



New strategy for amperometric determination of nabam pesticide by using potential assisted surface oxide regeneration method

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ABSTRACT

This project demonstrates a novel amperometric method to measure the concentration of nabam through a process of potential assisted surface oxide on the copper based electrode. Based on the strong cuprous ion chelating capability of nabam, the cuprous oxide layer is dissolved away from the electrode surface. A subsequent oxidative current induced from regeneration of the oxide layer was used to reflect the concentration of the nabam. Under a set of optimal conditions with operating potential at -125 mV in the 50 mM phosphate buffer, pH 5.75 , a suitable dynamic range of nabam from 0.2 μ M to 10 μ M (or 0.051 – 2.56 ppm) was achieved, which meets the requirement of the Joint FAO/WHO Meeting on Pesticide Residues. This method is free from the environmental interferences. However, most of all metal ions show a negative deviation due to the formation of other metal–nabam complexes. Here, a simple cation exchange column was used to release the chelated nabam molecule. Finally, two successful real applications were demonstrated by using a standard addition procedure after this cation exchange pretreatment.

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1. Introduction

Ethylene bisdithiocarbamate (EBDC) including nabam, mancozeb, mancozeb, mancozeb, and zineb are a group of organosulfur pesticides that are still frequently and extensively used as fungicides, bactericides, and algicides [1]. Investigation of these EBDC based pesticides is an important subject due to its carcinogenicity and teratogenicity of its metabolite, ETU [2]. According to the requirement of the Joint FAO/WHO Meeting on Pesticide Residues (JMPRs), the tolerance limits of EBDC pesticides are 7 ppm or less for most crops, and 25 ppm or less for lettuce, mustard greens, chard and spinach [3]. Due to its toxicity, several reports had reviewed the analytical methods for these EBDC pesticides [4–6]. Generally, the determination of the CS_2 molecule that converted from a hot-acid digested process is the most popular strategy to reflect the concentration of the EBDC pesticides. In this concept, early study used an iodine reagent to titrate the concentration of the CS_2 [7]. Following reports utilized the chelating property of the thiol functional group to measure the metal– CS_2 complexes through a visible spectrometer [8,9]. In addition, highly sensitive chemiluminescent methods for the determination of these metal– CS_2 complexes have

been reported by using several fluorochromes such as luminol [10] and ruthenium (II) tris (2, 2'-bipyridine) [11]. However, the aforementioned CS_2 based analytical schemes somehow lose the specific information on individual chemicals. Moreover, the tedious process is another difficulty in routine analysis. Direct measurement of the EBDC pesticides without digestion has been proposed by liquid chromatography coupled with an UV detector; however, a solid phase pre-concentrated process is required to improve its sensitivity [12]. Thus, an exceptionally sensitive analytical method for EBDC based pesticides is urgently needed.

Electroanalysis has been considered as an efficient tool in the determination of these EBDC pesticides. Early reports used a stripping voltammetric method for sensing the concentration of EBDC pesticides through its central metal ions [13,14]. Although the linearity of these reports is adequate for a real application, the concept of these stripping methods is measuring the metal ions rather than EBDC molecular itself, in other words, a positive deviation might be obtained if the test sample was polluted by the same metal ions. Moreover, the stripping technique requires a harmful mercury electrode to reach its high analytical performance; it is not an appropriate program in modern analysis. Direct oxidation of the thiol functional group with a bare glassy carbon or platinum electrode is a possible way for the determination of these EBDC pesticides; however, this electrochemical reaction might suffer from the slow kinetic rate and a significant surface fouling effect [15,16]. To the best of our knowledge, only cobalt phthalocyanine modified electrode has been reported to direct measurement the nabam by

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using a differential pulse voltammetry, successfully [17]. However, a scheme with feasibility in connection with a separation tool is still an urging issue dealing with a complicated sample.

Recently, we have demonstrated an amperometric scheme of potential assisted surface oxide regeneration (PASOR) on the copper electrode to determinate several electrochemical inactive analytes such as polyamines and creatinine [18,19]. We found out that this amperometric method is particularly useful in the determination of a molecule with chelating property for cuprous or cupric ion. The dithiol group of nabam is a famous bi-dentate functional group for the cupric ion [20]. Here, we extend our previous effort to develop an amperometric method to monitor the nabam molecule itself. This method possesses several advantages over the previous reports, such as less time consuming, relatively reasonable linear range with an excellent sensitivity. The detail experimental designs, analytical performances of the proposed scheme and its real application are also described in the following sections.

2. Experimental

2.1. Apparatus

Cyclic voltammetric experiments, electrochemical deposition, and amperometric detection in the flow injection analysis (FIA) system were carried out with a CHI 832B electrochemical workstation from CH Instruments (Austin, TX, USA). Ag/AgCl, 3 M NaCl, reference electrodes were used in this study. The flow rate of the FIA system was controlled with a syringe pump (74900 series, Cole-Parmer Instrument Company, IL, USA). A home-made ionic exchange column by filling with 30 g cation ionic exchange resin slurry (Dowax® 50WX8, Aldrich) into a buret was used to remove the metal ions from the real sample. This column was regenerated by passing through 1 M HCl, 50 mL, and washed with excess pure water.

2.2. Reagents

Nabam stock solution (2 mM) was prepared by dissolving of nabam (Chem Service, West Chester, PA, USA) in the relevant phosphate buffer solutions. The stock solutions of iron chloride, zinc chloride, magnesium sulfate, calcium carbonate, aluminum potassium sulfate dodecahydrate, camphor and humic acid (Aldrich, Milwaukee, WI, USA), sodium dodecyl sulfate (SDS) (Mallinckrodt, Paris, KY, USA), manganese chloride (Riedel-deHaen, RDH, Seelze, Germany) were prepared in 50 mM phosphate buffer solution, pH 5.75. All of the prepared stock solutions were stored in a refrigerator at 4 °C. The conductive carbon ink (C10903D14) was obtained from Gwent Electronic Materials Ltd. (Pontypool, UK). De-ionized water prepared by a Barnstead water purification system with a resistance of 18.3 M Ω -cm (Easypure Ro and Easypure UV/UF, Dubuque, IW, USA) was used to make up all the buffer solutions.

2.3. Fabrication and conservation of the copper plating electrode

The copper plating layer was deposited electrochemically by immersing a platinum electrode into a 0.1 M CuSO₄ (dissolved in 0.1 M H₂SO₄ solution) and a constant potential of –200 mV was applied for 60 s. Subsequently, this plating copper electrode was polished with 0.05 μ m alumina carefully until getting a mirror like surface. This electrode was subsequently preserved in a nitrogen rich container. Before each experimental run, this copper electrode was pretreated by applying a potential of –1 V in the buffer solution for 1 min [21].

2.4. Mechanism study and dual electrode flow injection analysis

All the electrochemical results with the exception of the cyclic voltammogram were obtained from a FIA system. A dual platinum thin-layer flow detector (Φ = 2 mm) with copper plating layer on the upstream electrode and a conductive carbon ink on the downstream one was used to investigate the detail mechanism. In the mechanism study, the operating potential of the upstream electrode was investigated from –200 mV to 50 mV, at the mean time, a constant potential of 100 mV was applied at the downstream electrode to monitor the amount of cuprous–nabam complex that produced from the upstream one.

2.5. Procedure of the real sample application

The application of real sample was demonstrated by using a standard addition method. Two water samples that collected from local pond and field were filtrated with filter papers and 0.45 μ m filters. Subsequently, 2.5 and 5 μ M nabam was added to form an artificial polluted water sample, respectively. The filtrations were purified through a home-made cation exchange column and diluted with 0.1 M phosphate buffer, pH 5.75 with a ratio of 1:1 (V/V). Finally, 2, 4, and 6 μ M nabam were added into the respective artificial sample and evaluated the recovery rate by using the standard addition method, respectively.

3. Result and discussion

3.1. Study of the amperometric detection principle

In order to demonstrate the feasibility of this scheme in the determination of the nabam, the voltammograms of the nabam obtained from a glassy carbon and a copper plating electrode were studied and compared. Fig. 1A shows the typical voltammograms of buffer obtained from a glassy carbon electrode (a), spiked with 250 (b), and 500 (c) μ M nabam. It is noticed an oxidative peak around 400 mV increased with the concentration of nabam. Apparently, this electrochemical reaction is attributed to oxidation of the CSSH functional group of nabam molecule. Fig. 1B shows the voltammograms of blank obtained from a copper plating electrode (d), a sharp reduction peak and a broad oxidative peak is believed as the self redox behaviors of the copper electrode. However, after sequential addition of 50 (e), and 100 (f) μ M nabam, a significant oxidative peak at 75 mV is obtained, and the intensity was almost twenty-five times than that obtained from the glassy carbon. Moreover, a slight decrement at the reduction peak was observed. According to the electrochemical phenomenon of these voltammograms, a typical EC reaction seems to be a possible mechanism [22]. However, our previous reports have shown that a similar electrochemical behavior can be also obtained if a cupric-complex generated on the electrode surface [18,19]. In order to define the actual mechanism of this scheme, a dual electrode with a copper based upstream electrode and downstream carbon ink was prepared on a flow injection system to perform a generation-collection experiment. In this study, the operating potential of the upstream electrode was investigated over a range from –200 to 50 mV while the downstream one was kept at a constant potential of 100 mV. Since the Cu–nabam complex was generated in the PASOR process and no reduction reaction was found in Fig. 1A, the surface reaction can be ensured by evaluation of the signal from the downstream electrode. Fig. 2 shows the current responses obtained from the upstream (A) and downstream (B), respectively. Interestingly, both the upstream and the downstream electrodes performed an oxidation reaction during this potential study. In addition, the current intensity obtained from the downstream electrode is closely related to the upstream

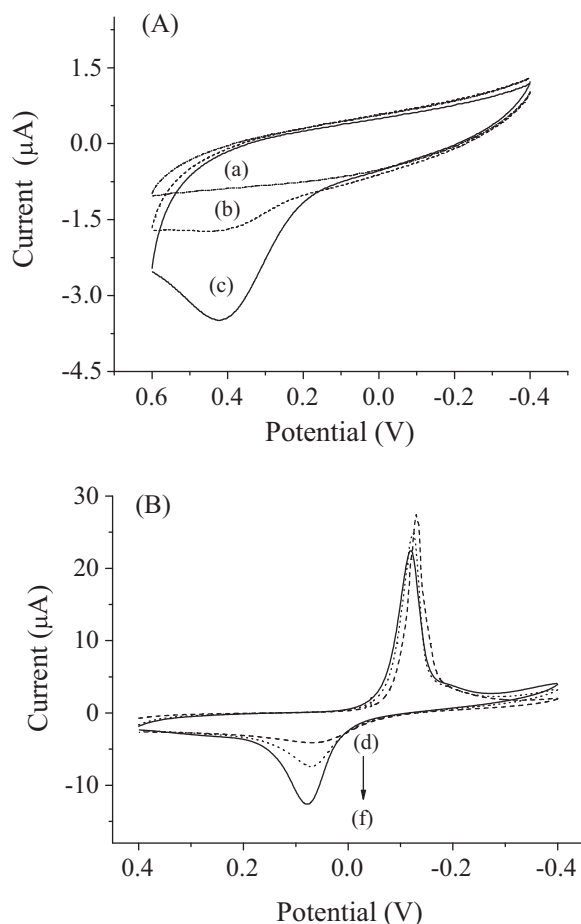


Fig. 1. Typical voltammograms of nabam on the glassy carbon electrode (A) and copper plating electrode (B). The concentrations of nabam are at 0 (a), 250 (b), 500 μM in (A), respectively, and (c), 0 (d), 50 (e), 100 (f) μM in B, respectively. The buffer conditions: 50 mM phosphate, pH 5.0. Scan rate: 50 mV/s.

one. Because both the operating potential of the downstream electrode and the concentration of the nabam solution were maintained constant, the oxidative peak recorded on the downstream electrode should be attributed to further oxidation of the cuprous–nabam complex rather than nabam itself. According to the aforementioned discussion, a PASOR process shown in Fig. 3 might be more suitable

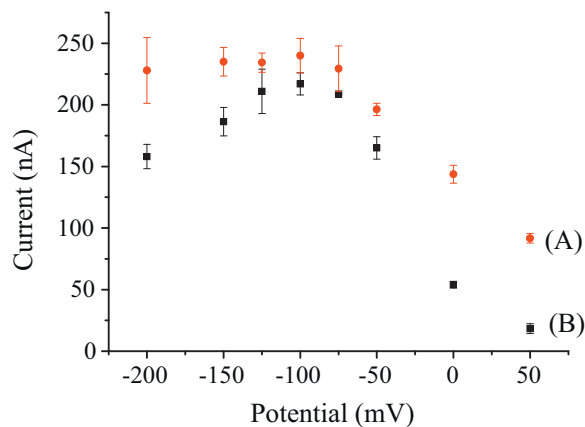


Fig. 2. Typical generation-collection study by using an upstream copper electrode (A) and a downstream carbon ink electrode (B). The applied potential of the upstream copper electrode was held between 50 and –200 mV, and the downstream carbon ink electrode (collected electrode) was held a constant potential of 100 mV. Other conditions: test sample: 10 μM nabam with 50 μL. The carrier buffer: 50 mM PBS pH 6.0, and the flow rate: 0.5 mL/min.

to represent this surface reaction between copper plating electrode and nabam. Briefly, the copper plating electrode would be oxidized to cuprous oxide after applying an adequately anodic potential. In the absence of nabam, this cuprous oxide passive layer would be accumulated above the copper layer gradually, which would hinder the inside copper layer from reacting with the electrolyte to form cuprous oxide, therefore, the background current would reach its steady state. However, in the presence of nabam, a portion of this cuprous oxide layer is dissolved away by forming a complex with nabam. This reaction exposes a fresh copper metal surface and the electrochemical surface oxidation proceeds on uninhibited by surface passivation as compared with nabam-free samples. Thus, this oxidation current is used to reflect the concentration of nabam in this study. In addition, because portion of the oxide layer dissolved in the process of PASOR, which also causes the decrement of the reductive peak as shown in Fig. 1B.

3.2. Optimization of the operating potential

Before optimization, several voltammetric methods including linear voltammetry, differential pulsed voltammetry, and triple pulsed amperometry were considered as the possible analytical

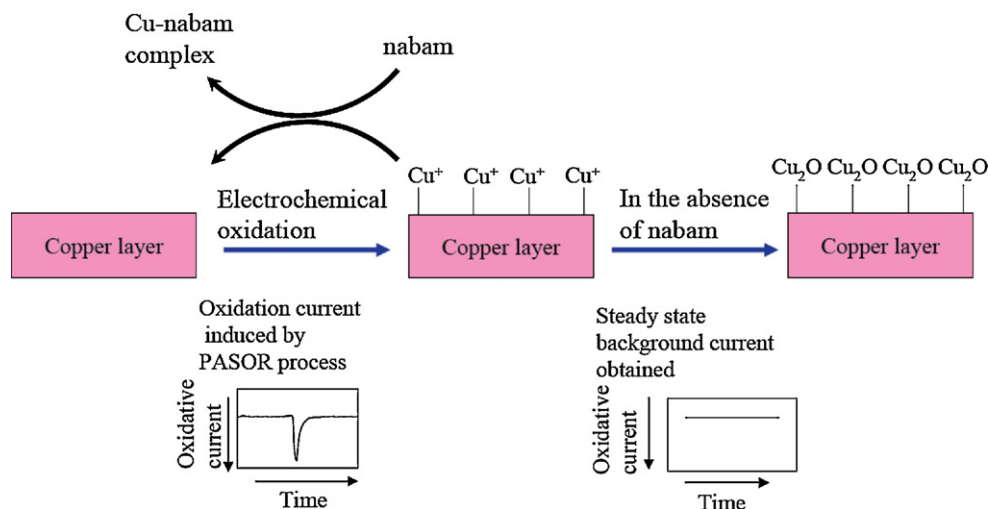


Fig. 3. Possible mechanism of PASOR used in the nabam determination.

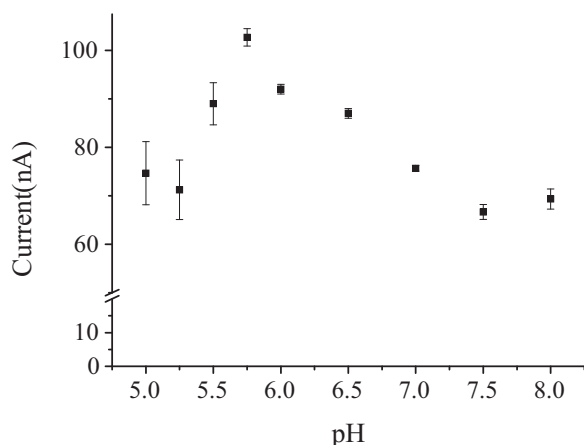


Fig. 4. Influence of pH in nabam determination. Current responses in FIA of the injection of 10 μM nabam in a 0.05 M phosphate solution at various pH ranging from 5 to 8. The potential was optimized at -0.125 V, other conditions are as in Fig. 2.

techniques. We found that the sensitivities of these dynamic potential scanning methods are better than the amperometric one; however, these techniques suffered from a huge variation and poor reproducibility between run-to-run operations (data not shown). Considering the reproducibility, a flow injection system coupled with the amperometric technique was used in the subsequent study. In the process of optimization, the operating potential was chosen as the first parameter to be investigated due to its concerns of the composition of the passive oxide layer. The influence of operating potential was studied from 0 to -200 mV by successive injection of 5 μM nabam. The trend of this potential study was similar to that of Fig. 2 and the best sensitivity was appeared at -125 mV (see Supplementary S1). According to the voltammogram of Fig. 1A, the applying potential for the electrochemical oxidation of the nabam should be more positive than 200 mV. Thus, this oxidative current appeared at -125 mV should be absolutely resulted from the process of PASOR rather than a direct oxidation of the nabam molecule. Interestingly, the intensity decreased in the higher operating potential, in other words, this oxidative reaction does not benefit from the higher operating potential. Similar result has been shown in our previous reports [18,19]. We found the process of PASOR cannot be performed when the cupric oxide is the major component on the electrode. Since this scheme can be seen as a ligand replaced reaction, this result may be attributed to the high stability of cupric oxide, which decreased the rate of replacement. In addition, although the sensitivity showed a slight decrement in the lower operating potential range, however, it suffered from the simultaneous reduction of dissolved oxygen, which caused a significant fluctuation of background current. Thus, -125 mV was chosen as the optimal potential condition in the subsequent studies.

3.3. Optimization of the buffer condition

The acidity of a buffer solution is another important factor in this measurement. Fig. 4 shows the average signal from three successive injections of a 5 μM nabam between the pH ranging from 5 to 8, a maximum sensitivity was found at pH 5.75. A poor reproducibility in the lower pH region is observed, this result may attribute to the unstable cuprous oxide in the lower pH region. It is conceivable that hydroxyl ion can be seen as another ligand to the metal ions and the decrement of current intensity in the high pH region may attribute to the hydroxyl ions competition with the chelating site of the cuprous ion, which decreased the formation rate of cuprous–nabam complex. Thus, a buffer solution with pH 5.75 was

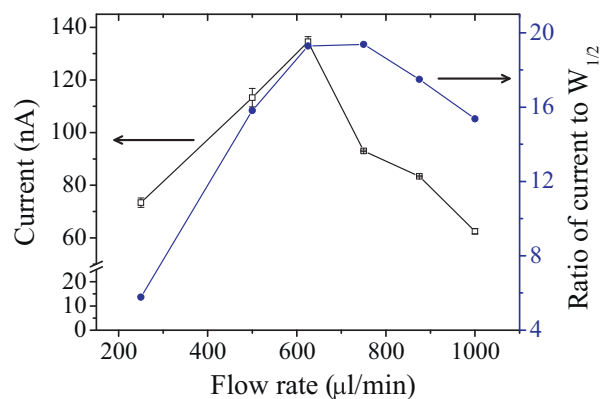


Fig. 5. Flow rate study. Current sensitivity (hallow square) and the relative ratio of half peak width (solid circle) in the flow rate ranging from 0.25 to 1 mL/min. Other conditions as in Fig. 3.

chosen as the optimal acidity. Subsequently, the composition of the buffer was evaluated; several candidates including phosphate, acetate, imidazole and tris (hydroxymethyl) aminomethane at pH 5.75 were investigated. After a careful investigation, a 50 mM phosphate buffer was chosen as the optimal buffer due to its ultimate sensitivity and reproducibility (see Supplementary S2 and S3).

3.4. Optimization of the flow rate and injected volume

In the optimization of a flow injection analysis based method, it is known that the flow rate and injected volume affects both the sensitivity and peak resolution. A suitable experimental condition is necessary to improve the analytical performance. The injected volume was studied between 10 μL and 200 μL in a flow rate of 0.5 mL/min. We found the signal intensity increased with the sample volume and approached its plateau after 50 μL sample injection (see Supplementary S4). Although it was still a slight enhancement when larger volume was injected, a peak tailing effect was also noticed. Thus, 50 μL was chosen as the optimal sample loading volume. Fig. 5 shows the influence of the flow rate from 0.25 to 1 mL/min. Here, not only the current intensity but also the peak width at half height was considered simultaneously. It was certain that a flow rate of 0.625 mL/min shows a best result for both of two factors, thus, we chose flow rate at 0.625 mL/min as the optimal flow rate.

3.5. Analytical performance

The analytical performance of proposed scheme was evaluated by using the optimal conditions as described in the above sections. Fig. 6 shows the calibration curve of nabam and the actual responses of 0.5 (a), 1 (b), 2 (c), and 3 (d) μM nabam were also shown. A suitable dynamic range from 0.2 μM to 10 μM (or 0.051–2.56 ppm, with $R^2 = 0.999$) with a sensitivity of 32.9 nA/ μM was achieved. The detection limit estimated from the slope of calibration plot and the standard deviation of the blank was only 0.063 μM or 16.13 ppb ($S/N = 3$). This linear range is adequate to meet the requirement of FAO (7 or 25 ppm for the crops). The RSD of the twenty-one successive measurements of 2 μM nab am was 1.3%, which indicates an excellent reproducibility of this scheme. Table 1 summarizes a simple comparison of various prior publications for determination of nabam. Although the limit of quantitation (LOQ) is not as low as the SPME/HPLC/UV method, however, this method provides an easier, rapid and cost-effective scheme to monitor nabam without a further concentrated manipulation.

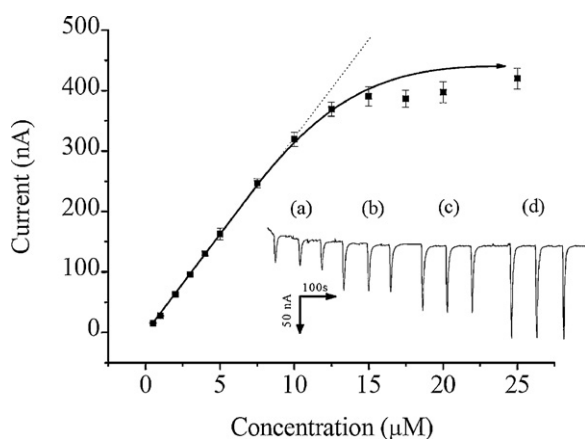


Fig. 6. Typical calibration curve of the nabam. The inset diagram is the actual responses of sequentially injected nabam with concentration of 0.5(a), 1 (b), 2 (c), and 3 (d) μM .

Table 1
Simple comparison of current scheme with prior sensing scheme for nabam.

Nabam sensing method: critical technique	Linear range	reference
FIA, fluorimetry, treat with the thallium reagent	1–10 μM	[23]
SPME for 30 min/HPLC/UV 254 nm	80 nM–2.4 μM	[12]
CoPC/ink electrode, DPV, FIA, Cu electrode, amperometry, -0.125 V	2.5–36 μM 0.2–10 μM	[17] This work

3.6. Interference and real sample application

In order to understand the influences of the environmental interferences to this scheme, the effect of the possible interferences was evaluated, respectively. Table 2 summarizes the influence of several environmental pollutions including humic acid, sodium dodecyl sulfate, and camphor in nabam determination. None of them caused a significant influence to this scheme, which indicates this scheme is not suffering from these typical organic pollutions. However, most of all metal ions cause a negative deviation to this scheme. Since the nabam is a well-known chelating reagent, furthermore, the signal of this scheme was also attributed to the thiol functional group of nabam that induced the process of PASOR; it is conceivable that the chelating site may be also occupied with other metal ions. Thus, the concentration of the free nabam may reduce, which caused a significant negative deviation. These results imply that metal ions are the major interferences of this scheme. Also, the

Table 2
Interference study. The 5 μM organic or 10 ppm metallic interferences was measured in the presence of 5 μM nabam.

Test compound	Current (nA)	Ratio (%) ^a
5 μM Nabam	129.2 \pm 3.1	100.0
5 μM Humate	134.7 \pm 3.7	104.3
5 μM camphor	129.2 \pm 3.9	100.1
5 μM SDS	128.0 \pm 2.7	99.1
10 ppm Cr^{3+}	131.9 \pm 2.6	102.1
10 ppm Mn^{2+}	123.9 \pm 3.4	96.1
10 ppm Ni^{2+}	91.8 \pm 3.2	71.1
10 ppm Zn^{2+}	99.4 \pm 3.0	77.0
10 ppm Co^{2+}	100.1 \pm 1.2	77.5
10 ppm Pb^{2+}	54.6 \pm 1.2	42.3
10 ppm Hg^{2+}	53.6 \pm 4.4	41.5
10 ppm Al^{3+}	120.4 \pm 2.6	93.2
10 ppm Mg^{2+}	121.7 \pm 3.2	94.2
10 ppm Ca^{2+}	122.2 \pm 2.7	94.6

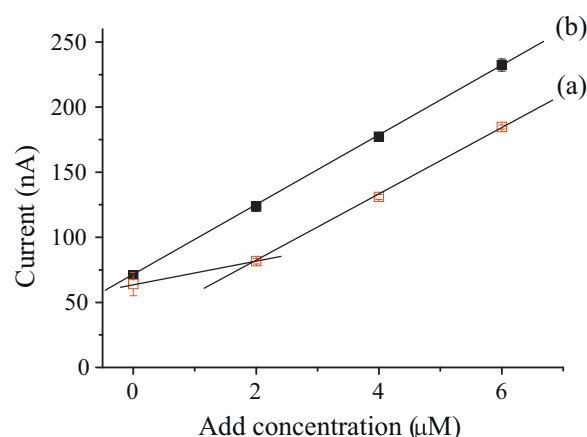


Fig. 7. Analytical result of an artificial sample by using the standard addition method without (a) and with (b) ion exchanged pretreated.

Table 3
Determination of nabam in pond and rice field sample. Each result is repeated for three times.

	Added (μM)	Found (μM)	Recovery rate
Pond sample	2.50	2.49	99.6%
Pond sample	5.00	5.27	105.4%
Field sample	2.50	2.58	103.2%
Field sample	5.00	5.10	102.0%

influence in real sample determination was investigated, as shown in Fig. 7a. It is noticed that the sensitivity diverted before and after addition of 2 μM nabam in the process of standard addition analysis. These data implied that trace amounts of metal ions may exist in the real sample. Therefore, the dried real sample was analyzed by EDX (see results in Fig. S5 in Supplementary Material), which revealed the existence of aluminum, magnesium and calcium ions, which might compete for the chelating site of nabam. In order to solve the influence of these metal ions, a cation ion exchange resin was used to extract the nabam. The analytical results after extraction is shown in Fig. 7b, an excellent linearity ($R^2 = 0.999$) in this standard addition analysis indicates the problem of the metal ions can be solved by this simple pretreatment. In addition, a similar slope of Fig. 7a and b from 2 to 6 μM nabam also supports our hypothesis that the metal ions are the major interference in the real sample. Table 3 shows the analytical results of four artificial polluted water samples. After a simple cation pretreatment, the recovery rates of four artificial samples are ranged from 99.6 to 105.4%. In addition, there is no negative deviation effect as Fig. 7a in each batch of standard addition analysis indicates this method coupled with a simple cation ion exchange column is a reliable method of determining of the environmental samples.

4. Conclusion

In this work, we have demonstrated the PASOR behavior is a reliable scheme in the determination of the nabam pesticide. The dynamic range of the current scheme meets the requirement of the JMPR Meeting. Compared to the conventional CS_2 titration or photometric measurement schemes, our method provided efficient and straightforward measurement of nabam in the environmental samples without tedious digestion. However, the nature of the measured mechanism makes this scheme would be affected by the metal ions and which issue can be avoiding by using a cation exchanged pretreatment is required to ensure the accuracy. Moreover, because the nabam is the basic component for several EBDC pesticides such as zineb and maneb, these pesticides can be

converted to yield a nabam molecule promptly after a simple treatment with EDTA solution. The detail analytical evaluations of these pesticides and total concentration of the EBDC pesticides are still under continuous investigation in our laboratory now.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.snb.2012.06.074>.

References

- [1] D.R. Cooley, W.J. Manning, *Environmental Pollution* 88 (1995) 315–320.
- [2] World Health Organization, *Environmental Health Criteria*, vol. 78, World Health Organization, Geneva, 1988.
- [3] FAO/PL:1967/M/11/1, WHO/Food Add./68.30.
- [4] G. Crnogorac, W. Schwack, *Trends in Analytical Chemistry* 28 (2009) 40–50.
- [5] A.K. Malik, W. Faubel, *Pesticide Science* 55 (1999) 965–970.
- [6] O.H.J. Szolar, *Analytica Chimica Acta* 582 (2007) 191–200.
- [7] D.G. Clarke, H. Baum, E.L. Stanley, W.F. Hester, *Analytical Chemistry* 23 (1951) 1842–1846.
- [8] W.K. Lowen, *Analytical Chemistry* 23 (1951) 1846–1850.
- [9] M. Petsch, J. Seipelt, B.X. Mayer-Helm, *Analytica Chimica Acta* 516 (2004) 119–124.
- [10] A. Waseem, M. Yaqoo, A. Nabi, *Analytical Sciences* 25 (2009) 395–400.
- [11] K. Tsukagoshi, N. Okuzono, R. Nakajima, *Journal of Chromatography A* 958 (2002) 283–289.
- [12] J.S. Aulakh, A.K. Malik, R.K. Mahajan, *Talanta* 66 (2005) 266–270.
- [13] L. Mathew, M.L.P. Reddy, T.P. Rao, C.S.P. Iyer, A.D. Damodaran, *Talanta* 43 (1996) 73–76.
- [14] M.S. Lin, B.I. Jan, H.J. Leu, J.S. Lin, *Analytica Chimica Acta* 388 (1999) 111–117.
- [15] Y.D. Zhao, W.D. Zhang, H. Chen, Q.M. Luo, *Sensors and Actuators B* 92 (2003) 279–285.
- [16] C. Deng, J. Chen, X. Chen, M. Wang, Z. Nie, S. Yao, *Electrochimica Acta* 54 (2009) 3298–3302.
- [17] M.S. Lin, J.S. Wang, *Electroanalysis* 16 (2004) 904–909.
- [18] M.S. Lin, C.H. Chen, Z. Chen, *Electrochimica Acta* 56 (2011) 1069–1075.
- [19] C.H. Chen, M.S. Lin, *Biosensors and Bioelectronics* 31 (2012) 90–94.
- [20] A.V. Ivanov, T. Rodyna, O.N. Antzutkin, *Polyhedron* 17 (1998) 3101–3109.
- [21] R.W. Frei, W. Th Kok, H.B. Hanekamp, P. Bos, *Analytica Chimica Acta* 142 (1982) 31–45.
- [22] A.J. Bard, L.R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*, 2nd ed., John Wiley & Sons Inc., New York, 2001.
- [23] P.R. Tomás, M.L. Carmen, V. Tomás, C. Rocio, *Talanta* 43 (1996) 193–198.

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