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Original Article

Ultrasensitive determination of melamine in milk products and biological fluids by luminol-hydrogen peroxide chemiluminescence

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ABSTRACT

An ultrasensitive chemiluminescence (CL) method for determination of melamine at picogram level was reported in a flow system. It was found that melamine can accelerate the electrons transferring rate of excited 3-aminophthalate with notable enhanced CL intensity of luminol-hydrogen peroxide reaction. The increased CL intensity was proportional to the concentration of melamine in the range from 2.5 to 250 pg mL^{-1} ($R^2 = 0.9953$), with a detection limit of 0.9 pg mL^{-1} (3σ) and the relative standard deviations lower than 5.0%. The proposed method was successfully applied to determine the melamine in liquid milk, yogurt, human urine and serum samples with the recovery of 98–105%, 93–105%, 98–105%, 97–109%, respectively.

1. Introduction

Melamine (1,3,5-triazine-2,4,6-triamine, MW 126) is a nitrogen-rich chemical used in the manufacture of laminates, plastics, coatings, commercial filters, glues or adhesives, dishware and kitchenware. Although it is not a permissible substance in foods at any level, some unethical manufacturers adulterated it into animal feeds or milk products to fraudulently elevate the protein content of these products, which resulted in pet deaths and infants kidney diseases (WHO/FAO, 2008). In September 2008, the outbreaks of nephrolithiasis and acute kidney injury among children in China attracted widespread public attention, leading to strict control of melamine in foods. Various methods for the analysis of melamine and related compounds in foods for human consumption and animal feeds have been reported, including high-performance liquid chromatography (HPLC) or gas chromatography (GC) combined with a selective detection technique, such as tandem mass spectrometry (MS/MS) (Andersen et al., 2008; Buu et al., 2010; Michael et al., 2008; Tittlemier, 2008; Varelis and Jeskelis, 2008), single-stage mass spectrometry (MS) (Litzau et al., 2008; Zhu et al., 2010) diode array detection (DAD) (Muniz-Valencia et al., 2008) and ultraviolet absorption (UV) (Bradley et al., 2005; Chou et al., 2003), enzyme-linked immunosorbent assay (ELISA) (Kim et al., 2008; Wang et al., 2010), capillary electrophoresis (CE) (Wang and Chen, 2009; Yan et al., 2009), electrochemistry (EC) (Cao et al., 2009), spectroscopic methods (Lin et al., 2008; Mauer et al., 2009; Rima et al., 2009), nuclear magnetic resonance (NMR) spectroscopy (Lachenmeier et al., 2009), chemiluminescence (CL) (Wang et al., 2009), sweeping-micellar electrokinetic chromatography(SMEC) (Tsai et al., 2009), and molecularly imprinted polymer film (MIP) (Pietrzyk et al., 2009).

CL coupled with the flow injection (FI) system, offering sensitivity, instrumental simplicity, sampling efficiency and reduced consumption, is an attractive analytic method. Recently, our group has developed a new approach using luminol–myoglobin CL system to determine melamine, ranging from 0.01 to 50.0 ng mL^{-1} with detection limit of 3 pg mL^{-1} (3σ) (Wang et al., 2009). It is well known that the reaction of luminol with hydrogen peroxide is a good CL system, which has been applied to determine organic and inorganic species in different science fields. In the previous work of our group, the determinations of sudan I in hot chilli sauce (Liu et al., 2007) and patulin in apple juice (Liu et al., 2008) using luminol-hydrogen peroxide CL system were reported, with the detection limits of 3 pg mL^{-1} and 10 pg mL^{-1}, respectively. In this paper, it was first

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found that melamine could greatly enhance the CL reaction between luminol and hydrogen peroxide. The enhancement of CL intensity was proportionate to the concentration of melamine ranging from 2.5 pg mL^{-1} to 250 pg mL^{-1} (R^2 = 0.9953) with the detection limit of 0.9 pg mL^{-1} (3σ). Therefore an ultrasensitive, economical, simple as well as rapid procedure was designed to determine melamine.

2. Materials and methods

2.1. Apparatus

A peristaltic pump of the IFAS-E Luminescence Analyzer (Xi'an Remax Electronic Science-Tech. Co. Ltd., Xi'an, China) was used to deliver all streams. PTFE tubing (1.0 mm i.d.) was used throughout the manifold for carrying the CL reagents. A six-way valve with a loop of 100 μL was used for sampling. The flow cell was made by coiling 15 cm of colorless glass tube (1.0 mm i.d.) into a spiral disk shape with a diameter of 2 cm and placed close to the photomultiplier tube (PMT). The CL signal produced in the flow cell was detected without wavelength discrimination, and the PMT output was recorded by PC with an IFAS-A client system (Remax, Xi'an, China).

2.2. Reagents

All chemicals used were of analytical reagent grade. Water purified in a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout. Standard solution of melamine was supplied by the Xi'an Supervision and Inspection Institute of Product Quality. Luminol (Fluka, Biochemika, Buchs, Switzerland) was obtained from Xi'an Medicine Purchasing and Supply Station, China. Hydrogen peroxide was purchased from Xi'an Chemical Reagent Plant.

Stock solutions of melamine (1.00 mg mL^{-1}) was prepared in 20% methanol and stored at 4 °C. Working standard solutions of melamine were prepared daily from the above stock solution by appropriate dilution as required. Luminol (2.5×10^{-2} mol L^{-1}) was prepared by dissolving 0.44 g of luminol in 100 mL of 0.01 mol L^{-1} NaOH solution in a brown calibrated flask. A 0.1 mol L^{-1} stock standard solution of hydrogen peroxide was prepared by dissolving the solution in distilled water.

2.3. Procedures

As shown in Fig. 1, flow lines were inserted into hydrogen peroxide, luminol, water carrier, sample and sodium hydroxide solutions, respectively. The pump was started at a constant speed of 2.0 mL min^{-1} to wash the whole system until a stable baseline was recorded. Then 100 μL luminol and hydrogen peroxide solution was injected into the water carrier stream by injection valve, merged with the solution stream of melamine. The mixed solution in an alkaline medium was delivered into the CL cell, producing CL emission, detected by the PMT and luminometer. The concentration of the sample was quantified by the increment of CL intensity ($\Delta I = I_s - I_o$), where I_s and I_o were CL signals in the presence and in the absence of melamine, respectively.

2.4. Sample preparation

Yili ultra heat treated full cream milk and Yinqiao plain yogurt were purchased from the local market and performed through below procedure. Known quantities of melamine were spiked into 2.00 g liquid milk and yogurt. After homogenization, the spiked samples were placed into a 50 mL PTFE centrifuge tube, and then 15 mL of 1% trichloroacetic acid was added to promote protein precipitation. The mixture was dissolved ultrasonically for 20 min and then centrifuged at 4000 rpm for 10 min (Standard of the People's Republic of China, GB/T 22388-2008). Then the upper clear solution was filtered through a 0.45 μm membrane filter and diluted to the mark in a 25 mL calibrated flask. Suitable aliquot samples from this solution were taken for determination.

The urine samples collected from three volunteers and the serum samples supplied by the Hospital of Northwest University were spiked before determination. To prepare the spiked samples, known quantities of melamine were spiked into 1.0 mL of urine or serum. After homogenization, the spiked samples were diluted 1.0×10^5-fold. The samples were directly determined by the proposed method.

3. 3 Results and discussion

3.1. CL intensity–time profile

The CL intensity–time profile of luminol-hydrogen peroxide with melamine using flow procedures was given in Fig. 2. It could

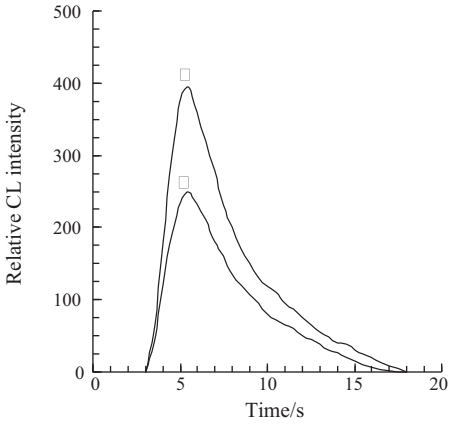

Fig. 2. Kinetic CL intensity–time profile. (I) CL intensity in the absence of melamine; (II) CL intensity in the presence of melamine (75 pg mL^{-1}). CL: chemiluminescence.

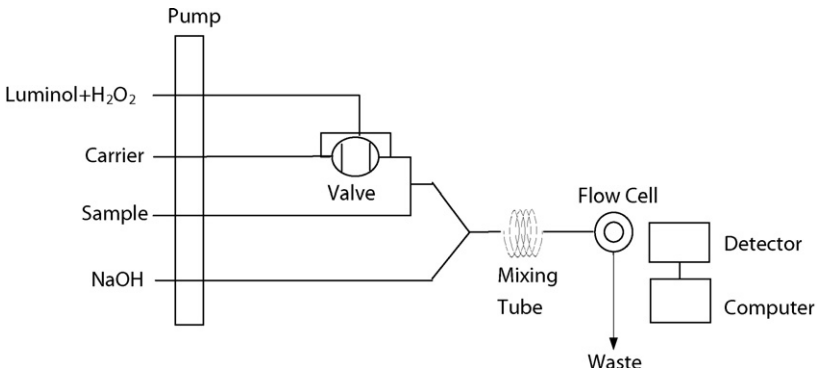

Fig. 1. Schematic diagram of the FI–CL for determination of melamine.