

Electrochemical Determination of Melamine on Static Mercury Drop Electrode and on Gold Nano Particle Modified Carbon Paste Electrode

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Abstract

An electrochemical method was developed for the determination of melamine (2,4,6 triamino-1,3,5-triazine) on static mercury drop electrode (SMDE) and on gold nano particle modified carbon paste electrode. Interaction of melamine with Cu^{2+} ion was investigated and the decrease of Cu stripping peak was measured for the determination of melamine using SMDE. Oxidation property of melamine was improved using gold nano particle modified carbon paste electrode. Direct determination of melamine was proposed from the oxidation peak with three sigma detection limit of $0.43 \mu\text{ML}^{-1}$. Recovery in milk powder sample has shown good recovery of melamine using the modified carbon paste electrode.

Keywords

Gold Nano Particles (AuNPs), Carbon Paste Electrode, Melamine, Voltammetry

1. Introduction

Melamine is a nitrogen rich compound with around 66% of nitrogen, which has attracted its use as adulterant in milk and milk products. Melamine is reported to be toxic and damages kidney by formation of needle like crystal when it combines with cyanuric acid. It has lots of industrial uses like, in packaging industry, synthesis of resins and in fire retardant products. Thus, it has been a concern to determine melamine in milk and milk related samples and in water sample. There are some reports of determination of melamine based on chromatography,

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[1]-[3] colorimetry [4] [5] and electrochemistry [6]-[8]. Most of the electrochemical procedures reported are indirect determination, based on the variation of electrochemical signal of any other electrochemically active compound in presence of melamine. Cao *et al.* [6] have reported the decrease in the ferricyanide signals due to the presence of melamine on oligonucleotide modified gold electrode for the determinations of melamine. Liao *et al.* [7] have reported the oxidation of uric acid as the recognition signal on screen printed electrode for the determination. In another report, electrochemical signal of ascorbic acid was used as the recognition signal in carbon nano tube (CNT) modified electrode for the determination [8]. Response from the direct electron transfer process in melamine is quite poor [9]. In the present investigation SMDE was used for the indirect determination of melamine in which the change in the stripping current due to the interaction between Cu^{2+} and melamine was used for the measurements. Carbon paste electrode was modified with AuNPs and it was used for the direct electrochemical signal generation from melamine. Recently there is an article on the determination of melamine from the decrease in the electrochemical signal from potassium ferrocyanide on gold nano particle modified indium tin oxide (ITO) electrode [10]. Determination of melamine using electrochemical techniques is becoming popular [11] [12]. Melamine was determined from the reduction signal of bismuthyl chloride by pre-concentrating it on a carbon composite electrode by solid phase micro-extraction [11] [13]. It was also determined in quail egg and milk samples by capillary zone electrophoresis [12] [14]. In the present study, gold nano particle modified electrode is used but the measurements are based on direct oxidation of melamine which is the primary objective of the present work. Easy renewability of the carbon paste electrode in the present case has the additional advantage to use the method in regular analysis.

2. Experimental

2.1. Instrumentations and Procedures

Electrochemical studies were carried out using the cell assembly with a three-electrode configuration consisting of the SMDE or modified carbon paste electrode as the working electrode and saturated calomel electrode (SCE) as the reference electrode. The potentials applied, measured and reported in this study were with respect to the SCE reference electrode. A platinum rod served as the counter electrode. All the experiments were carried out at room temperature (298 K). Electrochemical measurements were performed using an Eco Chemie Potentiostat/Galvanostat, Autolab-302N; the data acquisition and analysis were carried out by GPES 4.9 software. For the measurements with SMDE, 663 VA stand from Metrohm was used. Prior to the electrochemical scan, voltammetric solution was purged with high purity nitrogen gas for 10 min.

2.2. Reagents and Solutions

Melamine was purchased from E. Merck India Ltd. Acetic acid, boric acid and phosphoric acid were also of E Merck A R Grade. Auric Chloride was of high purity BDH Aristar grade. Ultra pure water was used for preparation of all the solutions.

3. Results and Discussion

Electroanalytical method was developed for the determination of melamine in two ways first by using SMDE as the working electrode and second by using modified carbon paste electrode and the methods were described in the following sub-sections.

3.1. Determination of Melamine Using SMDE Using It's Interaction with Cu^{2+}

Melamine has strong interaction with Cu^{2+} , which generates electrochemical signals. These electrochemical signals could be a measure of melamine concentration [9]. However a clear electrochemical signal for quantitative detection of melamine was not attempted previously. We have explored for the quantitative determination of melamine by monitoring the stripping signal of copper in linear sweep stripping voltammetry using SMDE. Anodic stripping voltammetry of copper was carried out with deposition potential of -1.0 V for 10 s and then the potential was scanned from -1.0 V to 0.2 V. The stripping peak due to the oxidation of Cu was observed at around 0 V as shown in **Figure 1**. Melamine was gradually added in the test solution containing $3.3 \mu\text{M}$ of Cu^{2+} . During the electrochemical scan Cu was stripped into the solution as Cu^{2+} ion. The original Cu stripping peak current was gradually decreased with the addition of melamine and a new pre-peak at more negative potential

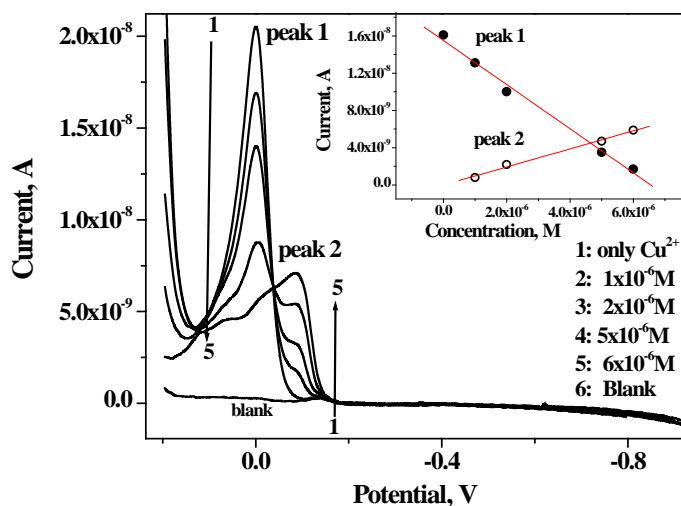


Figure 1. Linear sweep anodic stripping voltammetry of 3.3 μM⁻¹ of Cu²⁺ with different concentration of melamine. Deposition potential was -1.0 V for 10 s. Inset: corresponding linear calibration plot of peak 1 and peak 2.

to that of the Cu stripping peak was appeared. A calibration plot was constructed from the decrease in the stripping peak of copper which was shown in the inset of **Figure 1** (peak 1). The calibration plot followed the linear regression equation $i_p = 1.55 \times 10^{-8} - 2.37 \times 10^{-3} C$ with correlation coefficient of 0.998 and standard deviation of 6.15×10^{-10} , C in molar concentration. The three sigma detection limit from the linear calibration plots was obtained as $0.778 \mu\text{M L}^{-1}$. For indirect determination of melamine this measurement was most workable procedure to determine melamine as it is very easy to obtain reproducible mercury drop for regular analysis. The new peak which was appeared at -0.09 V was found to be increased proportionately with the melamine concentration, corresponding linear calibration plot was shown in the inset of **Figure 1** (peak 2) The calibration plot followed the linear regression equation as $i_p = -7.05 \times 10^{-12} + 9.71 \times 10^{-4} C$ with correlation coefficient of 0.996 and standard deviation of 2.503×10^{-10} , C in molar concentration. The three sigma detection limit from the linear calibration plots was obtained as $0.773 \mu\text{M L}^{-1}$. It was observed that both the decrease in the stripping peak and the increase in the pre-peak generated similar proportionality and the detection limits obtained from both the ways were similar. This indicated that the type of interaction responsible for the decrease in the stripping current of Cu and generation of a new pre-peak was the same. Though melamine could be detected indirectly by monitoring the stripping signal of Cu, our aim was to detect the direct oxidation signal of melamine. Thus we have investigated further to measure the oxidation signal directly generated from the oxidation in the melamine centre. Corresponding results were discussed in the following sub-section.

3.2. Determination of Melamine from Its Direct Oxidation on Modified Carbon Paste Electrode

Electro-oxidation of melamine produces poor electrochemical response. Previous reports claimed at weak electrochemical response due to the oxidation of melamine, which was observed only at neutral to alkaline solutions [9]. Gold has strong interaction with melamine [13]-[16]. However the oxidation peak due to melamine was not observed on bulk gold electrode. Thus, AuNPs modified electrode was preferred for the investigation with the aim to improve the electrochemical response of melamine, especially its oxidation signal. Carbon paste was used as the substrate due to the advantage of easy renewability on regular applications. Carbon paste electrode was prepared in our laboratory and procedure was followed as reported by the previous reports and also the reports from our group [17]-[22]. Carbon paste electrode was modified with AuNPs by electrodeposition method. Electrodeposition of AuNPs over carbon paste electrode surface was carried out from auric chloride solution of 1 mM concentration. Deposition was carried out at -0.3 V for 300 s. Deposits were characterized using SEM; corresponding micrograph was shown in **Figure 2**. Sizes of most of the deposits were in the range of 100 to 120 nm with some occasional smaller deposits of around 30 nm.

This AuNPs modified carbon paste electrode was used for melamine oxidation, corresponding square wave voltammetric plots were shown in **Figure 3**. Schematic representation of the oxidation process of melamine on AuNPs modified carbon paste electrode was shown in **Scheme 1**. Voltammetric signal was increased with increase in the melamine concentration in the test solution. Calibration plot was shown in the inset of the **Figure 3**. Two calibration regions were observed one at low and the other at high concentrations. Three sigma detection limits using this procedure was obtained as $0.43 \mu\text{ML}^{-1}$. Present method was free from interferences from the metal ions like, Cu^{2+} , Ag^{1+} , Pb^{2+} , Bi^{3+} , U^{6+} , and Hg^{2+} at 100 fold higher concentration than melamine. This is the biggest advantage of detection of melamine using its direct oxidation signal. In the case of indirect determination using Cu stripping signal the method has the problem interference due to the presence of Ag^{1+} , Pb^{2+} and Bi^{3+} at 10 times higher concentrations.

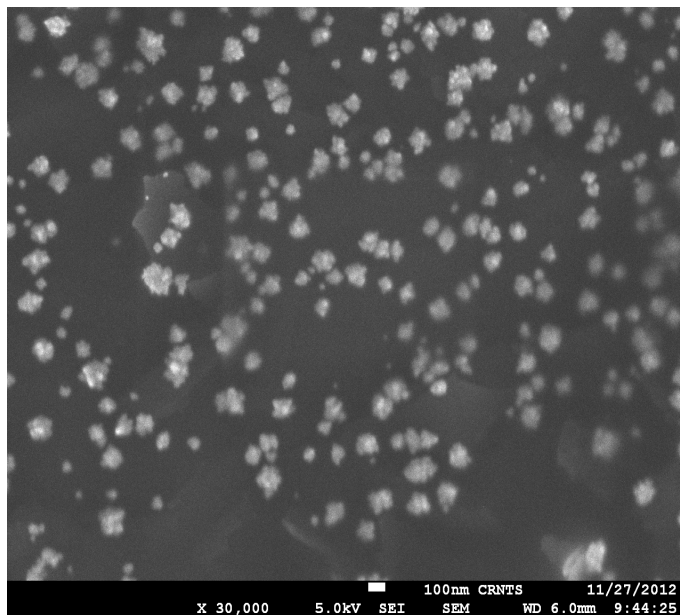


Figure 2. SEM micrograph of gold nano particles electrodeposited on carbon paste electrode substrate.

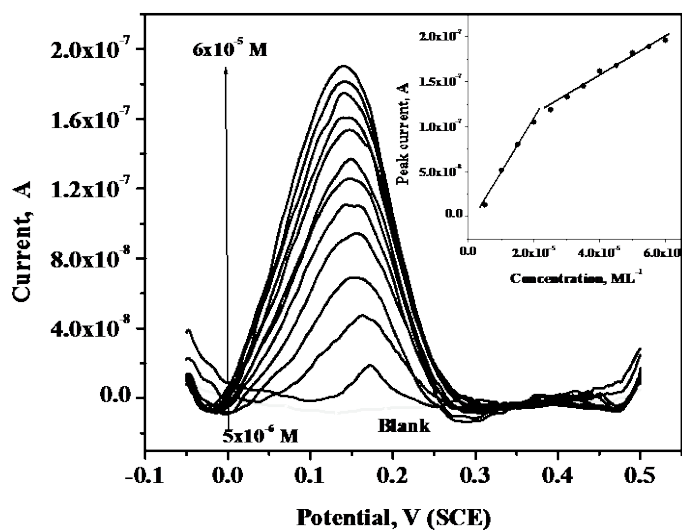
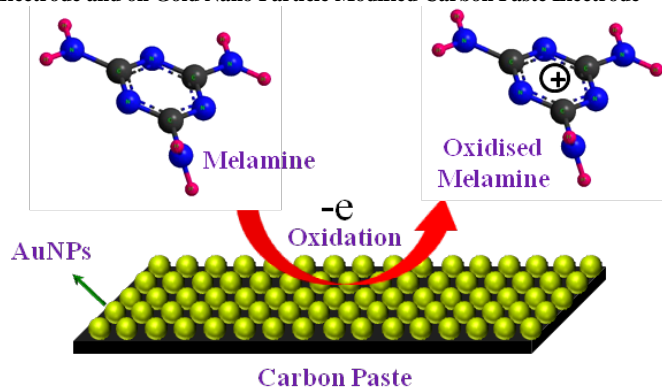


Figure 3. Voltammogram of melamine on AuNPs modified carbon paste electrode with successive addition of $0.5 \mu\text{M}$ melamine. Inset: Corresponding calibration plot.

Electrochemical Determination of Melamine on Static mercury Drop Electrode and on Gold Nano Particle Modified Carbon Paste Electrode



Scheme 1. Schematic representation of the oxidation of melamine over AuNPs modified carbon paste electrode.

Table 1. Recovery test result of melamine in milk sample using gold nano particle modified carbon paste electrode.

Melamine added	Melamine recovered	% Recovery
2.0 μML^{-1}	2.2 μML^{-1}	110%
5.0 μML^{-1}	5.4 μML^{-1}	108%

Recovery test was carried out by adding known amount of melamine in milk powder sample. Milk sample was treated by taking 1 g of milk powder mixing with 5 mL 0.1 ML^{-1} CaCl_2 , then 5 mL of 0.5 ML^{-1} trichloroacetic acid and 35 mL of 5 ML^{-1} methanol was added. After 10 min of ultra-sonication, this mixture was centrifuged and the solution was filtrated, through a 0.45 μm membrane to obtain the samples for detection of melamine [23]. Electrochemical measurements were carried out using this filtrate. Recovery test results were shown in **Table 1**. Recovery test result has shown good recovery of melamine in milk sample, recovery within 10%.

4. Conclusion

We have developed analytical methods for the determination of melamine in two ways. On SMDE the advantage was the very easy regeneration of the electrode using Metrohm VA 663 stand. This method is useful for the determination of melamine in the laboratory. However, this procedure was based on the indirect measurements using the interaction of melamine with Cu^{2+} . Oxidation response due to melamine was enhanced with the use of gold nano particle modified carbon paste electrode. Monitoring the oxidation signal has produced a high sensitive method of determination of melamine with three sigma detection limit as 0.43 μML^{-1} . Measurements based on the AuNPs modified carbon paste electrode have the potential to use as potable sensor of melamine and for field application.

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