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Electrochemical behaviors and determination of melamine in neutral and acid aqueous media

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Abstract The electrochemical behaviors of melamine (MEL) were studied at paraffin-impregnated graphite electrode in PBS (pH 7.0) and 0.5 M H_2SO_4. Various methods including UV–vis thin-layer spectroelectrochemistry, infrared spectra (IR) and electrochemicatry have been performed to investigate the characteristics. In 0.1 M PBS (pH 7.0), MEL loses two electrons to form a dication, which couples head-to-head with a neutral molecule of MEL to form a dimer accompanying the production of azocompound, the dimer plays a role of a monomer in the following polymerization. In 0.5 M H_2SO_4, unstable MEL mostly hydrolyzes to form ammeline, ammelide, s-triazine-2,4,6-trion, and tricyanic acid, respectively; The hydrolysis could be accelerated by electrochemical method; Meanwhile, MEL associates tricyanic acid to give a plane molecule cake by hydrogen bonding. The spectra responses of MEL at 205 and 234 nm are linearly increasing in a same concentration range of 1.0×10^{-7}–1.0×10^{-5} M in 0.5 M H_2SO_4 (determination limit, 1×10^{-8} and 3×10^{-8} (3σ)). The proposed method was successfully applied to the determination of MEL in real sample.

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Introduction

Recently researches have proved that combination from melamine (MEL) and cyanuric acid could cause the formation of insoluble crystals in the kidneys of cats [1, 2] and infant [3]. Most countries have prohibited the addition of MEL in child and pets's food; it was presumed that MEL was still deliberately added to increase the measured nitrogen content of diluted dairy products according to Kjeldahl testing [4]. For example, pet food ingredients contaminated by MEL and its analogues resulted in a major outbreak of renal disease and associated deaths in cats and dogs in the USA in March of 2007 [2]. High concentration of MEL was reported in contaminated Chinese infant formula in September of 2008. More than 51,900 infants and young children in China were hospitalized for urinary problems, possible renal tube blockages, and kidney stones due to the consumption of MEL-contaminated infant formula and related dairy products [2].

Various methods have been applied to the determination of MEL. The methods involved high-performance liquid chromatography (HPLC) [5], liquid chromatography/tandem mass spectrometry (LC/MS/MS) [2, 6], gaschromatography/mass spectrometry (GC/MS) [3], colorimetry [7], and electrochemical analysis [8]. Among these, the electrochemical method is interesting in terms of facility and economy. But the reports are rare, Zhao et al. revealed that MEL might interact with oligonucleotides mainly through electrostatic and hydrogen bonding using electrochemical probe of ferricyanide. The interactions can lead to the increase in the peak currents of ferricyanide, which could be used for MEL sensing [8]. Qi et al. report a potentiometric sensor based on molecularly imprinted polymer for the determination of MEL in milk [9]. Zhu et

al. developed a rapid, simple and sensitive electrochemical sensor based on Cu–MEL complex [10].

In our opinion, the electrochemical study of MEL has not been achieved so far. A thorough investigation is significant in terms of its electrochemical mechanism and determination. In present work, we revealed the electrochemical behaviors of MEL at paraffin-impregnated graphite electrode (WGE) in PBS (pH 7.0) and 0.5 M H_2SO_4. The determination of MEL was performed by spectrumelectrochemical method.

Experiment

Apparatus

All electrochemical experiments were performed with a CHI 660B electrochemical workstation (Chenhua, Shanghai, China). A conventional three-electrode electrochemical system was used for all electrochemical experiments, which consisted of a working electrode, a twisted platinum wire counter electrode and a saturated calomel reference electrode (SCE). A homemade WGE with formal surface area of 0.125 cm^2 was used as the basal working electrode. All potentials reported are versus SCE. UV–vis thin-layer spectroelectrochemistry was performed as described previously at 401PC spectrophotometer (Shimadzu, Japan) [11, 12]. Infrared spectra were measured at IR 200 (America, Nicolet).

Chemicals and solutions

MEL, trichloroacetic acid, and methanol were obtained from Chemical Reagent Company of Shanghai (China). All other reagents used were of analytical grade. Phosphate-buffered saline (0.1 M PBS 7.0) solutions were prepared by mixing two stock solutions of 1 M KH_2PO_4 and K_2HPO_4. Solutions of 0.01 M MEL/0.5 M H_2SO_4 and 0.01 M MEL/0.1 M PBS

were prepared. All aqueous solutions were prepared in doubly distilled, deionized water.

Procedure

UV–vis thin-layer spectroelectrochemistry measurements were carried out in a 0.01 M MEL/0.5 M H_2SO_4 or 0.01 M MEL/0.1 M PBS (pH 7.0). A 1-ml volume of MEL solution was injected into the thin-layer compartment of the cell through its reference electrode chamber. In this way, the solution entered the outer cell and removed any air bubbles in the thin-layer compartment. The UV–vis absorption spectra were measured with a similar cell put in the reference beam of the spectrophotometer. After the thin-layer solution was electrolyzed at a steady potential for 5 min, each spectrum was measured. All measurements were performed at room temperature.

Results and discussion

Electrochemical behaviors of MEL in PBS (7.0)

Figure 1a shows the cyclic voltammetry (CV) of MEL at WGE in 0.01 M MEL/0.1 M PBS (7.0). There are a pair of peaks for the first (0.35/−0.13 V, a), one cathodic peak and reoxidation peaks (0.06/−0.13 V) for the second (a and b), which are similar to the electrooxidation of aromatic aniline compounds [11, 13]. MEL loses two electrons to form a dication (0.35 V) during oxidation, which undergoes head-to-head coupling with a neutral molecule of MEL with simultaneous removal of two protons to form azocompound (−0.13 V); This dimer could be reoxidized matching to the peak (0.06 V); Meanwhile with the continuous formation of dimer, it probably plays a role of a monomer in the following polymerization after 30 cycles (b and c) [11, 13], as a result a new pair of peaks can be observed at 0.52/0.24 V. An electrochemistry-chemistry-electrochemistry process (ECE)

Fig. 1 **a** Cyclic voltammetries (CVs) of 0.01 M melamine (MEL) on paraffin-impregnated graphite electrode (WGE) at the first and the second (*a*), the 30th (*b*), and the 90th (*c*) cycle in 0.1 M PBS (pH 7.0). **b** CVs of the MEL-modified WGE at the first (*a*), the 30th (*b*), and the hundredth in blank 0.1 M PBS (pH 7.0). Scan, 50 mV S^{-1}
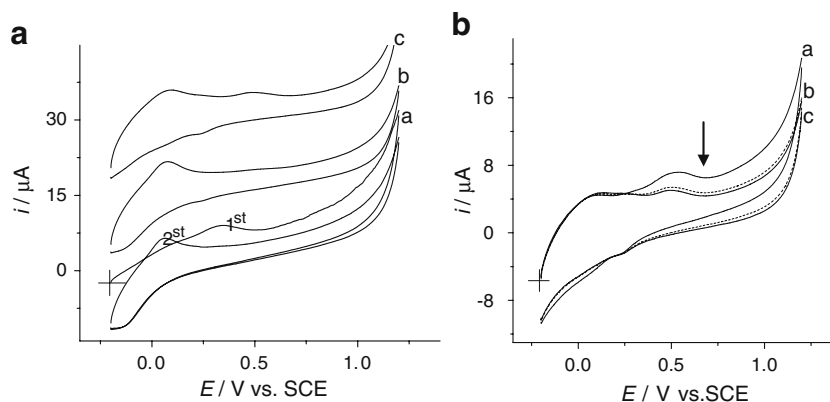
could be suggested in Scheme 1. Figure 1b shows the CVs of the polymelamine-modified WGE (MEL/WGE) in blank 0.1 M PBS (pH 7.0), the peaks can still be seen after nonstop 200 scanning cycle to remove simple adsorption, suggesting a stable modification. Scheme 1 could be proved by following the experiments further.

Figure 2a shows the UV–vis thin-layer spectroelectrochemical absorption spectra of 0.1 mM MEL for 0 (a), tenth (b), 50th (c), and 90th (d) cycle in 0.1 M PBS (pH 7.0). The spectra curves show two absorption peaks at 202 and 235 nm related to the electronic transition of MEL (a) [14]. Three new peaks at 265, 365 and 450 nm can be observed after repeated cycle for ten cycles (b), which involve the electronic transition of −N=N− [11, 14, 15], the peak at 450 nm matches to the azo bond in the electrooxidation product substituted azocompounds [15]. It is notable that the peaks at 235, 270, 365, and 450 nm are increase with the decrease of peak at 202 nm in continuous potential scanning, suggesting the formation of azocompounds (c and d).

The inset in Fig. 2a shows the IRs of untreated MEL (a); 0.01 M MEL/0.1 M PBS (pH 7.0) before (b), after repeated 300 cycles on WGE (c), and in 0.1 M PBS (pH 7.0) (d). The double peaks at 3,468 and 3,418 cm^{-1} are ascribed to the symmetrical and asymmetrical stretching vibrations of NH_2 group in MEL molecules (a). The absorptions at 1,650–1,437 cm^{-1} are caused by vibration of the framework in MEL molecules. The peak at 810 cm^{-1} is relative to the out-of-plane ring deformation [16]. The absorptions at 3,468–3,418 cm^{-1} and 1,650–1,437 cm^{-1} are overlapping before repeated cycle (b). An obvious increase at 1,640 cm^{-1} could be seen in curves c and d compared with that in curve b after repeated 300 cycles, the reason probably related to the overlapping of the stretching

vibrations of −N=N− and amide-I [11, 14]. Moreover, there is no obvious change for other spectrums in curves c and d.

Electrochemical behaviors of MEL in H_2SO_4

Figure 3 shows that the WGE was conducted under CV conditions in 0.5 M H_2SO_4 (a) and 0.01 M MEL/0.5 M H_2SO_4 for the first (b), second (c), third (d), and fourth cycle (e). There is not any peak at bare WGE (a). However, a pair of peaks (1.45/0.65 V, b) can be seen in 0.01 M MEL/0.5 M H_2SO_4 in the first cycle, then, a new reoxidation peak (0.70 V, b) was observed except the pair of peaks in the second cycle. The pair of peaks (0.70/0.65 V) are gradually enhanced even up to the 90th cycle (no show). The phenomena are different from the result in Fig. 1a. The reason could be as follows: MEL is oxidized to form s-triazine-2,4,6-trion at a higher potential (1.45 V) firstly, which is reduced to form tricyanic acid (0.65), then tricyanic acid can be reoxidized at a lower potential to give s-triazine-2,4,6-trion (0.70 V, reaction 1) [13]. Actually, MEL is unstable in 0.5 M H_2SO_4, which can be hydrolyzed to form ammeline, ammelide, tricyanic acid, and s-triazine-2,4,6-triol (reaction 2). Meanwhile, MEL probably associates tricyanic acid to form "plane molecule cake" by hydrogen bonding [17]. Therefore, an ECE can be suggested in Scheme 2. The pair of peaks (0.70/0.65 V) gradually fades out after nonstop 40 cycle in blank 0.5 M H_2SO_4 in the inset in Fig. 3, implying an unstable modification. Moreover, it is noticed that some small insoluble crystals gradually appear in successive cycle in 0.5 M H_2SO_4.

The UV–vis thin-layer spectroelectrochemistry of MEL was performed to prove the Scheme 2 in 0.5 H_2SO_4

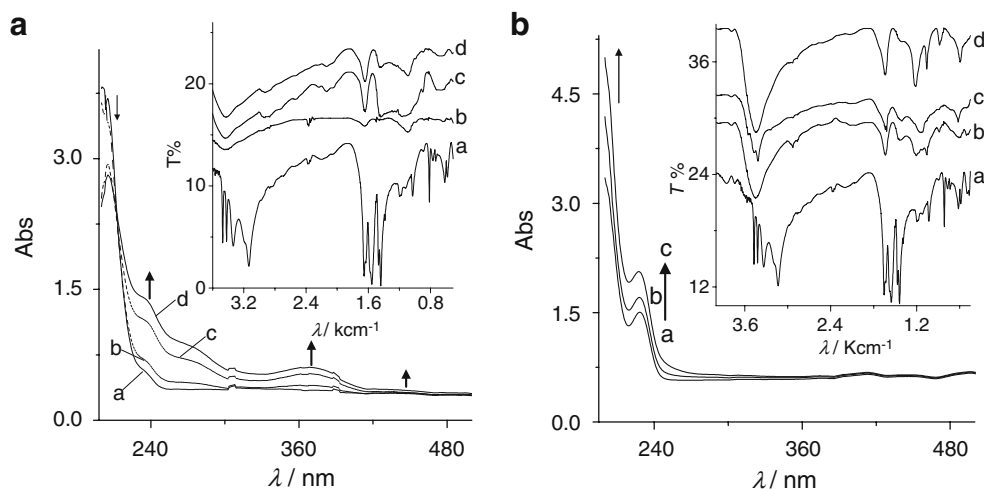Scheme 1 MEL in 0.1 M PBS (pH 7.0)
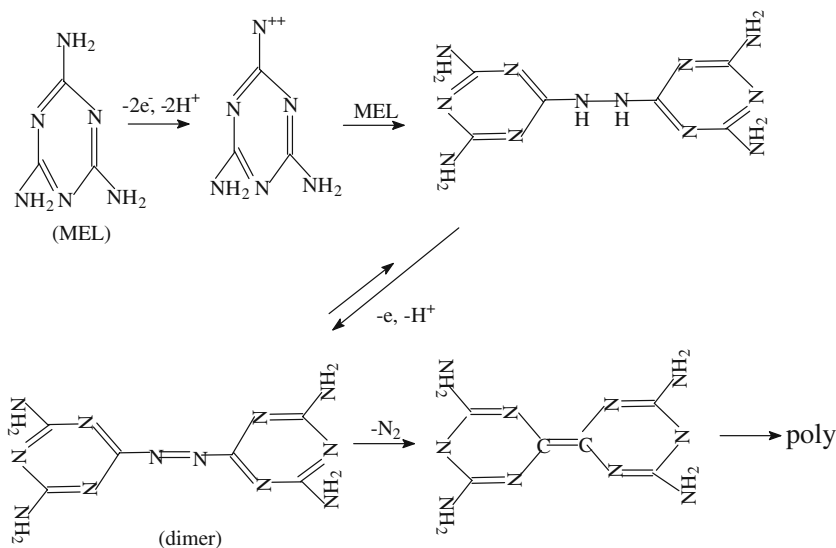
Fig. 2 **a** UV–vis thin-layer spectroelectrochemical absorption spectra of 0.1 mM melamine (MEL) cycled at paraffin-impregnated graphite electrode (WGE) for 0 (*a*), tenth (*b*), 50th (*c*), and 90th (*d*) cycles. *Inset*, IR of untreated MEL (*a*); of 0.01 M MEL before (*b*), and after repeated 300 cycles on electrode (*c*) and in solution (*d*). Buffer, 0.1 M

PBS (pH 7.0). **b** UV–vis thin-layer Spectroelectrochemical absorption spectra of 0.01 M MEL cycled at WGE for 0 (*a*), ten (*b*), and 50 (*c*) cycles. *Inset*, IR of untreated MEL (*a*); of 0.01 M MEL before (*b*), and after repeated 300 cycles on electrode (*c*) and in solution (*d*). Buffer, 0.5 M H_2SO_4

(Fig. 2b), There are two absorption peaks at 202 and 230 nm (a) before the oxidation [13]. After repeated cycle from ten (b) to 50 (c) cycles, the maximal adsorption peak at 202 nm shifts to 205 nm due to the formation of tricyanic acid and s-triazine-2,4,6-triol, the other at 230 nm shifts to 227 nm with the production of ammeline [17]; they are increasing with continuous scan; it is probably attributed to a stimulative effect of CVs towards the hydrolysis of MEL.

The inset in Fig. 2b shows the IR of untreated MEL (a); 0.01 M MEL before (b) and after repeated 300 cycles on

electrode (c), and in 0.5 M H_2SO_4 (d). The absorptions at 3,468–3,418 and 1,650–1,437 cm^{-1} are overlapping (b) compared with that of untreated MEL (a). In comparison to that of curve b, some obvious shifts from 1,650 to 800 cm^{-1} can be found in continuous 300 cycles (d). These shifts include the change from 1,650 to 1,640 cm^{-1}due to the stretching vibrations of −C=O, and 810 to 870 cm^{-1} due to the association of MEL and tricyanic acid. There are two new peaks at 1,208 and 1,060 cm^{-1}, the former one relates to the characteristic absorptions of the traditional synthesis of melamine cyanurate [18], and the latter one relates to the stretching of −COH [19]. All the changes mentioned above indicate that MEL probably interacts with tricyanic acid by hydrogen bonding or electrical charge attraction. Moreover, the peaks at 1,200–1,040 cm^{-1} in curve c become over-lapping compared with that in curve d. It illustrates an advantage of acid media towards the formation of tricyanic acid accompanying the association of MEL and tricyanic acid [14, 15].
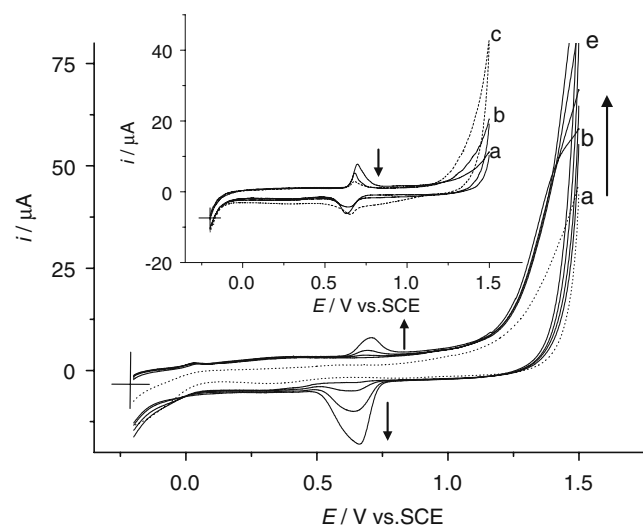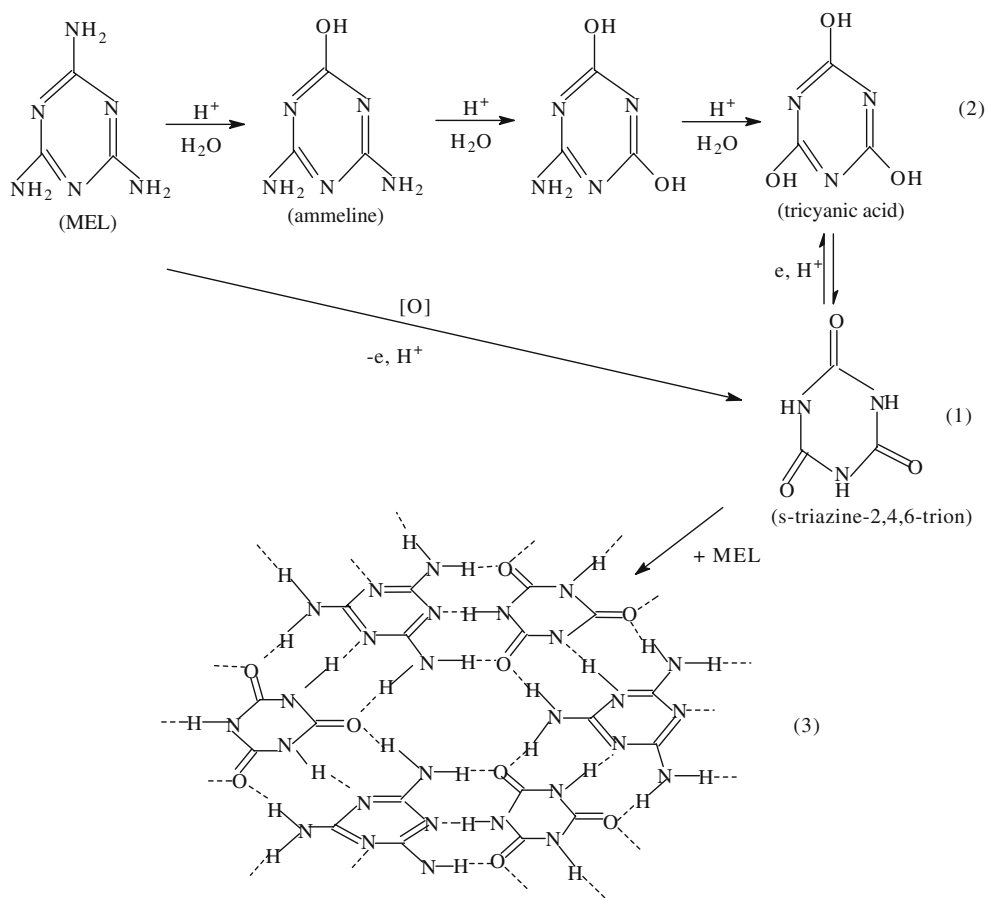
The morphology of MEL/WGE film formed in 0.1 M PBS (pH 7.0) and 0.5 M H_2SO_4

The SEMs of MEL/WGE were carried out to investigate the structure (Fig. 4). The origin WGE shows a typical porous graphite layer (a). However, The film formed in 0.5 M H_2SO_4 gives an overlapping leaves structure (b), and the film formed in 0.1 M PBS (pH 7.0) is porous with a diameter of 35–160 nm (c), they are in line with the presumptions of Schemes 1 and 2. The present images prove a significant difference for the films formed in neutral and acid media.

Fig. 3 Cyclic voltammetries (CVs) of 0.01 M melamine (MEL) for first (*b*), second (*c*), third (*d*), and fourth (*e*) cycle at paraffin-impregnated graphite electrode (WGE); and CV of WGE (*a*) in 0.5 M H_2SO_4. *Inset*, CVs of MEL/WGE in 0.5 M H_2SO_4. Scan, 50 mV S^{-1}

Scheme 2 MEL in 0.5 M H$_2$SO$_4$

Determination of MEL in 0.5 M H$_2$SO$_4$

The hydrolysis of MEL could be furthered by CV in 0.5 M H$_2$SO$_4$ in the above work. Here same result could be observed in Fig. 5 and inset, after MEL undergone a constant potential preparation of 0.9 (b) V in 0.5 M H$_2$SO$_4$, the spectra are obviously increase compared with that in no preparation (a). The spectra are enhancing with the increasing of potential from 0 to 0.8 V; they reached a maximum at 0.9 V and tend to a flat from 0.9 to 1.3 V. Then an obvious decrease could be seen after 1.4 V, implying the disintegration of MEL. Thus, 0.9 V could be an optimum potential. Moreover, the optimum reaction time was investigated too; the spectra were enhancing with the
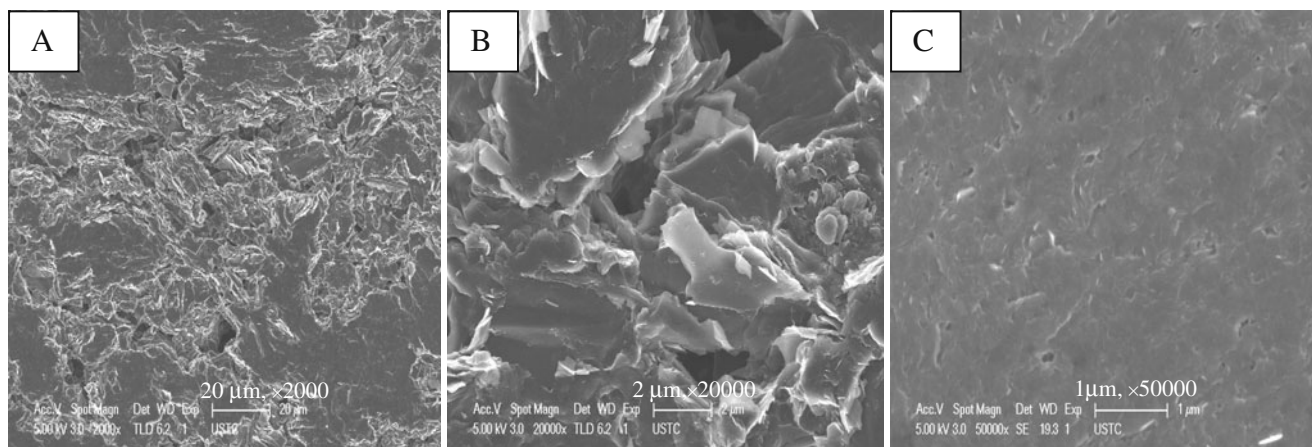

Fig. 4 FE-SEM of bare WGE (**a**) and scanned in 0.01 M melamine (MEL)/0.5 M H$_2$SO$_4$ (**b**) 0.01 M MEL/0.1 M PBS (**c**) in 50 CVs
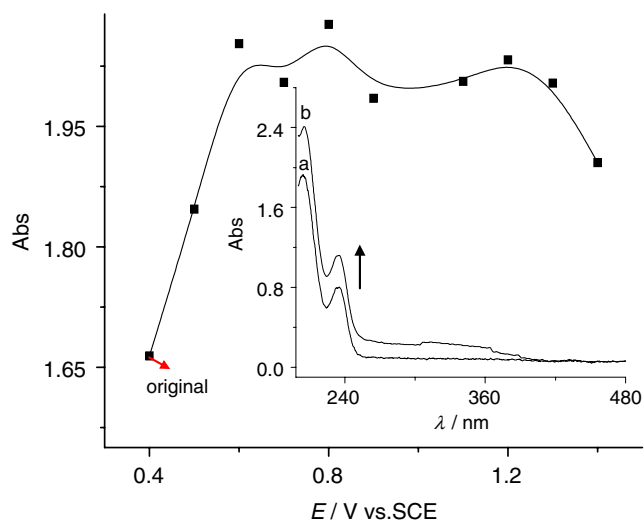
Fig. 5 Absorption spectra of 7.0 μM MEL depend on the oxidation potential. Inset: UV–vis thin-layer spectroelectrochemical absorption spectra of 7.0 μM MEL at 0 V (**a**) and 0.9 V (**b**) in 5 min. Buffer, 0.5 M H$_2$SO$_4$. Accumulation time, 5 min
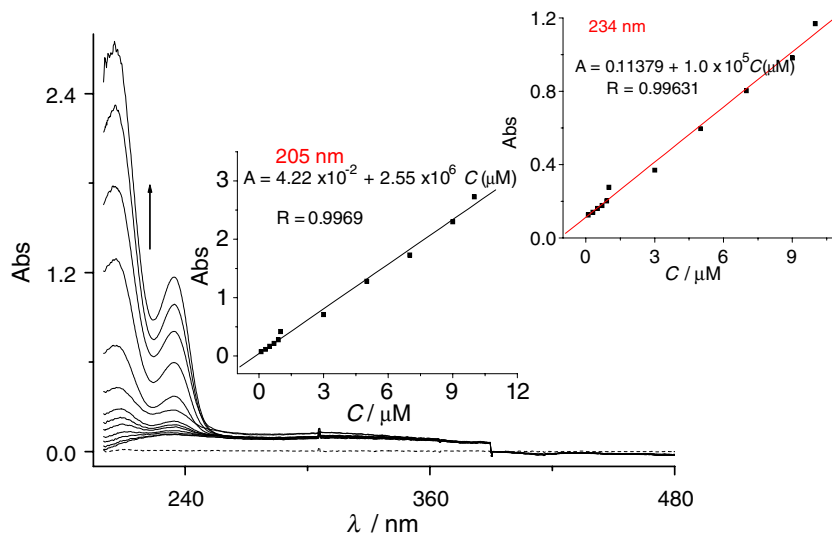
Table 1 Application of the proposed method to determine melamine (MEL) in milk powder samples spiked with different amounts of melamine

Sample	Concentration of MEL (μM)		Recovery (%)
	Amount added	Amount found[a]	
1	0.8	0.76±0.035	94
2	1.0	0.99±0.02	99.4
3	6.0	6.22±0.04	107
4	9.0	8.70±0.03	96

[a] Average value of three determinations±standard deviation

delaying the time from 1 to 5 min, they displayed a maximum at 5 min, then, reached a flat (no shown). Actually, the spectra are gradually increase without the electrochemical preparation, but they could reach maximum after 2 h (no show) in 0.5 M H$_2$SO$_4$

On the optimum conditions, the MEL was detected (Fig. 6), the spectra value at 205 and 234 nm are increase depending on the concentration of MEL in a large range from 1×10^{-7} to 1×10^{-5} M (For 205 nm: $A = 4.22 \times 10^{-2} + 2.55 \times 10^6 c(\mu M)$), $R = 0.9969$, LDS $= 1 \times 10^{-8}$M(3σ). For 234 nm, $A = 1.14 \times 10^{-2} + 1.05 \times 10^6 c(\mu M)$), $R = 0.9963$, LDS $= 3 \times 10^{-8}$M(3σ), $n=3$). The analysis of MEL

in real sample milk powder was carried out to evaluate the proposed method. The milk powder was pretreated according to the general procedure [10]; 1.0 g milk powder was mixed with 5 mL 0.1 M CaCl$_2$ (against the interference of glucose) first, then, 5 mL of 0.5 M trichloroaceticacid, 35 mL of 5 M methanol. After 10-min sonication, the mixture was centrifuged and the supernatant was filtrated. Then the filtrate was condensed to give a total volume of 5 mL and filtered through a 0.45 μm filter membrane to obtain the samples for detection. Because the existing milk powder in the market is free of MEL, the sample was spiked with different amounts of MEL standard solution directly. It is notable that trichloroacetic acid and methanol solution have spectra response from 205 to 240 nm, a blank comparison solution should been given. The concentration of MEL was calculated by standard addition method. Table 1 shows that recovery rate is in the range of ±10%, demonstrating the accuracy of the proposed method.

The influences of some foreign substances on the determination of 3×10^{-6} MEL were investigated. The

Fig. 6 UV–vis thin-layer spectroelectrochemical absorption spectra of MEL depend on the concentration (1×10^{-8} and 5×10^{-8}; 1×10^{-7}–9×10^{-7}, interval, 2×10^{-7}; 1×10^{-6}–9×10^{-6}, interval, 2×10^{-6}; 1×10^{-5}). *Inset*, the absorption spectras of MEL depend on the concentration. Oxidation potential, 0.9 V; buffer, 0.5 M H$_2$SO$_4$. Accumulation time, 5 min

proposed method showed a good selectivity for MEL detection. For example, after preparation using above method, 3,000-fold K^+, Na^+; 100-fold NH_4^+, Cl^-, PO_4^{3-}, and Ac^-; 50-fold glucose, ascorbic acid, alanine, arginine, tyrosine, and monosodium glutamate barely interfere in the determination of MEL.

Conclusions

This study revealed for the first time that the process of redox of melamine is complex, pH, and potentially dependent, and most steps are reversible based on spectroelectrochemistry. Actually, MEL undergoes different redox scheme in neutral and acid aqueous media. In 0.1 M PBS (pH 7.0), MEL can be electropolymerized at WGE with a strong adherence, and the final product is electroactive. In 0.5 M H_2SO_4, the hydrolysis of MEL can be accelerated in continuous scan or constant potential preparation, MEL can associate with tricyanic acid through hydrogen bonding at WGE with an unstable modification. The spectra are obviously increase at 205 and 234 nm in a constant potential preparation of 0.9 V. The particularly, the method was successfully applied to the determination of MEL in real sample.

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