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Short communication

A sensitive spectrofluorimetric method for the quantification of melamine residue in milk powder using the Mannich reaction in aqueous solutions

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ABSTRACT

The objective of this study was to develop a spectrofluorimetric method for the quantitative determination of melamine. The method was based on the complexation of melamine with a mixture of formaldehyde and chemicals including a ketone group, as described by the Mannich reaction. The complex was determined by spectrofluorimetric measurement as it is characterized by specific spectroscopic properties that are related to the chromophore of the ketone compounds. 1,3-Diphenylpropane-1,3-dione (DPPD) was tested as a ketone compound. The fluorescence spectrum of the complex presented a maximum of absorption at 325 nm.A quenching of the fluorescence occurred when melamine was added into the solution. The kinetic of fluorescence quenching was followed to determine quantitatively the melamine concentration. An internal standard was added to quantify melamine. The method was tested to determine the level of melamine in contaminated milk powder. The recovery value was 97% and the limit of detection was 0.007 μg $mL^{-1.}$

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1. Introduction

Melamine (2,4,6-triamino-1,3,5-triazine) is a chemical used in manufacturing plastics and fertilizer products. Several recent studies reported numerous cases of nephrolithiasis and, in some instances, renal failure in Chinese babies following consumption of melamine-contaminated infant formula (milk powder). Some manufacturers illegally used melamine as an adulterant to increase the food products apparent protein content. For the same purpose, melamine was added to animal feeds and thus the industrial chemical was detected in eggs and in all food categories that use milk powder as an ingredient.

Previously reported methods for the quantitative determination of melamine include enzyme immunoassay (EIA), gas chromatography mass spectrometry (GC–MS), liquid chromatography mass spectrometry (LC–MS), and high-performance liquid chromatography (HPLC) with UV detection [1–4]. Standard methods enacted by the Chinese government for determining melamine in raw milk and dairy products included HPLC–UV, LC–MS, and GC–MS methods [GB/T 22388 2008, GB/T 22400 2008]. However, the high cost of operation and maintenance of GC/LC–MS systems as well as the labor intensive derivatization that GC–MS requires limits their use in milk product factories.

For the quantitative determination of melamine as a chemical contaminant in food such as lard, potato proteins, food-stimulants and beverages, only few methods have been reported, such as spectrophotometry [5], liquid chromatography[6–8] and gas chromatography. Several studies [9–11] used HPLC/MS to determine the melamine in pet food by enzyme immunoassay. GC–MS (gas chromatography–mass spectrometry) has also been used after trimethylsilylation for the determination of melamine and its analogs in wheat gluten and pet food matrices.

This latter method has been recommended by the European Commission to analyze consignments of wheat gluten, corn gluten, corn meal, soy protein, rice bran and rice protein concentrate originating from developing countries, in particular from China. Melamine has been detected using liquid chromatography in beverages at levels of 0.54, 0.72, 1.42 and 2.2 mg kg^{-1} in coffee, orange juice, fermented milk and lemon juice, respectively, with a limit of detection of 0.05 mg L^{-1}. These levels are due to the migration of melamine from the cup, made of melamine–formaldehyde resin, into the beverage under acidic conditions [8].

In our previous work [12] we measured melamine in Chinese fish by a simple

spectrophotometric method using the Mannich reaction resulting from interaction between melamine formaldehyde and a ketone compound.

The objective of this work was to develop a new spectrofluorimetric method, more sensitive than the spectrophotometric one for the quantitative determination of melamine in powdered milk. The

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fluorescence spectroscopy was used to monitor the complex of melamine, formaldehyde and DPPD, as described by the Mannich reaction.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and solvents used were of analytical grade or of a higher grade, when available. Formaldehyde, 1,3-Diphenylpropane-1,3-dione (DPPD), melamine, ethyl acetate, were purchased from Fisher, (MA, USA). Ultra pure water was prepared using a multi-Q filter system (Millipore, MA, USA).

2.2. Instruments

The solutions were monitored by Cary UV spectrophotometer (Varian Inc., CA, USA). The fluorescence was measured by a HITACHI 7000 Spectrofluorimeter. Measurement of pH was done using a Mettler Toledo (OH, USA) pH-meter.

2.3. Standard solutions

Stock aqueous solution of melamine was transferred into a volumetric flask to produce a solution with a concentration of 36 μg mL^{-1}. The solution was shaken for 20–30 min until complete dissolution of the melamine crystals. Samples were prepared for analysis by mixing 1 mL of aqueous DPPD solution 12 μg mL^{-1}, 1 mL of pure formaldehyde and different volumes of melamine stock solutions (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.8 mL). De-ionized water was transferred to each sample to reach a final volume of 5 mL. The final concentration of DPPD was 2.4 μg mL^{-1} and the melamine concentrations varied from 0.72 μg mL^{-1} to 5.76 μg mL^{-1}.

Table 1 presents the composition of samples used for melamine analysis.

2.4. Extraction procedure

About 10 g of milk powder, polluted by 0.15 mg of melamine, were transferred into a 100 mL polypropylene centrifuge tube to which 50 mL of extraction solvent (diethylamine:water:acetonitrile/ 10:40:50) was added. After mixing to thoroughly wet the entire sample, the mixture was centrifuged for 20 min at 5000 rpm. In order to remove fatty acids from the milk sample, 20 mL of the supernatant extraction solvent were shaken with dichloromethane which was then discarded using a separator funnel.

Thus, the aqueous layer containing melamine was ready to be analyzed. To fortify the samples, different concentrations of melamine were added to the same volume of extracted milk powder sample. Table 2 summarizes the volumes of different chemicals used in different mixtures.

Table 1
Composition of the samples used for melamine analysis.

Volume of melamine [36 μg mL^{-1}] (ml)	Volume DDPD [12 μ gmL^{-1}] (ml)	Volume of formaldéhyde (ml)	Volume of water added (ml)
0.1	0	1	3.9
0.1	1	1	2.9
0.2	1	1	2.8
0.3	1	1	2.7
0.4	1	1	2.6
0.6	1	1	2.4
0.8	1	1	2.2

Table 2
summarizes the volumes of different chemicals solutions used in the internal standard addition method for the melamine determination in the polluted milk powder.

Volume of sample extracted from the milk (ml)	Volume of melamine added (ml)	Volume of DDPD (ml)	Volume of formaldehyde (ml)	Volume of water added (ml)
1	0	1	1	2
1	0.5	1	1	1.5
1	1	1	1	1
1	1.5	1	1	0.5
1	2	1	1	0

2.5. Calibration curves and recovery

Stock solutions were used to prepare solutions of lower concentrations to build the calibration curve using fluorescence measurements. Linearity was performed with melamine working solutions within the range 0.72–5.76 μg mL^{-1}.

Recovery experiments were performed by standard addition method with fortifying melamine working solution added to samples. The percentage of recovery (%*R*) was calculated as follows:

$$\%R = [(C_r - C_f)/C_r]$$

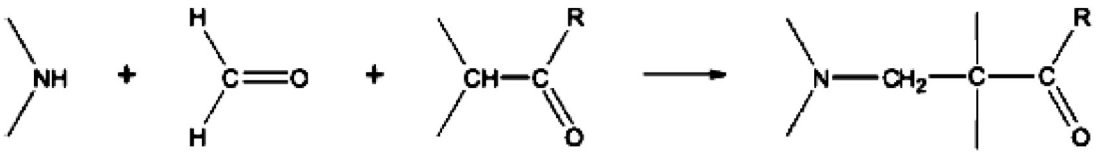
C_r = Real concentration of melamine in the fortified samples;
C_f = Concentration of melamine obtained by the internal standard addition curve.

3. Results and discussion

3.1. Identification of the melamine–formaldehyde–DPPD complex

The Mannich reaction (Eq. 1) consists of an amino alkylation of an acidic proton placed next to a carbonyl functional group with formaldehyde and ammonia or any primary or secondary amine.

The final product is a β-amino-carbonyl compound. Reactions between aldimines and



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