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Colorimetric determination of melamine in milk using unmodified silver nanoparticles

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ABSTRACT

Melamine is nitrogen rich chemical compound used as an adulterant in dairy products by unscrupulous people to increase the apparent protein content. This incident prompted the researchers to develop simple methods for easy detection of melamine in food samples. In the present paper, we report a simple and sensitive colorimetric method for detection of melamine in milk based on silver nanoparticles. This method relies upon the principle that melamine causes the aggregation of silver nanoparticles, resulting in abrupt color change from yellow to red under optimized conditions. The concentration of melamine in adulterated sample can be quantitated by monitoring the absorption spectra of silver nanoparticles using ultraviolet–visible (UV–Vis) spectrometer. The present colorimetric method which utilizes silver nanoparticles of 35 nm can reliably detect melamine down to a concentration of 0.04 mg l^{-1}.

1. Introduction

Melamine is a synthetic chemical compound widely used in the manufacture of melamine-formaldehyde resin in chemical industries. It is also used in production of adhesives, flame retardant material, and thermosetting plastics [1]. Melamine is rich in nitrogen (66%), and hence was used to adulterate protein rich products like pet foods, milk and infant formula to increase the apparent protein content [2,3]. In 2007 and 2008, cases of melamine adulteration were reported in pet food and infant formula, respectively. The origin of melamine adulteration was traced to mainland China which has caused tremendous health effects and devastating economic loss. Consumption of melamine contaminated products caused development of kidney stones and other renal problems. The adulterated products were exported to other countries also, making it an international issue. The methods (Kjeldahl and Dumas) used for protein estimation in food samples, measure nitrogen content and not directly the protein content, thus found unsuitable to distinguish between nitrogen of protein source or non-protein source (melamine) [4,5]. Therefore, melamine adulterated food products easily passed the quality checks.

At that time, it was challenge to find methods for detection of melamine in food and feed products as it was never thought that such incident could happen. Later on, researchers developed various methodologies based on gas chromatography/mass spectrometry (MS) [6,7] high-performance liquid chromatography/MS [8–11], capillary zone electrophoresis/MS [12,13], chemiluminescence [14,15] and immunoassay [16] methods. These methods are sensitive and reliable, but involve high cost, sophisticated instrumentations and are time consuming.

In recent years, use of nanotechnology is gaining momentum to develop very fast, accurate and cost effective tests for adulteration detection in food products. Nanoparticles are successfully applied to develop colorimetric probe for detection of microbial contamination and metal ions [17–20]. Nanoparticle based colorimetric methods have also been reported for the detection of melamine [21–27]. Nanoparticles are very suitable entity to develop colorimetric probe because of their strongly distance-dependent optical properties and extremely high extinction coefficient.

In the present work, we report a simple and cost effective colorimetric test for the determination of melamine in milk using silver nanoparticles. Silver nanoparticles are straw yellow in color when dispersed in solution, but upon aggregation caused by melamine changes to red/orange in color.

2. Materials and methods

2.1. Chemicals

Melamine (99%) and sodium borohydride ($NaBH_4$) were purchased from Sigma-Aldrich (St Louis, MO, USA). Sodium citrate was purchased from Glaxo Laboratories (India) Ltd. (Mumbai, India). Sodium hydroxide (NaOH) was purchased from Thermo-Fisher Scientific India Pvt. Ltd. (Mumbai, India). Silver nitrate ($AgNO_3$) was purchased from

Abbreviations: Ag NPs, silver nanoparticles; TEM, transmission electron microscopy; DLS, dynamic light scattering; LOD, limit of detection; LOQ, limit of quantification.

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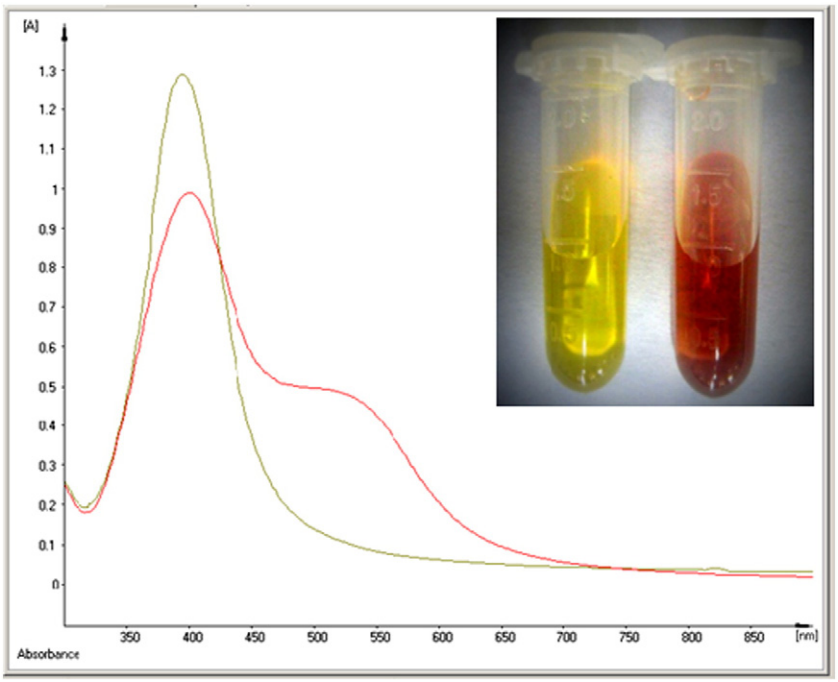

Fig. 1. Absorption spectra of Ag NPs in the absence of melamine (green line) and in the presence of melamine (red line). Insert is the photograph showing visual color change of silver nanoparticles. Experimental conditions: 600 μl Ag NPs + 400 μl H_2O, 600 μl Ag NPs + 400 μl melamine; level of addition of melamine was 1 mg/l and incubation time was 20 min.

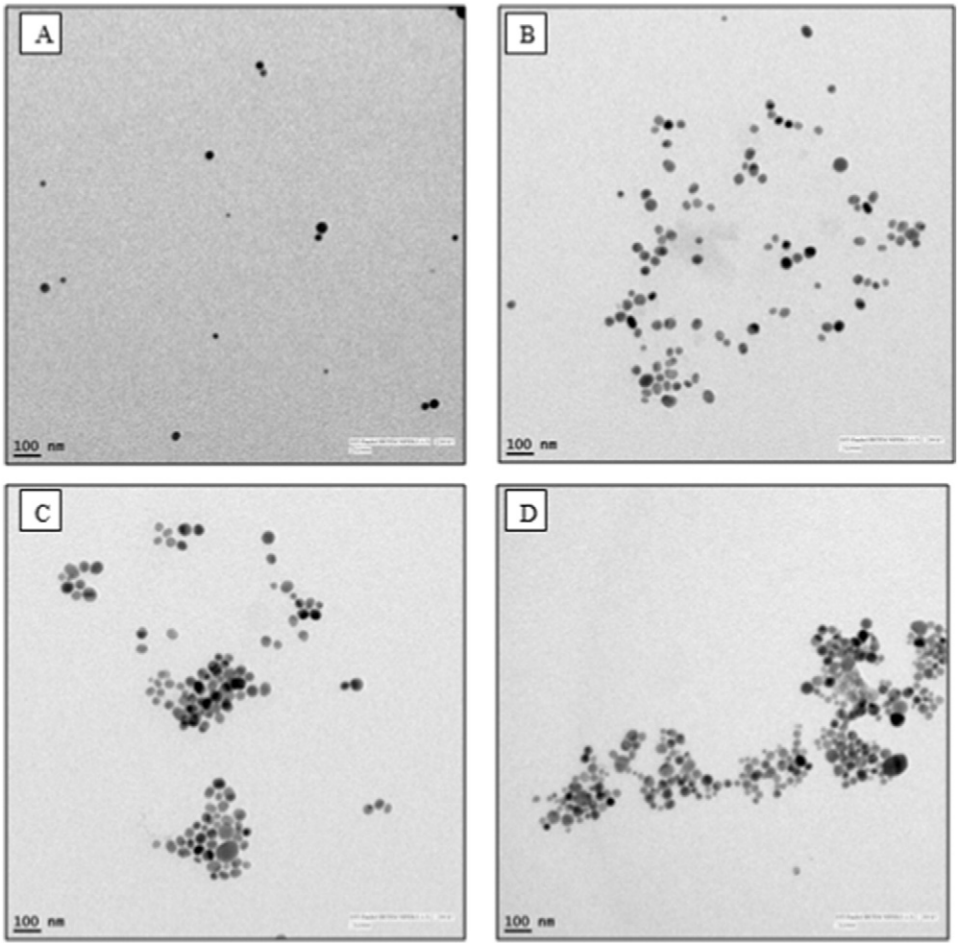

Fig. 2. TEM images of Ag NPs A. 600 μl Ag NPs + 400 μl H_2O (dispersed); B. 600 μl Ag NPs + 400 μl melamine (0.5 mg/l); C. 600 μl Ag NPs + 400 μl melamine (1 mg/l); and D. 600 μl Ag NPs + 400 μl melamine (10 mg/l) (aggregated).