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Simple spectrophotometric method for determination of melamine in liquid milks based on green Mannich reaction

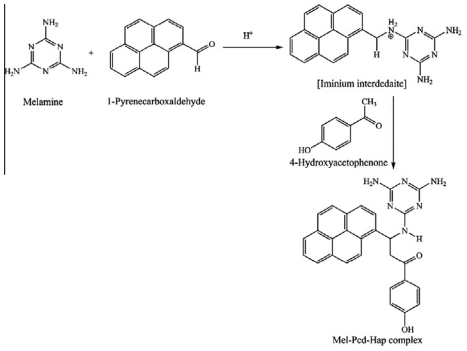
Woravith Chansuvarn [a,*], Sirirat Panich [a], Apichat Imyim [b]

[a] Division of Science, Faculty of Science and Technology, Rajamangala University of Technology Phra Nakhon (RMUTP), Bangkok 10800, Thailand
[b] Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

HIGHLIGHTS

- We adapted the green Mannich reaction using Hap and Pcd to form complex with melamine.
- A simple and rapid spectrometric method has been developed for melamine monitoring.
- Developed and validated method has been used for melamine in liquid milk samples.

GRAPHICAL ABSTRACT



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ABSTRACT

A new and simple spectrophotometric method has been developed and validated for measuring the contamination of melamine in different milk products. The method is based upon measuring the absorption of the complex formed from melamine, 4-hydroxyacetophenone (Hap) and 1-pyrene carboxaldehyde (Pcd), which was adapted from the Mannich reaction. Quantitative analysis was done at a wavelength of 236 nm within a few minutes. The reaction was optimized by focusing on both obtaining high performance of the method and to concern the volatility and toxicity of used reagents. This method provided a linear dynamic range, limit of detection and limit of quantification of 0.100–3.78, 0.08 and 0.14 mg L^{-1}, respectively. The relative standard deviation (RSD) was 3.6% (n = 10). The recoveries of melamine spiked liquid milk samples, with melamine concentrations of 0.63, 1.26, 1.89 and 2.52 mg L^{-1}, were 87.7 ± 3.7%, 91.1 ± 8.8%, 89.2 ± 4.4% and 90.6 ± 3.6% (n = 3), respectively.

Introduction

Melamine (1,3,5-triazine-2,4,6-triamine) contains a substantial amount of nitrogen which accounts for about 66% of its mass. It is commonly used in an industrial chemical in the production of melamine–formaldehyde polymer resins, such as plastics, glues or adhesives, flame retardant, laminate and fertilizer. Due to its high nitrogen content, melamine has been illegally used as non-protein nitrogen additive in order to increase its "false" apparent protein content in food products. Melamine is able to bind with cyanuric acid to form an insoluble melamine cyanurate crystal in kidneys, causing renal failure [1]. The World Health Organization (WHO) has also recommended the tolerable daily intake for melamine to be 0.2 mg kg^{-1} body weight per day, while, the US Food and Drug Administration (FDA) has updated the maximum residue levels of melamine in infant formula to be 1.0 mg kg^{-1} and 2.5 mg kg^{-1} for milk and other milk products, respectively [2]. At low concentrations, melamine caused an acute oral toxicity, but its high

* Corresponding author. Tel.: +66 2913 2424x195; fax: +66 2913 2424x194.
E-mail address: woravith.c@rmutp.ac.th (W. Chansuvarn).

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concentrations can also induce renal pathology and even death, especially in babies and children [3,4].

A number of potential methods for the determination of melamine in feed and food products have been widely reported, such as high performance liquid chromatography (HPLC) [5–7], ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) [8,9] and gas chromatography/mass spectrometry (GC/MS) or gas chromatography/tandem mass spectrometry (GC/MS/MS) [10] with the detection limit of 2, 0.01, and 0.05 mg kg^{-1}, respectively. Although these instrumental methods offer several advantages, such as high sensitivity, high throughput and high potential for melamine monitoring, they necessarily require various extraction and preparation techniques. Aside from chromatographic methods, other analytical approaches, such as enzyme immunoassay couple with HPLC-diode array detection [11], capillary electrophoresis coupled with ESI–MS [12], electrochemical sensor and/or chemiluminescence [13] have also been developed. However, the methods mentioned above are not widely used in basic analytical laboratories because they require complicated sample preparation and more expensive instrumentation, and they are more time consuming. Spectrophotometry is widely used for quantitative analysis because of its common availability in laboratories, the simplicity of spectrophotometric procedures, and for its precision and accuracy. Rima et al. [14] reported a spectrophotometric method for detection of melamine in fish samples based on the Mannich reaction [15], which is the reaction of melamine with formaldehyde and uranine. Its complex can absorb the UV radiation at a maximum wavelength of 214 nm. The lowest concentration for melamine detection was 0.06 mg L^{-1} with a relative standard deviation of 3%. The maximum absorption bands of melamine (λ_{max} = 202 nm) and melamine complex (λ_{max} = 214 nm) strongly resemble one another, resulting in interference with quantitative melamine detection. Nevertheless, the toxicity of formaldehyde has been of particular concern to human and environments. The International Agency for Research on Cancer (IARC) has recommended that there is sufficient evidence for the carcinogenicity of formaldehyde both in humans and in experimental animals. Formaldehyde is therefore considered to be carcinogenic to humans [16]. Recently, Ding et al. [17] reported a colorimetric method for melamine detection in dairy products based on the determination of residual H_2O_2 using Fe_3O_4 magnetic nanoparticles as a catalyst. However, the reaction between melamine and H_2O_2 can generate an additional compound, one which is stable at high temperature (~100 °C). A solution containing H_2O_2, 2-2′-azino-bis (3-ethylbenzothioazoline-6-sulfonic acid) diammonium salt (ABTS), Fe_3O_4 and acetate buffer (pH 4) was incubated at 45 °C for 10 min and then kept in an ice-bath for 10 min in order to stop the reaction completely. After removing the magnetic Fe_3O_4 nanoparticles, the solution was diluted with water and its absorption was measured at 417 nm. The lowest concentration of detectable melamine was 0.25 mg L^{-1}. In addition, gold nanoparticles have been widely used in visual and colorimetric detection of melamine based on their aggregation, resulting in a changing solution color due to their surface plasmon resonance property [18–21].

In this work, we introduce a new, simple and sensitive direct spectrophotometric method for determination of low level melamine in liquid milk products. The reaction was adapted from the Mannich reaction with three-component reactions involving the primary amines of melamine, the aldehyde functional group of 1-pyrene carboxaldehyde (Pcd) and the ketone group of 4-hydroxyacetophenone (Hap) in an aqueous solution. The complex of Mel–Pcd–Hap displayed a characteristic band which was quantitatively measured at a wavelength of 236 nm.

Experimental

Apparatus

Absorption spectra were acquired on a UV–vis spectrophotometer (Shimadzu UV-1700, Japan) at room temperature using ultra-pure water as the blank for background correction. Ultra-pure water (Milli-Q) was produced using a model TKA, Germany.

Chemicals and reagents

All chemicals used in the experiments were of analytical grade and used as received. All solutions were prepared with ultra-pure water (Milli-Q). 1,3,5-Triazine-2,4,6-triamine (Mel), 4-hydroxyacetophenone (Hap), 1-pyrene carboxaldehyde (Pcd), 2-acetonaphthone (AN) and formaldehyde were purchased from Fluka, China. Acetonitrile, trichloroacetic acid (TCA) and absolute ethanol were obtained from Merck, Germany. The melamine test kit (Hients) was purchased from Higher Enterprises Co., Ltd. (Thailand). The glassware used in the experiment was cleaned with aqua regia (3:1 (v/v) HCl:HNO_3) and rinsed thoroughly with ultra-pure water.

Standard solutions

A stock standard melamine solution (6.30 mg L^{-1}) was prepared with ultra-pure water. The mixture was shaken with a Vertex mixer and ultrasonication for 20–30 min until complete dissolution of the melamine crystals and then stored at 4 °C.

A stock solution of 4-hydroxyacetophenone (6.30 mg L^{-1}) and 2-acetonaphthone (6.30 mg L^{-1}) was prepared in 5% (v/v) absolute ethanol. A stock solution of 1-pyrene carboxaldehyde (6.30 mg L^{-1}) was prepared by dissolution in 10% (v/v) absolute ethanol and nitric acid. After shaking and ultrasonication for 5 min, the solutions were stored at 4 °C.

Sample preparation

Due to melamine contaminated milk cannot be obtained from market, milks were directly spiked with the appropriate amounts of melamine standard solution. The sample preparation was carried out according to the method recommended by the National Standard of China (GB/T22388-2008) [18]. Briefly, 5 mL of liquid milk and 5 mL of 10% (w/v) TCA were added into 1 mL of acetonitrile. After 15 min sonication and 10 min shaking, the mixture was centrifuged at 15,000 rpm for 10 min, and the supernatant was filtered through a 0.45 μm filter membrane to obtain the sample solution for detection.

Analytical method for real sample

A 3.50 mL sample solution was transferred in a test tube containing 0.50 mL of 1.60 mg L^{-1} Hap and 0.10 mL of 1.60 mg L^{-1} Pcd and then corrected to a total volume of 5.00 mL with ultra-pure water. The solution was shaken for 2 min and then transferred into a quartz cell for measurements. The absorption spectra were also monitored from 200 to 400 nm with 1 nm interval against a reagent blank.

Results and discussion

According to the Mannich reaction [15], ammonia or any primary or secondary amines are able to react with carbonyl functional group of aldehydes and ketones to form a β-amino-carbonyl compound as depicted in Scheme 1. In this work, the reactions of aldehyde and ketone reagents, such as formaldehyde (Fd),