (IV) Nitrate

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Enzymeless flow injection analysis of 2,4,6-trichlorophenol (2,4,6-TCP) based on preoxidation by ammonium cerium (IV) nitrate will be presented in this work. A preoxidation scheme is applied for 2,4,6-TCP determination without any enzyme treatment. This preoxidation scheme can provide a determination method with low cost and non-conductive polymerization reaction of 2,4,6-TCP. In proposed scheme, the 2,4,6-TCP is oxidized to 2,6-dichloro-1,4-benzoquinone by ammonium cerium (IV) nitrate and the 2,6-dichloro-1,4-benzoquinone can be detected at low reduction potential. The linear range of 2,4,6-TCP determination was 0.4 to 750 μM with correlation coefficient (R^2) 0.9999 and the estimated detection limit (S/N=3) was 40 nM which were demonstrated by flow injection analysis. Twenty successive detect of 100 μM 2,4,6-TCP showed the relative standard deviation was 1.56%. Several 2,4,6-TCP structure-like compounds were studied as interferences including 2,4-dichlorophenol, 2-chlorophenol, phenol and 4-aminophenol. No obviously influences were observed. Two water samples which were collected from local farm and pool were adopted as analytical application. The recoveries of two water samples are 105.2% and 107.5%, respectively. An easy operation and enzymeless treatment detection scheme of 2,4,6-TCP is illustrated in this work.

Keywords: enzymeless, flow injection analysis, trichlorophenol, ammonium cerium (IV) nitrate

1. INTRODUCTION

Chlorophenol compounds is one of the major group of pollutants in the environment because of their toxicity and commonly use in wood preservatives, herbicides, and pesticides. Among these

chlorophenol compounds, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol are the most toxic particularly. Moreover, the 2,4,6-TCP is considered a priority pollutant by both the US Environmental Protection Agency (USEPA) and the European Union (EU). Thus, the residue of 2,4,6-TCP amounts in our living environment regard as very important determination. Many official methods were published for determination of 2,4,6-TCP including EPA methods 604[1] and 8270[2]. Many researchers also have reported chromatography methods for determination of chlorophenols, including high performance liquid chromatography (HPLC)[3,4], GC-mass spectroscopy (GC-MS)[5,6] and GC-electron capture detector (GC-ECD)[7,8]. These methods present a good way of sensitivity and selectivity but complicated procedures are also found in these works. Thus, several electrochemical biosensors have developed for shorten procedures in 2,4,6-TCP determination, such as tyrosinase[9], laccase[10], polyphenol oxidase[11], peroxidase[12] and chloroperoxidase[13]. The enzyme is a basic biosensor which provides a sensitivity, selectivity and less time consuming scheme in 2,4,6-TCP determination. However, the low stability and high cost of the enzyme may be limited the application of the biosensor. In this work, we provide a preoxidation scheme for 2,4,6-TCP determination. The present scheme can detect 2,4,6-TCP that is using a bare glassy carbon electrode. The long linear range of 2,4,6-TCP was being observed and was better than the prior biosensors. Several 2,4,6-TCP structure-like compounds were studied as interferences, including 2,4-dichlorophenol, 2-chlorophenol, phenol and 4-aminophenol. No influences were observed obviously. In the analytical application, two water samples which were collected from local pool and farm were demonstrated, and the results showed good recoveries. An easy operation scheme of 2,4,6-TCP determination is illustrated in this work.

2. EXPERIMENTAL; MATERIALS AND METHODS

2.1. Instruments and chemicals

Voltammetric measurements and FIA experiments were carried out with a CHI 750 electrochemical workstation (Austin, TX, USA). A three-electrode cell assembled with a glassy carbon working electrode, an Ag/AgCl reference electrode (RE-5, BAS), and platinum disc auxiliary electrode were used. Since oxygen did not interfere the analysis at the detection potentials and also no deaeration was performed in this study. The FIA system consists of a carrier reservoir, a Cole Parmer Syringe pump drive, a Rehodyne 7125 sample injection valve (20 μL loop), interconnecting Teflon tubing, and a BAS CC-5 thin layer electrochemical detector with a BAS electrochemical detector (West Lafayette, IN, USA).

2,4,6-TCP was purchased from Chem Service (PA, U.S.A.). Ammonium Cerium(IV) nitrate, 4-aminohenol, 2-chlorophenol, phenol and 2,4-dichlorophenol were purchased from Ridel-deHaën (Seelze, Germany) and all other compounds (ACS-certified reagent grade) were used as received. Aqueous solutions were prepared with doubly distilled deionized (DI) water. A fresh pH 3, 0.05 M phosphate solution (PBS) and 2,4,6-TCP solution were prepared daily.

2.2. Preoxidation of 2,4,6-TCP

The standard solution of 2,4,6- TCP was adjusted to a suitable concentration by the PBS buffer and then it was pretreated with 5 mM ammonium cerium (IV) nitrate. After stirring for 5 min, 3 ml of 0.2 M sodium dihydrogen phosphate solution was added and precipitated excess Ce^{4+}. After centrifuging, the resulting solution was injected into the FIA system. Every parameters of FIA were repeated five times and used to reproducibility calculation.

2.3. Real Sample Assay

Two water samples were collected from the pool in the campus and farm in Taipei. The samples were filtered with 0.22 μm filter paper and then the solutions were adjusted to 0.05 M, pH 3 PBS. The 20 ppm of 2,4,6-TCP were spiked in the solutions and the recoveries were calculated by the standard addition method.

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetry of 2,4,6-TCP

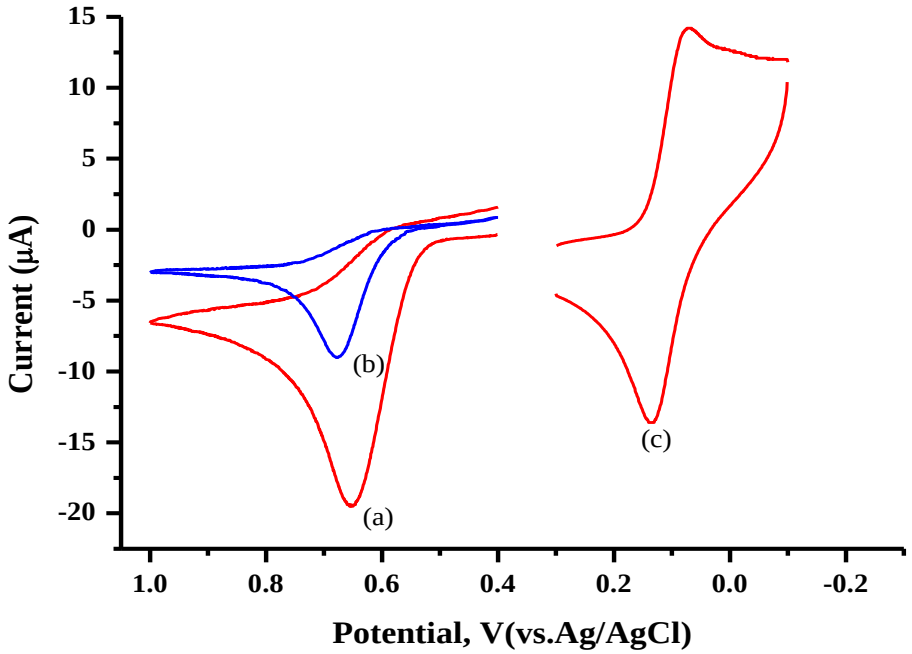

Figure 1. The cyclic voltammogram of 2,4,6-TCP. The cyclic voltammogram of (a) first scan, (b) tenth scan and (c) scan after oxidation in 2,4,6-TCP solution.

The cyclic voltammogram of bare glassy carbon electrode in 0.05 M, pH 3 PBS buffer containing 1 mM 2,4,6-TCP is shown in Fig. 1(a) The 2,4,6-TCP can be oxidized at potential higher

than +0.65 V (vs. Ag/AgCl) on glassy carbon electrode. After 10 consecutive cycles, the oxidation current of 2,4,6-TCP is reduced 50% as shown in Fig. 1(b). The non-conductive electropolymerization behavior in phenol and phenol derivate compounds is observed in 2,4,6-TCP as well. It causes irreversible reaction of electropolymeration and bad reproducibility for 2,4,6,-TCP determination. Many researchers have reported similar behaviors in phenol and phenol derivate compounds[14]. Fig.1(c) represents a cyclic voltammogram of 0.5 mM 2,4,6-TCP which is preoxidized by ammonium cerium (IV) nitrate, a redox couple is observed at +0.13 and +0.075 V (vs. Ag/AgCl), respectively. The purposed oxidation product of 2,4,6-TCP is 2,6-dichloro-1,4-benzoquinone. An UV/Vis spectroscopy is used to identify the product of this reaction as shown in Fig.2. A absorption peak is observed at 273 nm after 25 μM of 2,4,6-TCP is preoxidized by 1 mM ammonium cerium (IV) nitrate, this peak is assigned as the absorption of 2,6-dichloro-1,4-benzoquinone and has illustrated by the prior work. According to the preoxidation scheme of 2,4,6-TCP, our method presents no irreversible reaction from eletropolymeration and good reproducibility of 2,4,6-TCP detection as shown in Fig.3.

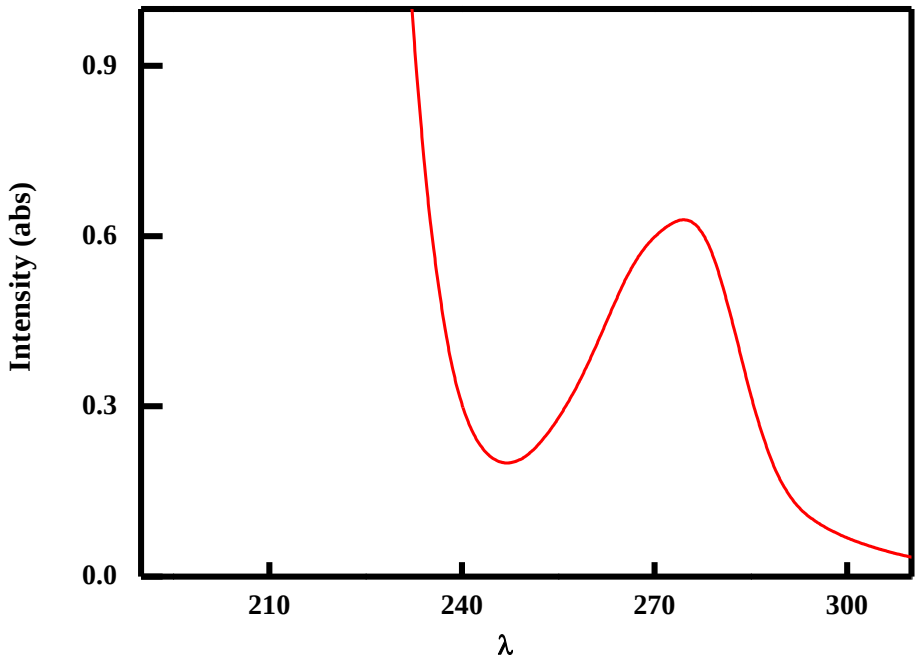

Figure 2. The UV/Vis spectroscopy of 2,4,6-TCP. The 2,4,6-TCP is preoxidized by ammonium cerium (IV) nitrate.

3.2. Flow Injection Analysis of 2,4,6-TCP

In the preliminary work, the FIA is used to study the repeatability during 2,4,6-TCP determination and it is with the pre-oxidation step and also it is without the pre-oxidation step. After twenty successful detect 1.0 mM 2,4,6-TCP, the response current of 2,4,6-TCP reduce 71% without pre-oxidation step and no obviously change with pre-oxidation step as shown in Fig.3. It is clear evidence that pre-oxidation step can avoid the non-conductive electropolymerization during 2,4,6-TCP

determination. The typical plots of detection potential and flow rate are shown in Fig.4. The detection potentials were studied from +0.3 to -0.1 V (vs.Ag/AgCl). High reduction current is observed at more negative potential but the relative standard deviation (R.S.D) is increased after –0.05 V shown in Fig 4(a). Thus, the –0.05 V was adopted as a suitable detection potential for further studies. The flow rates of carrier solution were studied from 0.1 ml min^{-1} to 1.2 ml min^{-1} that is showed in Fig.4(b). At low flow rate, the mass transfer between 2,4,6-TCP and electrode interphase is relative to be low, so the broader peak and worse resolutions is observed. The well-defined peaks are observed while flow rate higher than 0.8 ml min^{-1}. After 1.0 ml min^{-1}, the electron transfer and mass transfer rate are not quiet difference. The 1.0 ml/min is adopted a best flow rate of carrier solution in 2,4,6-TCP determination for acceptable resolution and sensitivity. Other FIA conditions are also studied including sample loop and pH of buffer solution are showed at Fig.5. The optimum conditions of FIA were described as following: detection potential -50 mV, flow rate 1.0 ml/min, sample loop 20 μL in pH 3 0.05 M phosphate buffer. The calibration plot of this work is showed at Fig.6 and inset plot is actual FIA graph. The linear range of this work is 0.4~750 μM with correlation coefficient (R^2) is 0.9999, the linear range is better than prior enzyme based biosensor. The limit of the estimated detection of 2,4,6-TCP is 40 nM (S/N=3). After 20 times consecutive inject of 100 μM 2,4,6-TCP, the R.S.D is 1.56%. The SDS, camphor, humic acid, 4-aminohenol, 2-chlorophenol, phenol and 2,4-dichlorophenol were used as interferences. The results are shown in table1. As it can be seen at table 1, the ammonium cerium nitrate applies are more selectivity than cerium sulfate as pre-oxidant. No obvious influences are observed in this system especially in 2-chlorophenol and 2,4-dichlorophenol. Finally, the system was extended to assay two water samples which were collected from local pool and farm. The 20 ppm of 2,4,6-TCP were spiked in the solutions and the recoveries were calculated by the standard addition method. The recoveries of two water samples are 105.2% and107.5% respectively.

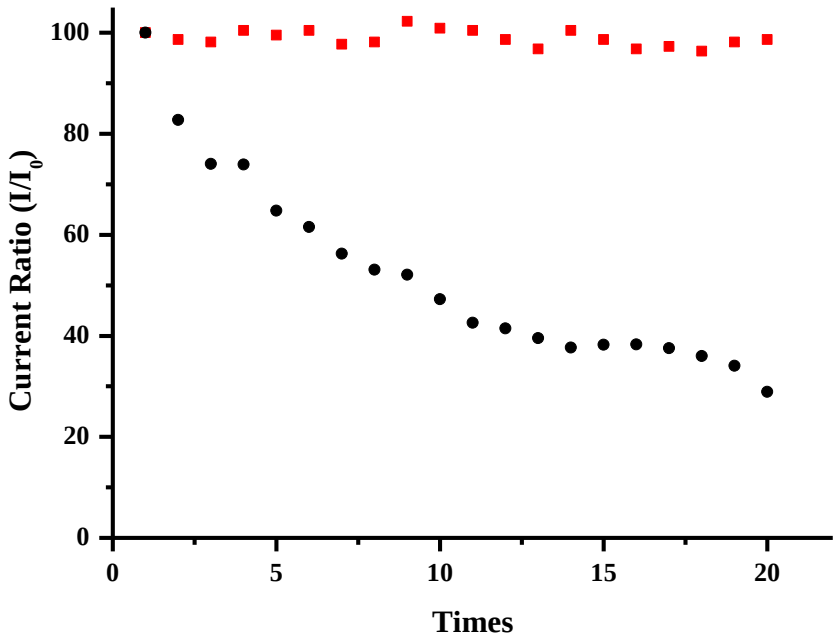

Figure 3. The reproducibility of 2,4,6-TCP detection.

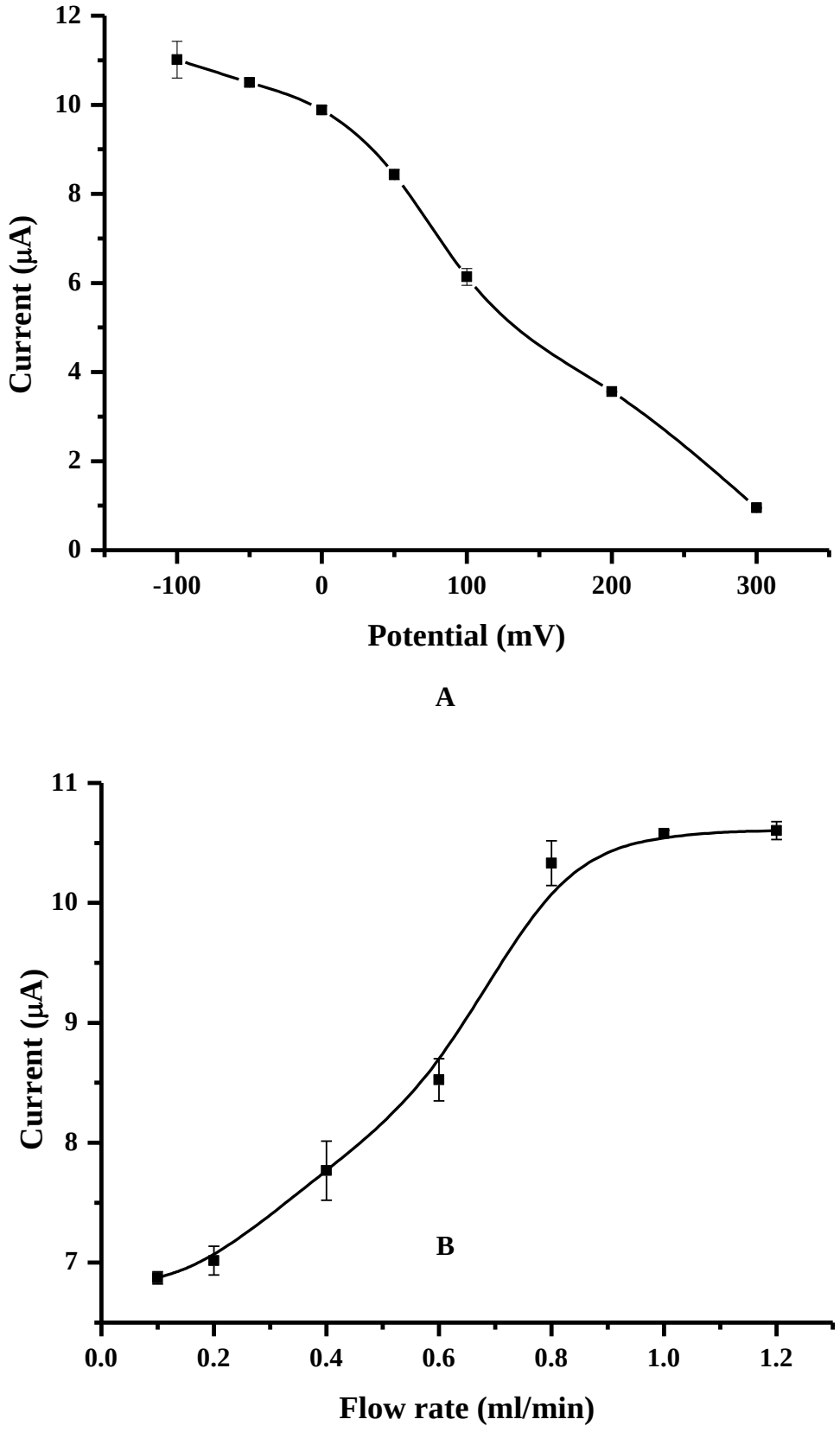

Figure 4. The study of applied potential (a) and flow rate (b) effect of 2,4,6-TCP determination.

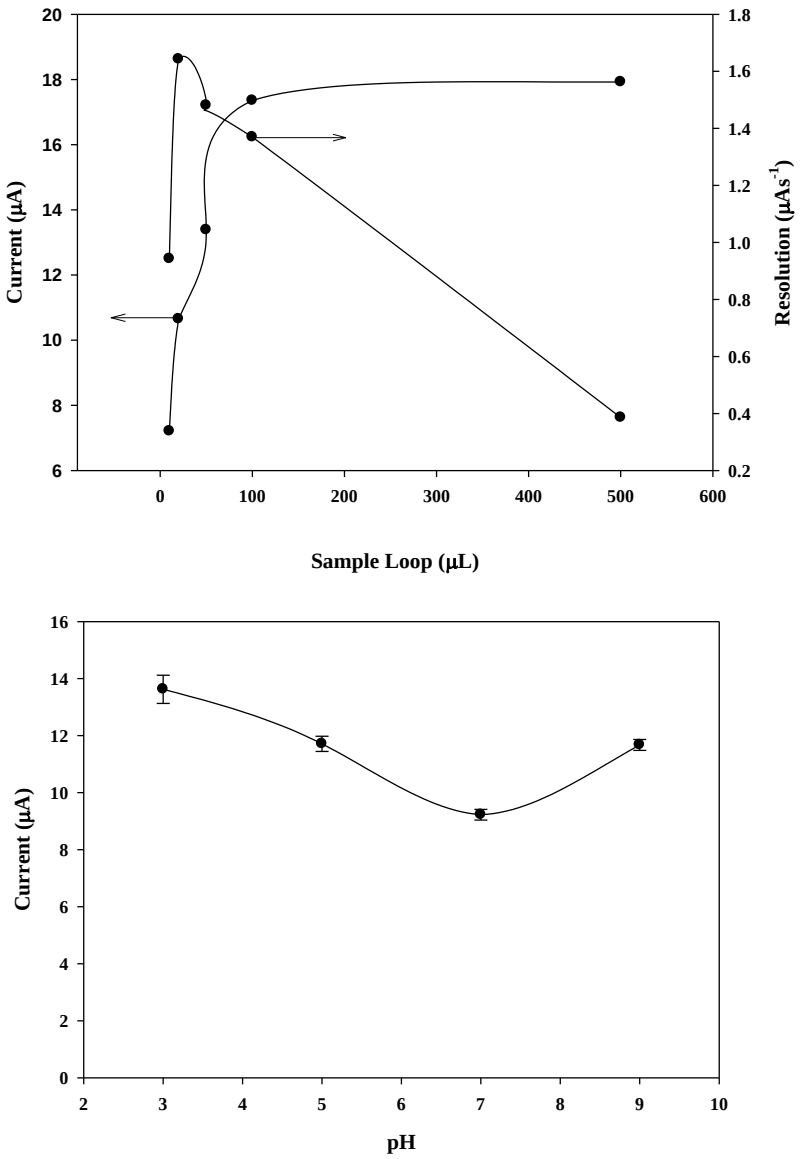

Figure 5. The study of sample loop (a) and pH (b) effect of 2,4,6-TCP determination.

Table 1. The interferences study for 2,4,6-TCP determination

Interferences	Error (%)
Humic acid	-9.79
SDS	-2.99
Camphor	0.98
2,4-dichlorophenol	7.85
2-chrolophenol	5.80
Phenol	4.89
4-aminophenol	5.30

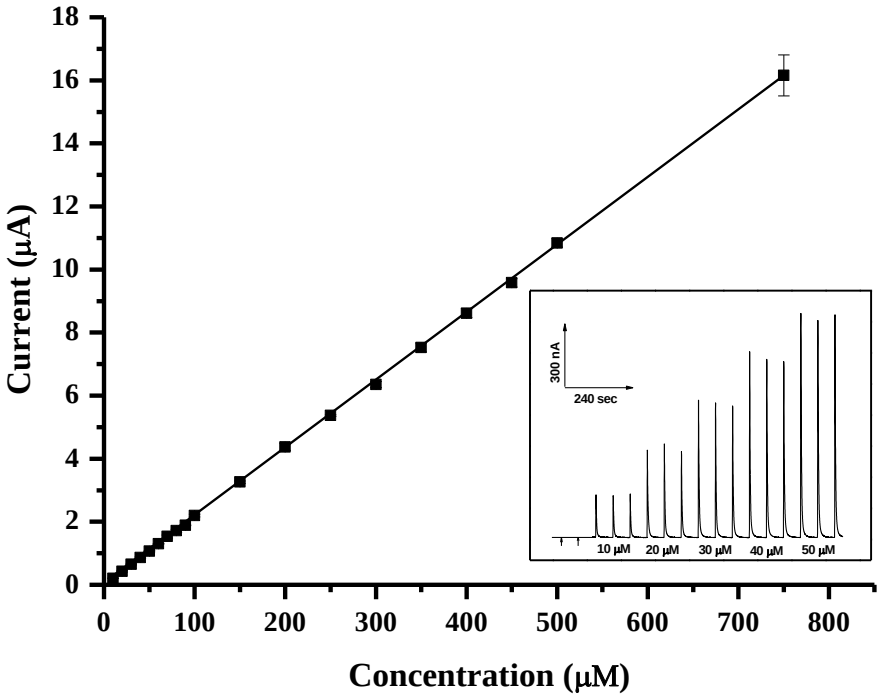

Figure 6. The calibration plot of 2,4,6-TCP.

4. CONCLUSION

A preoxidation reaction is demonstrated in 2,4,6-TCP determination without any enzyme treatment. The results show a long linear range and low detection limit of 2,4,6-TCP determination. A selective preoxidation reaction is observed in the result. Two water samples are studied as real samples and good recoveries that has been achieved. A enzymeless approach of 2,4,6-TCP determination is illustrated in this work.

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