**American Journal of Analytical Chemistry, 2015, 6, 263-273**
Published Online March 2015 in SciRes. http://www.scirp.org/journal/ajac
http://dx.doi.org/10.4236/ajac.2015.64025

# Voltammetric Study of the Interaction between Melamine and DNA on a Carboxylic Acid-Functionalized Nano-$Fe_3O_4$ Modified Electrode and Its Analytical Application for Melamine Determination in Liquid Milk Samples

**Ke Chen[1], Haoyu Shen[1*], Zhejun Wang[1], Amen Zhou[1], Qinghua Xia[2]**

[1]Biological and Chemical Engineering School, Ningbo Institute of Technology, Zhejiang University, Ningbo, China
[2]Hubei Collaborative Innovation Center for Advanced Organochemical Materials, & Ministry-of-Education Key Laboratory for the Synthesis and Applications of Organic Functional Molecules, Hubei University, Wuhan, China
Email: *hyshen@nit.zju.edu.cn

Received 9 January 2015; accepted 26 February 2015; published 3 March 2015



## Abstract

**Carboxylic acid-functionalized nano-sized magnetic composite polymers (COOH-NMPs) were synthesized and used for the preparation of the modified glassy carbon electrode, *i.e.,* COOH-NMPs/GCE and DNA/COOH-NMPs/GCE. The electrochemical behaviors of melamine (MM) were investigated on COOH-NMPs/GCE by cyclic voltammetry (CV) in both cases of DNA in the solution and immobilized on the electrode surface. The electron transfer coefficient ($\alpha$) and the rate constant ($k_s$) kept unchanged in the absence and presence of DNA. Based on the electrochemical properties of the interaction of MM on the surface of the DNA/COOH-NMPs/GCE, a direct method for the determination of MM in liquid milk was established. The detection limit of this method was 2.0 ng·L$^{-1}$, with average recoveries at 95.9% - 104.2% and RSD at 4.5% - 8.2%. The proposed method was provided to have a good accuracy, high stability and good reproducibility with a simple and environmental friendly process. 10 kinds of liquid milk samples bought from the market randomly were tested, and only 1 of them was found at relatively low level of MM residue with the amount of 0.12 ug·L$^{-1}$.**

*Corresponding author.

**How to cite this paper:** Chen, K., *et al.* (2015) Voltammetric Study of the Interaction between Melamine and DNA on a Carboxylic Acid-Functionalized Nano-$Fe_3O_4$ Modified Electrode and Its Analytical Application for Melamine Determination in Liquid Milk Samples. *American Journal of Analytical Chemistry*, **6**, 263-273. http://dx.doi.org/10.4236/ajac.2015.64025

## Keywords

**Carboxylic Acid-Functionalized, Nano-Sized Magnetic Composite Polymers (NMPs), Melamine (MM), Herring Sperm DNA (HS-DNA), Interaction, Analytical Application, Determination of Melamine**

## 1. Introduction

Melamine (MM), with a chemical formula of $C_3H_6N_6$, is an organic base and a trimer of cyanamide, with a 1,3,5-triazine skeleton. It is widely used in industrial as retardants, pigments, colorants and crude materials for resins, etc. [1]. Since it contains 66.6% of nitrogen by mass, MM is sometimes illegally added to food products in order to increase the apparent protein content. After the 2007's pet food incident and the following year's milk powder scandal, great attention has been paid to MM. Various analytical methods are available for the determination of melamine in dairy products [2]-[19]. Conventional determination of melamine is always achieved by high performance liquid chromatography (HPLC) with UV detection (HPLC-UVD) [2]-[5], liquid chromatography/tandem mass spectrometry (LC/MS/MS) [6]-[8], gas chromatography/mass spectrometry (GC/MS) [9], and gas chromatography/tandem mass spectrometry (GC/MS/MS) [10]. In October 2008, the Standardization Administration of China released the national standard for the determination methods for melamine in milk and milk products, GB/T 22388-2008, in which the quantification limits by HPLC-UV or DAD, HPLC-MS/MS and GC-MS or GC-MS/MS methods were 2, 0.01, and 0.05 $mg \cdot kg^{-1}$, respectively [11]. Some other methods reported recently including enzyme-immunoassay [12], electrophoresis [13] [14], Raman spectroscopy [15], electrochemical sensor [16], sweeping-micellar electrokinetic chromatography [17]. All these methods mentioned above are not widely available in ordinary analytical laboratories. The expensive instrumentation and complex operation hinder their application. Besides, analysis of melamine in dairy samples always requires complex sample pretreatment such as solid-phase extraction [18] [19]. And ion-pair reagent is a prerequisite to retain the high polar melamine when reversed-phase liquid chromatography is employed [3] [18]. Therefore, it is still necessary to develop the approaches that can be more easily managed to determining melamine with simple and easy preconcentration procedures.

Recently, DNA-modified electrode has been used for investigating the interactions between DNA and small molecules [20]-[25]. With the rapid development of nano-materials in recent years, synthesis of nano-composites and their application on modified electrodes have been reported continuously [26]-[28]. In this paper, carboxylic acid-functionalized nano-sized magnetic composite polymers (COOH-NMPs) were synthesized first. COOH-NMPs modified glassy carbon electrode (COOH-NMPs/GCE) was fabricated. DNA was covalently immobilized to the COOH-NMPs modified GCE surface with the aid of coupling activator, subsequently. The direct electrochemistry of MM and its interaction with DNA were studied by cyclic voltammetry (CV) on COOH-NMPs/GCE and DNA/COOH-NMPs/GCE. DNA/COOH-NMPs/GCE was further used for the determination of MM in liquid milk. Some favorable results obtained.

## 2. Experimental

### 2.1. Chemicals and Reagents

Melamine (MM) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) with purity of 99%. Herring Sperm DNA (HS-DNA) was purchased from Shanghai Bio Life Science and Technology Co., Ltd. (Shanghai, China). The stock solution of DNA was prepared by dissolving DNA in 0.01 $mol \cdot L^{-1}$ of Tris buffer at pH 7.2 (0.01 $mol \cdot L^{-1}$ of tris(hydroxymethyl)aminomethane (Tris) with NaCl concentration at 0.01 $mol \cdot L^{-1}$) and dialyzing exhaustively against the same buffer for 24 h, and used within 5 days. A solution of DNA gave a ratio of UV absorbance at 260 and 280 nm more than 1.8, indicating that DNA was sufficiently free from protein. The DNA concentration of the stock solution was determined by UV spectrophotometry, in properly diluted samples, using the molar absorption coefficient 6600 $L \cdot mol^{-1} \cdot cm^{-1}$ at 260 nm; the stock solution was stored at 4˚C. A stock solution of MM ($1 \times 10^{-4}$ $mol \cdot L^{-1}$) was prepared by dissolving an appropriate amount of MM in Tris buffer. A fresh working solution was prepared daily by diluting the stock solution with Tris buffer and used

for different studies. Other used chemicals were of analytical reagent grade.

COOH-NMPs with core-shell nanostructures were prepared according to our previous work [29].

### 2.2. Apparatus

The cyclic voltammetry studies were carried out by using LK-2006 electrochemical system (Lanlike Co. Ltd., Tianjing, China). Electrochemical cell consisted of a glass container with a cap having holes for introducing electrodes and nitrogen. The cell was then maintained oxygen free by passing nitrogen over the solution. The reference electrode used was saturated calomel electrode (SCE), while the auxillary was platinum foil electrode and working electrode was glassy carbon electrode (GCE), or COOH-NMPs/GCE and DNA/COOH-NMPs/GCE. In a typical cyclic voltammetric experiment, a stream of nitrogen was passed over them and the reaction mixture was thermostated. The three electrodes were connected to a computer controlled potentiostat and required potential scan rate, current sensitivity, initial potential and final potential were given and the resulting current was measured as a function of applied potential.

The morphology and dimensions of the COOH-NMPs were examined by transmission electron microscopy (TEM) (Hitachi H-7650) at 80 kV. Each sample was prepared by placing a very dilute particle suspension onto 400 mesh carbon grids coated with copper film. Magnetic behavior was analyzed by a vibrating sample magnetometer (VSM) (Lake Shore 7410). The structures of the COOH-NMPs were investigated by an X-ray diffractometer (XRD) (Bruker D8 Advance) at ambient temperature. The instrument was equipped with a copper anode generating Cu Kα radiation ($\lambda$ = 1.5406 Å).

### 2.3. Preparation of DNA/COOH-NMPs/GCE

10 μL 1 mg·L$^{-1}$ COOH-NMPs suspension was cast onto the pretreated GCE and dried in the air. The COOH-NMPs/GCE was immersed in a DNA solution containing 0.01 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide solution (EDC) and 0.01 M N-hydroxysuccinimide solution (NHS) for 24 h. Then the electrode was washed with Tris buffer and dried in the air, finally immersed in water for 4 h to eliminate the excessive salt on the electrode surface.

## 3. Results and Discussion

### 3.1. Characterization of COOH-NMPs

The TEM image of as-prepared COOH-NMPs was shown in **Figure 1**. It could be clearly seen that COOH-NMPs were multi-dispersed with an average diameter of around 150 nm. **Figure 2** showed the XRD analysis of COOH-NMPs as well as the bare $Fe_3O_4$. It indicated that COOH-NMPs had retained the spinel structure of $Fe_3O_4$, in which the identical peaks for $Fe_3O_4$ located at 30.1˚, 35.5˚, 43.1˚, 53.4˚, 57.0˚ and 62.6˚, corresponding to their indices (220), (311), (400), (422), (511) and (400) [30]-[32] appeared. The vibrating sample magnetometer (VSM) was applied to test the magnetic properties of COOH-NMPs as shown in **Figure 3**. The saturation magnetization of the four COOH-NMPs was found to be 10.66 emu·g$^{-1}$.

### 3.2. Electrochemical Sensing Characteristics of the DNA/COOH-NMPs/GCE

Electrochemical sensing characteristics of the DNA/COOH-NMPs/GCE were studied with $K_3Fe(CN)_6$ probe (as shown in **Figure 4**). The redox peak current value on DNA/COOH-NMPs/GCE (**Figure 4(b)**) is lower than that on COOH-NMPs/GCE (**Figure 4(a)**) because of the static repulsion between DNA and $[Fe(CN)_6]^{3-}$. The successful conjugation of DNA on the surface of COOH-NMPs/GCE was proved.

The effective working area of COOH-NMPs/GCE and a bare GCE was calculated according to Equation (1) [26].

$$I_p = 2.69\times10^5 An^{3/2} D_R^{1/2} Cv^{1/2} \quad (1)$$

where, $I_p$ refers to the peak current, $A$ refers to the effective surface area of electrode, $n$ refers to the electron number, $D_R$ refers to the diffusion coefficient, $C$ refers to the concentration of $K_3Fe(CN)_6$, $v$ refers to the scan rates. For $K_3Fe(CN)_6$, $n$ = 1, $D_R = 7.6\times10^{-6}$ cm$^2$·s$^{-1}$ (0.1 mol·L$^{-1}$ KCl), then the surface areas of the bare GCE, COOH-NMPs/GCE and DNA/COOH-NMPs/GCE electrode can be calculated to be 0.033, 0.055 and 0.031 cm$^2$, respectively. It indicated that the bare electrode was modified efficiently.

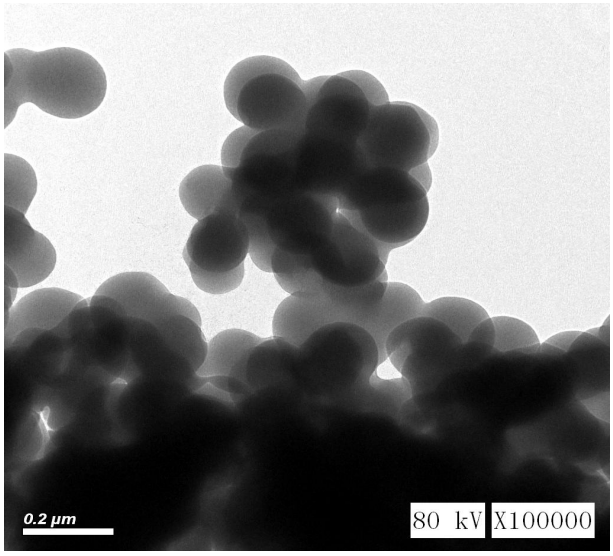



**Figure 1.** TEM of COOH-NMPs.

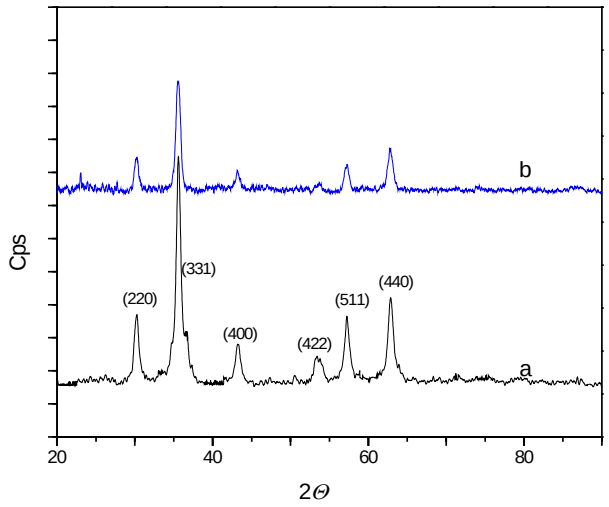



**Figure 2.** XRD spectra of (a) $Fe_3O_4$; (b) COOH-NMPs.

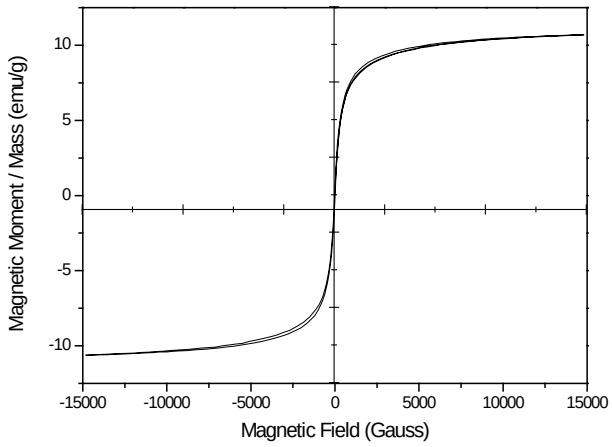



**Figure 3.** VSM of COOH-NMPs.

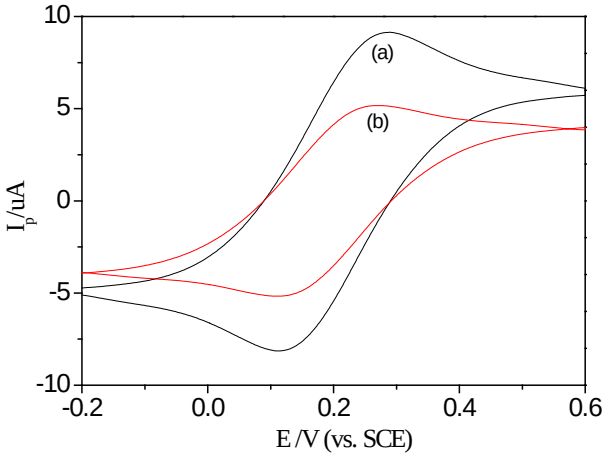



**Figure 4.** Cyclic voltammograms of $1.0 \times 10^{-3}$ mol·L$^{-1}$ $K_3Fe(CN)_6$ at COOH-NMPs/GCE (a) and DNA/COOH-NMPs/GCE (b), Scan rate *v*: 0.05 V·s$^{-1}$.

### 3.3. The Electrochemical Behaviors of MM and Interaction between MM and DNA

Typical cyclic voltammetric curves of the MM with concentration at $1.0 \times 10^{-5}$ mol·L$^{-1}$, carried out in Tris buffer (pH 7.2) on bare GCE and the proposed COOH-NMPs/GCE, were shown in **Figure 5(a)** and **Figure 5(b)**, respectively.

As shown in **Figure 5(a)**, a pair of redox peaks for MM appeared using a bare GCE in the range of −1.5 to +1.5 V (vs. SCE). The cathodic peak potential ($E_{pc}$) was at −0.59 V for MM with a scan rate of 0.1 V·s$^{-1}$, while the anodic peaks for MM was not obvious. The reduction peak current of MM was obviously much higher than the oxidation peak current. This result indicated that the electrochemical process of the MM at a bare GCE was quasireversible. When the CV·scans were conducted on COOH-NMPs/GCE (**Figure 5(b)**), increase in reduction peak currents of MM (from −16.44 μA to −38.96 μA) was observed, which indicated that the bare electrode was modified efficiently by COOH-NMPs.

DNA/COOH-NMPs/GCE was applied to explore the interaction of MM with DNA. Typical cyclic voltammetric curves of the MM with concentration at $1.0 \times 10^{-5}$ mol·L$^{-1}$ in 0.05 mol·L$^{-1}$ Tris (pH 7.2) with a scan rate of 0.1 V·s$^{-1}$ were shown in **Figure 5(c)**. The effect of the immersion time on peak current was investigated by CV and the experimental results showed that the peak current tended to be a constant after 10 min of immersion. The peak current decreased dramatically on the DNA/COOH-NMPs/GCE (from −38.96 μA to −20.97 μA), which implied that the interaction between DNA and MM occurred. This was consistent with the spectrophotometric and electrochemical results obtained in MM and DNA solutions in our previous work [23]-[25].

For kinetic investigation, cyclic voltammetric experiments of the MM on bear GCE, COOH-NMPs/GCE, and DNA/COOH-NMPs/GCE, in 0.05 mol·L$^{-1}$ Tris (pH 7.2), with varied scan rate were recorded and shown in **Figure 6**. It was observed that the reduction peak current ($I_{pc}$) of MM, at all the 3 kinds of situation, varied linearly with scan rate ($v$) rather than $v^{1/2}$. The results were summarized in **Table 1**, which indicated that the electrode processes were controlled by adsorption step [33].

For an adsorption controlled quasireversible electrochemical process, the relationship between the peak current ($I_p$) and the coulomb of adsorption ($Q_a$) obeys the following formula [34]:

$$I_p = n^2F^2A\Gamma v/4RT \quad (2)$$

$$\text{Since,} \quad Q_a = nFA\Gamma \quad (3)$$

$$\text{Then,} \quad I_p = nQ_aFv/4RT \quad (4)$$

where $R$ is the universal gas constant (8.314 J·K$^{-1}$·mol$^{-1}$), $T$ is the Kelvin temperature (T), $F$ is the Faraday constant (96,487 C mol$^{-1}$), $n$ is the number of electrons transferred in reaction, $A$ is the surface area of the working electrode (cm$^2$), $v$ is the scan rate (V·s$^{-1}$), $\Gamma$ is the surface concentration of adsorption (mol·cm$^{-2}$), $Q_a$ is the coulomb in the process of adsorption (C), and $I_p$ is the peak current (A).

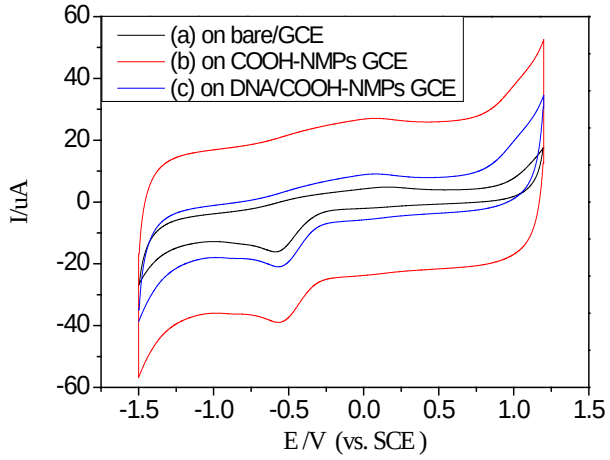
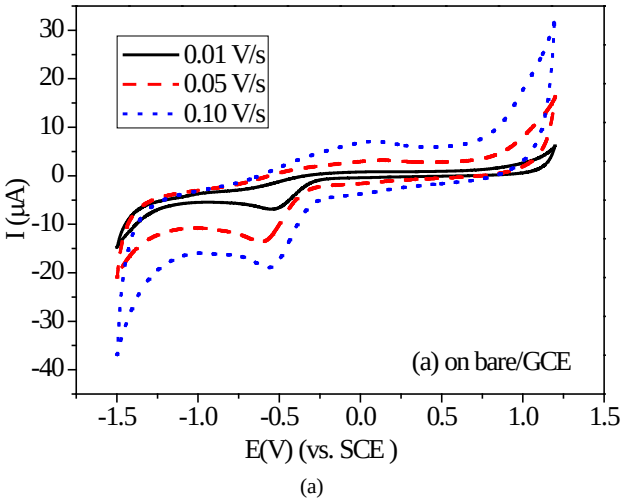
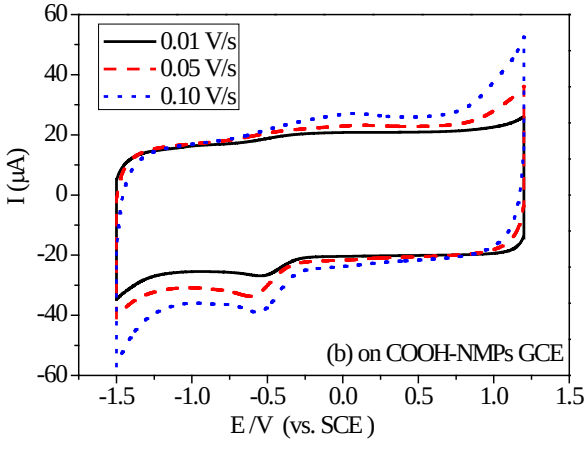


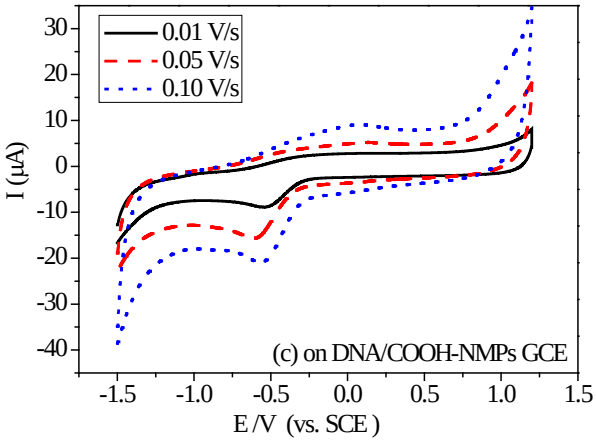

**Figure 5.** Cyclic voltammograms (CV) of MM: (a) on bear GCE; (b) on COOH-NMPs/GCE; (c) on DNA/COOH-NMPs/GCE, in 0.05 mol·L$^{-1}$ Tris (pH 7.2), with a scan rate of 0.1 V·s$^{-1}$.




(a)




(b)




(c)

**Figure 6.** Cyclic voltametry (CV) of MM (a) on bear GCE; (b) on COOH-NMPs/GCE; (c) on DNA/COOH-NMPs/GCE, at different scan rate.

**Table 1.** The relationship between $I_p$ and $v$, the number of electrons transferred per molecule ($n$) of MM on bear GCE, COOH-NMPs/GCE, and DNA/COOH-NMPs/GCE.

| GCE | $v$ (V·s$^{-1}$) | $I_p$ (μA) | $I_p \sim v$ | $Q$ (μC) | $n$ | $n_{av}$ |
|---|---|---|---|---|---|---|
| Bare | 0.01 | −6.84 | $I_p = -130.31v - 6.2297$ $R^2 = 0.9902$ | −35.32 | 1.99 | 2.05 |
| | 0.02 | −9.35 | | −23.55 | 2.04 | |
| | 0.05 | −13.14 | | −12.98 | 2.08 | |
| | 0.08 | −16.73 | | −11.02 | 1.95 | |
| | 0.1 | −18.97 | | −8.94 | 2.18 | |
| COOH-NMPs | 0.01 | −26.85 | $I_p = -130.43v - 25.764$ $R^2 = 0.9889$ | −124.27 | 2.22 | 2.08 |
| | 0.02 | −28.28 | | −71.92 | 2.02 | |
| | 0.05 | −33.08 | | −34.33 | 1.98 | |
| | 0.08 | −35.56 | | −21.54 | 2.12 | |
| | 0.1 | −38.96 | | −19.43 | 2.06 | |
| DNA-COOH-NMPs | 0.01 | −8.84 | $I_p = -130.99v - 7.7944$ $R^2 = 0.9901$ | −46.34 | 1.96 | 2.02 |
| | 0.02 | −10.35 | | −25.08 | 2.12 | |
| | 0.05 | −15.14 | | −15.18 | 2.05 | |
| | 0.08 | −17.73 | | −11.86 | 1.92 | |
| | 0.1 | −20.97 | | −10.56 | 2.04 | |

By integrating the peak areas of the reduction peaks, $Q$ can be obtained, therefore, the number of electrons transferred per molecule ($n$) were calculated as listed in **Table 1** The number of electrons transferred per molecule ($n$) was found to be ~2.

As discussed above, the reduction peak current ($I_{pc}$) of MM varied linearly with scan rate ($v$) rather than $v^{1/2}$, indicating that the electrode process was controlled by an adsorption step. According to Laviron theory [33], for an adsorption controlled process, the relationship between $E_p$ and lg$V$·should obey Equation (5)

$$E_p = E_p^{0\prime} + \frac{2.3RT}{(1-\alpha)nF}\log\frac{RTk_s}{(1-\alpha)nF} - \frac{2.3RT}{(1-\alpha)nF}\log v \quad (5)$$

where $E_p$ is the peak potential (V), $E_p^{0\prime}$ is the formal potential (V), which can be obtained from the intercept of the resulted lines by plotting of $E_p \sim v$ [33] [34], $R$ is the universal gas constant (8.314 J·K$^{-1}$·mol$^{-1}$), $T$ is the Kelvin temperature (T), $F$ is the Faraday constant (96,487 C mol$^{-1}$), $n$ is the number of electrons transferred in reaction, $k_s$ is the standard rate constant (s$^{-1}$), $\alpha$ is the charge transfer coefficient, $v$ is the scan rate (V·s$^{-1}$).

By plotting of $E_p \sim$ lg$v$, the charge transfer coefficient $\alpha$ and the standard rate constant $k_s$ of MM on bear GCE, COOH-NMPs/GCE, and DNA/COOH-NMPs/GCE, can be obtained. The results were listed in **Table 2**. The results showed that the values of $\alpha$ and $k_s$ were not much changed, which is consisted with the findings that the electrode process was controlled by an adsorption step [23]-[25]. These results were consistent with those found in solution in our previous work, in which the interaction systems of MM with DNA were exergonic ($\Delta H < 0$) and entropy favored ($\Delta S > 0$), and the interaction constants in solution were at ~10$^5$ L·mol$^{-1}$ determined by spectrophotometric, spectrofluorometric methods and cyclic voltammetric studies [23]-[25].

### 3.4. Analytical Application

Cyclic voltametric experiments of MM with the varied concentrations on DNA/COOH-NMPs/GCE were carried out. The results showed that the difference of the reduction peak current ($\Delta I_p$) increased linearly with the concentration of the MM in the range of 0.01 - 5.0 μg·L$^{-1}$ (shown in **Figure 7**). The detection limit was 2.0 ng·L$^{-1}$ (three times about the ratio of signal to noise), which was much lower then that reported using multi-wall carbon

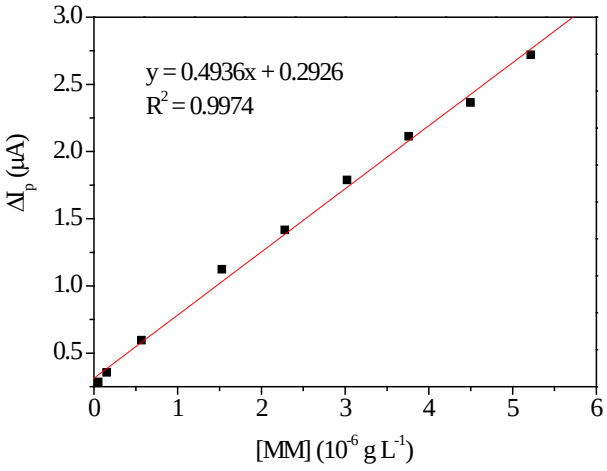



**Figure 7.** The linear relationship between the difference of the reduction peak current ($\Delta I_p$) and the concentration of the MM.

**Table 2.** The main electrochemical kinetic parameters of the MM and MM-DNA systems on bear GCE, COOH-NMPs/GCE, and DNA/COOH-NMPs/GCE.

| System | $E_p \sim v$ | $E_p^{0\prime}$ (V) | $E_p \sim \lg v$ | $\alpha$ | $k_s(s^{-1})$ |
|---|---|---|---|---|---|
| Bare GCE/MM | $E_p = -1.0662v - 0.5237$ $R^2 = 0.9504$ | −0.52 | $E_p = 0.1032\lg v - 0.7222$ $R^2 = 0.986$ | 0.28 | 0.26 |
| Bare GCE/ MM-DNA | $E_p = -0.8309v - 0.6627$ $R^2 = 0.9793$ | −0.66 | $E_p = -0.1244\lg v - 0.8668$ $R^2 = 0.9641$ | 0.24 | 0.28 |
| COOH-NMPs/MM | $E_p = -0.4337v - 0.5284$ $R^2 = 0.9735$ | −0.53 | $E_p = -0.0397\lg v - 0.6073$ $R^2 = 0.98241$ | 0.27 | 0.27 |
| DNA-COOH -NMPs/MM | $E_p = -0.4503v - 0.5288$ $R^2 = 0.9816$ | −0.53 | $E_p = -0.0410\lg v - 0.6103$ $R^2 = 0.9790$ | 0.29 | 0.28 |

nanotube/chitosan composite-modified electrode ($3.0 \times 10^{-9}$ mol·L$^{-1}$, *i.e.*, 3.78 μg·L$^{-1}$) [27]. Thus it can be seen that the modified electrode showed comparatively low detection limit, good sensitivity, rapid response, extreme simplicity, wide linear range over simpler electrodes. Reproducibility and stability of DNA/COOH-NMPs/GCE was also investigated. For 10 successive determinations of 2.5 μg·L$^{-1}$ MM at the same modified electrode, the relative standard deviation (RSD) was 3.9%. Fabrication reproducibility was estimated with five different electrodes, which were constructed by the same procedure, RSD was 6.78%.

The proposed method above mentioned was applied to analyze the concentration of MM in real milk. For the process of verification, MM spiked pure milk samples were prepared at the concentration levels of 0.10, 0.20 and 0.50 μg/L, respectively, where KCl was 0.1 mol·L$^{-1}$. The average recoveries were found to be at 95.9% - 104.2%, with RSD at 4.5% - 8.2%, as listed in **Table 3**, which demonstrating the proposed method had a good accuracy. The proposed method was also a simple and environmental friendly process, because the method did not need complex subsequent procedures like centrifugation and filtration and the use of toxic solvent such as trichloroacetic acid and methanol.

10 kinds of liquid milk samples were bought from the market randomly. The proposed method was used for this study. The result showed that only 1 of the 10 selected samples was found at relatively low level of MM residue at the amount of 0.12 ug/L, which is below the maximum allowed limit in food (2.5 mg·kg$^{-1}$) set by the United Nations' food standards body, Codex Alimentarius Commission and EU [35], as well as 1.0 mg·kg$^{-1}$ for infant formula milk powder in China [11], which implied that more and more attention has been paid to food safety issues, especially on MM. Very few samples were detected containing MM.

## 4. Conclusion

In summary, a carboxylic acid-functionalized nano-sized magnetic composite polymer (COOH-NMPs) was

**Table 3.** The recoveries and precision (RSD value) of the spiked pure milk.

| Spiked concentration (μg/L) n = 5 | 0.10 | 0.20 | 0.50 |
|---|---|---|---|
| Average found concentration (μg/L) | 0.1042 | 0.1972 | 0.4795 |
| Average recovery % | 104.2 | 98.6 | 95.9 |
| RSD % | 8.2 | 6.7 | 3.5 |

prepared and used for the preparation of COOH-NMPs modified glassy carbon electrode (COOH-NMPs/GCE) and DNA/COOH-NMPs/GCE. The electrochemical behaviors of MM and its interaction with DNA were investigated on COOH-NMPs/GCE and DNA/COOH-NMPs/GCE. No obvious change of electrochemical parameters was observed, indicating that MM and DNA formed a non-electroactive complex. The DNA/COOH-NMPs/GCE was used for the determination of MM in liquid milk. The detection limit of this method was 2.0 $ng \cdot L^{-1}$, with average recoveries at 95.9% - 104.2% and RSD at 4.5% - 8.2%. The proposed method had a good accuracy in practical analysis of real milk without complex procedures, which provided simplicity, low-cost, high stability and good reproducibility. 10 kinds of liquid milk samples bought from the market randomly were tested, and only 1 of the 10 selected samples was found at relatively low level of MM residue at the amount of 0.12 ug/L.

## Acknowledgements

We would like to thank the National Natural Science Foundation of Zhejiang Province (LY14B04003), the National Natural Science Foundation of Ningbo (2014A610092), the National College Students' innovation and entrepreneurship training program (201413022011), the Xinmiao Students' innovation training program of Zhejiang Province (2014R401190) for the financial support.

## References

[1] Suchy, P., Strakova, E., Herzig, I., Sta, J., Kalusoca, R. and Pospichalova, M. (2009) Tixicological Risk of Melamine and Cyanuric Acid in Food and Feed. *Interdisciplinary Toxicology*, **2**, 55-59. http://dx.doi.org/10.2478/v10102-009-0010-6

[2] Tan, J., Li, R. and Jiang, Z.T. (2012) Determination of Melamine in Liquid Milk and Milk Powder by Titania-Based Ligand-Exchange Hydrophilic Interaction Liquid Chromatography. *Food Analytical Methods*, **5**, 1062-1069. http://dx.doi.org/10.1007/s12161-011-9350-x

[3] Sun, H., Wang, L., Ai, L., Liang, S. and Wu, H. (2010) A Sensitive and Validated Method for Determination of Melamine Residue in Liquid Milk by Reversed Phase High-Performance Liquid Chromatography with Solid-Phase Extraction. *Food Control*, **21**, 686-691. http://dx.doi.org/10.1016/j.foodcont.2009.10.008

[4] Wang, S.S., Li, D.M., Hua, Z.D. and Zhao, M.P. (2011) Molecularly Imprinted Monolith Coupled On-Line with High Performance Liquid Chromatography for Simultaneous Quantitative Determination of Cyromazine and Melamine. *Analyst*, **136**, 3672-3679. http://dx.doi.org/10.1039/c1an15086c

[5] Wang, X.H., Fang, Q.X., Liu, S.P. and Chen, L. (2012) Preparation of a Magnetic Molecularly Imprinted Polymer with Pseudo Template for Rapid Simultaneous Determination of Cyromazine and Melamine in Bio-Matrix Samples. *Analytical and Bioanalytical Chemistry*, **404**, 1555-1564. http://dx.doi.org/10.1007/s00216-012-6200-7

[6] Braekevelt, E., Lau, B.P.Y., Feng, S., Ménard, C. and Tittlemier, S.A. (2011) Determination of Melamine, Ammeline, Ammelide and Cyanuric Acid in Infant Formula Purchased in Canada by Liquid Chromatography-Tandem Mass Spectrometry. *Food Additives & Contaminants*: *Part A*, **28**, 698-704. http://dx.doi.org/10.1080/19440049.2010.545442

[7] Fang, G.H., Lau, H.F., Law, W.S., Fong, S. and Li, Y. (2012) Systematic Optimisation of Coupled Microwave-Assisted Extraction-Solid Phase Extraction for the Determination of Pesticides in Infant Milk Formula via LC-MS/MS. *Food Chemistry*, **134**, 2473-2480. http://dx.doi.org/10.1016/j.foodchem.2012.04.076

[8] Wu, Y.T., Huang, C.M., Lin, C.C., Ho, W.A., Lin, L.C. and Chiu, T.F. (2009) Determination of Melamine in Rat Plasma, Liver, Kidney, Spleen, Bladder and Brain by Liquid Chromatography-Tandem Mass Spectrometry. *Journal of Chromatography A*, **1216**, 7595-7601. http://dx.doi.org/10.1016/j.chroma.2009.05.027

[9] Xu, X., Ren, Y., Zhu, Y., Cai, Z., Han, J., Huang, B. and Zhu, Y. (2009) Direct Determination of Melamine in Dairy Products by Gas Chromatography/Mass Spectrometry with Coupled Column Separation. *Analytica Chimica Acta*, **650**, 39-43. http://dx.doi.org/10.1016/j.aca.2009.04.026

[10] Miao, H., Fan, S., Wu, Y., Zhang, L., Zhou, P. and Li, J. (2009) Simultaneous Determination of Melamine, Ammelide,

Ammeline, and Cyanuric Acid in Milk and Milk Products by Gas Chromatography-Tandem Mass Spectrometry. *Biomedical and Environmental Sciences*, **22**, 87-94. http://dx.doi.org/10.1016/S0895-3988(09)60027-1

[11] Standardization Administration, the People's Republic of China, GB/T 22388-2008.

[12] Kim, B., Perkins, L.B., Bushway, R.J., Nesbit, S., Fan, T., Sheridan, R. and Greene, V. (2008) Determination of Melamine in Pet Food by Enzyme Immunoassay, High-Performance Liquid Chromatography with Diode Array Detection, and Ultra-Performance Liquid Chromatography with Tandem Mass Spectrometry. *Journal of AOAC International*, **91**, 408-413.

[13] Klampfl, C.W., Andersen, L., Haunschmidt, M., Himmelsbach, M. and Buchberger, W. (2009) Analysis of Melamine in Milk Powder by CZE Using UV Detection and Hyphenation with ESI Quadrupole/TOF MS Detection. *Electrophoresis*, **30**, 1743-1746. http://dx.doi.org/10.1002/elps.200800786

[14] Yan, N., Zhou, L., Zhu, Z. and Chen, X. (2009) Determination of Melamine in Dairy Products, Fish Feed, and Fish by Capillary Zone Electrophoresis with Diode Array Detection. *Journal of Agricultural and Food Chemistry*, **57**, 807-811. http://dx.doi.org/10.1021/jf803429e

[15] Lin, M., He, L., Awika, J., Yang, L., Ledoux, D.R., Li, H. and Mustapha, A. (2008) Detection of Melamine in Gluten, Chicken Feed, and Processed Foods Using Surface Enhanced Raman Spectroscopy and HPLC. *Journal of Food Science*, **73**, T129-T134. http://dx.doi.org/10.1111/j.1750-3841.2008.00901.x

[16] Liang, R., Zhang, R. and Qin, W. (2009) Potentiometric Sensor Based on Molecularly Imprinted Polymer for Determination of Melamine in Milk. *Sensors and Actuators B*, **141**, 544-550. http://dx.doi.org/10.1016/j.snb.2009.05.024

[17] Tsai, I.L., Sun, S.W., Liao, H.W., Lin, S.C. and Kuo, C.H. (2009) Rapid Analysis of Melamine in Infant Formula by Sweeping-Micellar Electrokinetic Chromatography. *Journal of Chromatography A*, **1216**, 8296-8303. http://dx.doi.org/10.1016/j.chroma.2009.06.008

[18] Lutter, P., Savoy-Perroud, M.C., Campos-Gimenez, E., Meyer, L., Goldmann, T., Bertholet, M.C., Mottier, P., Desmarchelier, A., Monard, F., Perrin, C., Robert, F. and Delatour, T. (2011) Screening and Confirmatory Methods for the Determination of Melamine in Cow's Milk and Milk-Based Powdered Infant Formula: Validation and Proficiency-Tests of ELISA, HPLC-UV, GC-MS and LC-MS/MS. *Food Control*, **22**, 903-913. http://dx.doi.org/10.1016/j.foodcont.2010.11.022

[19] Deng, X.J., Guo, D.H., Zhao, S.Z., Han, L., Sheng, Y.S., Yi, X.H., Zhou, Y. and Peng, T. (2010) A Novel Mixed-Mode Solid Phase Extraction for Simultaneous Determination of Melamine and Cyanuric Acid in Food by Hydrophilic Interaction Chromatography Coupled to Tandem Mass Chromatography. *Journal of Chromatography B*, **878**, 2839-2844. http://dx.doi.org/10.1016/j.jchromb.2010.08.038

[20] Sun, Y.J., Liu, R.T., Chi, Z.X., Qin, P.F., Fang, X.Y. and Mou, Y. (2010) Spectroscopic Investigation on the Toxic Interaction of Melamine with Herring Sperm DNA. *Journal of Biochemical and Molecular Toxicology*, **24**, 323-329. http://dx.doi.org/10.1002/jbt.20341

[21] da Silveira, V.C., Benezra, H., Luz, J.S., Georg, R.C., Oliveira, C.C. and da Costa Ferreira, A.M. (2011) Binding of Oxindole-Schiff Base Copper(II) Complexes to DNA and Its Modulation by the Ligand. *Journal of Inorganic Biochemistry*, **105**, 1692-1703. http://dx.doi.org/10.1016/j.jinorgbio.2011.09.016

[22] Shen, H.Y., Shao, X.L., Li, J. and Pan, S.D. (2011) *In Vitro* Study of DNA Interaction with Trichlorobenzenes by Spectroscopic and Voltammetric Techniques. *International Journal of Electrochemical Science*, **6**, 532-547.

[23] Shen, H.Y., Liu, Y.Q., Gao, J., Zheng, H.M., Zhu, N. and Li, J. (2011) *In Vitro* Study of DNA Interaction with Melamine and Its Related Compounds (MARCs). *DNA and Cell Biology*, **30**, 255-264. http://dx.doi.org/10.1089/dna.2010.1095

[24] Shen, H.Y., Zhu, F., Shi, W., Chen, Z. and Pan, Q.Q. (2010) Studies of Interaction between Melamine and Herring Sperm DNA. *Acta Chimica Sinica*, **68**, 1719-1725.

[25] Shen, H.Y., Zhang, Y., Lin, F., Xu, N.Y. and Zheng, H.M. (2012) *In Vitro* Study on the Interactions between Native Herring Sperm DNA and Melamine in the Presence of $Ca^{2+}$ by Spectroscopic and Voltammetric Techniques. *International Journal of Electrochemical Science*, **7**, 3817-3834.

[26] Bian, L., Zeng, Q.X., Yang, L.J., Xiong, H.Y., Zhang, X.H. and Wang, S.F. (2011) Voltammetric Studies of the Interaction of Rutin with DNA and Its Analytical Applications on the MWNTs-COOH/$Fe_3O_4$ Modified Electrode. *Sensors and Actuators B*, **156**, 615-620. http://dx.doi.org/10.1016/j.snb.2011.02.007

[27] Zhao, T.K., Liu, L.H., Li, G.M., Dang, A.L. and Li, T.H. (2012) Electrochemical Determination of Melamine with a Glassy Carbon Electrode Coated with a Multi-Wall Carbon Nanotube/Chitosan Composite. *Journal of the Electrochemical Society*, **159**, K141-K145. http://dx.doi.org/10.1149/2.065205jes

[28] Yardim, Y., Keskin, E., Levent, A., Ozsoz, M. and Senturk, Z.S. (2010) Voltammetric Studies on the Potent Carcinogen, 7,12-dimethylbenz[a]anthracene: Adsorptive Stripping Voltammetric Determination in Bulk Aqueous Forms and Human Urine Samples and Detection of DNA Interaction on Pencil Graphite Electrode. *Talanta*, **80**, 1347-1355.

http://dx.doi.org/10.1016/j.talanta.2009.09.035

[29] Shen, H.Y., Zhao, Y.G., Hu, M.Q. and Xia, Q.H. (2009) Preparation and Adsorption Properties of Polycarboxylate Nano-$Fe_3O_4$ Magnetic Composite Particles. *Acta Materiae Compositae Sinica*, **26**, 68-73.

[30] Pan, S.D., Shen, H.Y., Xu, Q.H., Luo, J. and Hu, M.Q. (2012) Surface Mercapto Engineered Magnetic $Fe_3O_4$ Nanoadsorbent for the Removal of Mercury from Aqueous Solutions. *Journal of Colloid and Interface Science*, **365**, 204-212. http://dx.doi.org/10.1016/j.jcis.2011.09.002

[31] Zhao, Y.G., Shen, H.Y., Pan, S.D. and Hu, M.Q. (2010) Synthesis, Characterization and Properties of Ethylenediamine-Functionalized $Fe_3O_4$ Magnetic Polymers for Removal of Cr(VI) in Wastewater. *Journal of Hazardous Materials*, **182**, 295-302. http://dx.doi.org/10.1016/j.jhazmat.2010.06.029

[32] Pan, S.D., Zhang, Y., Shen, H.Y. and Hu, M.Q. (2012) An Intensive Study on the Magnetic Effect of Mercapto-Functionalized Nano-Magnetic $Fe_3O_4$ Polymers and Their Adsorption Mechanism for the Removal of Hg(II) from Aqueous Solution. *Chemical Engineering Journal*, **210**, 564-574. http://dx.doi.org/10.1016/j.cej.2012.09.016

[33] Bonjean, K., de Pauw-Gillet, M.C., Defresne, M.P., Colson, P., Houssier, C., *et al*. (1998) The DNA Intercalating Alkaloid Cryptolepine Interferes with Topoisomerase II and Inhibits Primarily DNA Synthesis in B16 Melanoma Cells. *Biochemistry*, **37**, 5136-5146. http://dx.doi.org/10.1021/bi972927q

[34] Zhong, W., Yu, J., Liang, Y., Fan, K. and Lai, L. (2004) Chlorobenzylidine-Calf Thymus DNA Interaction II: Circular Dichroism and Nuclear Magnetic Resonance Studies. *Spectrochimica Acta Part A*, **60**, 2985-2992. http://dx.doi.org/10.1016/j.saa.2004.02.012

[35] World Health Organization. International Experts Limit Melamine Levels in Food. http://www.who.int/mediacentre/news/releases/2010/melamine_food_20100706/en/index.html