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Melamine detection in dairy products by using a reusable evanescent wave fiber-optic biosensor

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ABSTRACT

A sensitive, simple and rapid detection of trace amounts of melamine in milk products was achieved in a reusable, portable optical sensor system based on the principle of immunoassay and evanescent wave-excited fluorescence. The evanescent wave fiber-optic immunosensor (EWFI) employed a single-multi mode fiber optic coupler for light excitation and collection of fluorescence generated from the fiber optic probe. A reusable immunosurface of fiber probe was established to allow the performance of more than 300 assay cycles. Each assay cycle was less than 15 min. The typical calibration curves of the EWFI obtained for melamine (MA) had a detection limit of 5.14 μg/L with the detectable working range from 12.62 to 284.18 μg/L when the concentration of Cy5.5-labeled antibody was 0.2 μg/mL. The cross-reactivity against the organic compounds structurally similar to MA was negligible. The recoveries of MA in all sorts of dairy products ranged from 86.1% to 106.5% with relative standard deviation values of less than 4%, confirming the application potential in the measurement of MA in reality. The immunoassay performance of the EWFI was also validated with respect to conventional LC–MS/MS and the correlation between methods was in good agreement with the coefficient variation of less than 20% for majority samples.

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1. Introduction

Melamine (2,4,6-triamino-1,3,5-triazine, MA), with chemical formula $C_3H_6N_6$, has a wide variety of applications in industrial chemical, which is commonly used in the manufacture of the serviceable plastic tableware and flame retardants, such as MA–formaldehyde polymer resins for laminates, coatings, adhesives and plastics. But it is forbidden in food additive [1]. Nowadays, MA was illegally adulterated into dairy products as a bountiful supply of nitrogen compound (66.6% by mass) to fraudulently increase the apparent protein content [2]. In 2007, the illegal use of melamine was proved that many cats and dogs were victims of feeding the MA contaminated pet food in the United States [3]. In 2008, San Lu infant formula contaminated by melamine, which caused incidents of poisoning among children around the world suffer needlessly [4]. Therefore, developing an easy and sensitive method for portable detection of MA is of great importance for food safety and human public health.

Established analytical methods for MA monitoring include gas chromatography–mass spectrometry (GC–MS) [5], high performance liquid chromatography (HPLC) [6,7,8,9,10], liquid chromatography–mass spectrometry (LC–MS) [11–13], capillary electrophoresis (CE) [14,15], nuclear magnetic resonance (NMR) spectroscopy [16], matrix-assisted laser desorption/ionization with time-of-flight mass spectroscopy (MALDI-TOFMS) [17] etc. MA can be qualitatively and quantitatively monitored by the above-mentioned methods, which include complex, time-consuming purification and often require expensive apparatus and mazy operation procedures. On the contrary, immunoassays are generally accurate, highly sensitive, selective and cost-efficient. Recently, various enzyme linked immunosorbent assays (ELISA) have been proposed for MA detection [18–20]. However, these assays involve

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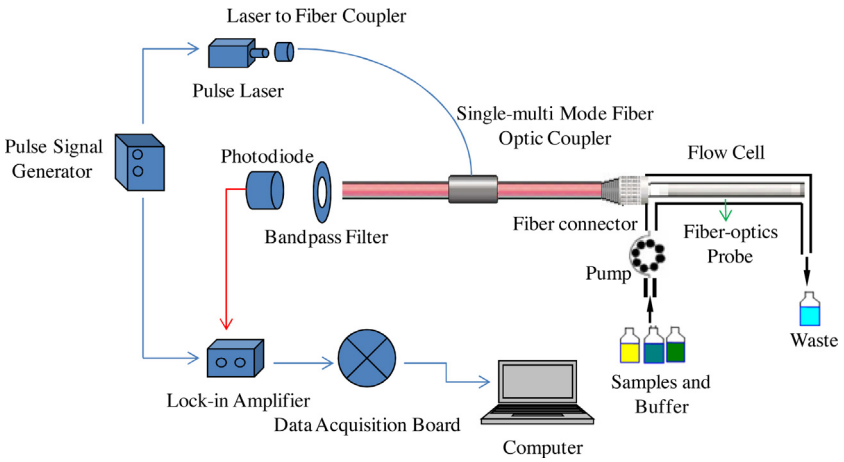

Fig. 1. Schematic set-up of portable and reusable evanescent wave fiber-optic immunosensor (EWFI).

heavy manual labor and need relatively large amounts of reagents. Therefore, it is extremely important and urgent to develop a rapid, low-cost, ultra-sensitive, routine and portable assay for the analysis of MA contamination in real samples.

Recently, immunosensors have received a lot of attention for the detection of pollutants at trace concentrations in food and environment monitoring. Immunosensors have a number of advantages such as reusability, rapidity, potential portability and low cost, as opposed to the disadvantages of traditional ELISA. In this paper, a portable and reusable evanescent wave fiber-optic immunosensor (EWFI) has been used for the ultrasensitive trace amounts of MA detection and analysis. On the platform of EWFI, both the transmission of the excitation light and the collection and transmission of the generated fluorescence were achieved by the fiber optics through the single-multi mode fiber optic coupler. Based on the principle of immunoassay and total internal reflection (TIR) fluorescence, the sensitivity, specificity and reusability of the proposed EWFI toward MA detection were fully investigated and evaluated in this study. Furthermore, the performance of EWFI was evaluated by research of the recovery of MA in all sorts of dairy products and validating with respect to the traditional LC–MS/MS method.

2. Experiments

2.1. Materials and chemicals

3-Mercaptopropyl-trimethoxysilane (MTS), bovine serum albumin (BSA), 1-ethyl-3 (-3dimethylamino-propyl) carbodiimide hydrochloride (EDC) and melamine were purchased from Sigma-Aldrich (Germany). Other reagents, if not specified, were supplied by Beijing Chemical Agents (China). All reagents were analytical grade and used without further purification. Deionized water was used throughout the experiments. 1 mg/mL MA stock solution was prepared in the deionized water and stored at 4 °C. Standard concentrations of the analyte were prepared from the stock solution by serial dilutions in 0.01 M phosphate buffer solutions (PBS, pH = 7.4, 137 mM NaCl + 2.7 mM KCl + 4.3 mM Na_2HPO_4 + 1.4 mM KH_2PO_4).

The MA monoclonal antibody and the hapten conjugate of MA and carrier protein was purchased from Shijiazhuang Solarpex Biotechnology Co. Ltd (China) and labeled with Cy5.5 (GE Healthcare Life Sciences) according to the procedure proposed by Mujumdar et al. [21].

2.2. Instrumentation

A slightly modified EWFI used in this study was previously described by our previous reported literature [26] and presented in Fig. 1. The pulse laser beam from a 635-nm pulse diode laser (RTR Optoeletronics Technology Co., China) with pigtail was directly launched into the single-mode fiber of the single-multi mode fiber coupler (Beijing Glass Research Institute, China). The design reduced the optical components and no longer required optical alignment. The laser light then entered the multi-mode fiber probe with the diameter of 600 μm and NA of 0.22 from the single-mode fiber. Afterwards, the excitation light from the laser, through the fiber connector, was coupled to a fiber probe. The incident light propagates along the length of the probe via total internal reflection. The evanescent wave generated at the surface of the probe then interacted with the surface-bound fluorescently labeled analyte conjugate, and caused excitation of the fluorophores. The collected fluorescence was subsequently filtered by means of a band pass filter (FF01-692/40, Semrock, US) and detected by photodiodes through lock-in detection (LIA-MVD-200-H, FEMTO Messtechnik GmbH, Germany).

The probe was embedded in a flow glass cell with a flow channel. Compared with our previous system (60 mm in length and 2 mm in diameter of the flow channel) [22], the new dimension was decreased to be 55 mm in length, which was preferred since they consumed less sample at the same bulk velocity and proved no effects on the assay results. Furthermore, the influent import and effluent export were adjusted perpendicular to the front end and back end of the flow channel, which effectively reduced the generation and accumulation of bubble gases during the measurement. Diagrams of the previous and new flow cell adopted in the EWFI are presented in Figure S1.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2014.08.023.

2.3. Probe preparation

The plastic-clad step-index silica optical fiber with the length of 8.5 cm and core diameter of 600 μm was purchased from Chunhui Science & Technology Industrial Co., China. The fiber cladding in length of 5.0 cm in the distal end was stripped away from the core to form a sensing region, which was immersed in a liquid sample with a refractive index less than that of the optical fiber cladding. In order to reduce the losses of fluorescence signal level coupling back into the fiber, as stated in the previous reports [23,24], the core radius was reduced along the uncladded region to form a combination tapered fiber, which was also found to have higher sensitivity than the continuous tapered fibers. The tube-etching method reported in our previous study was taken [25]. As the sensitivity of evanescent wave fiber-optic sensor heavily depends on the diameter of the core

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