Analytical Biochemistry 456 (2014) 43–49

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Colorimetric detection of melamine in milk by citrate-stabilized gold nanoparticles

Naveen Kumar *, Raman Seth, Harish Kumar

Dairy Chemistry Division, National Dairy Research Institute, Karnal 132001, Haryana, India

ARTICLE INFO

Article history:
Received 20 December 2013
Received in revised form 27 March 2014
Accepted 2 April 2014
Available online 13 April 2014

Keywords:
Gold nanoparticles
Melamine
Milk
Quantification
Limit of detection

ABSTRACT

Here, we report a simple and sensitive colorimetric method for detection of melamine in milk using gold nanoparticles (AuNPs). AuNPs of 21-nm size were synthesized by the citrate reduction method. The method is based on the principle that the melamine causes the aggregation of AuNPs and, hence, the wine red color of AuNPs changes to blue or purple. This change in color can be visualized with the naked eye or an ultraviolet–visible (UV–Vis) spectrometer. Under optimized conditions, AuNPs are highly specific for melamine and can detect melamine down to a concentration of 0.05 mg L^{-1}.

Nanotechnology has emerged as a new tool for solving food safety-related issues during recent years. Nanoparticles have been applied in making nanosensors for the detection of foodborne pathogens, microorganisms, and other contaminants. Nanoparticle-based sensing systems provide fast and reliable detection of contaminants with lower cost compared with chromatography and immunoassay-based analytical methods.

Melamine adulteration of infant formula is one recent example of food safety crisis that has raised worldwide concern about food products. Melamine is a synthetic chemical compound primarily used in the manufacture of laminates, plastics, coatings (including can coatings), commercial filters, adhesives, and dishware/kitchenware. It has high nitrogen content (66%) and, hence, is used for increasing apparent protein content of milk and other food products. The standard tests (Kjeldahl and Dumas) for estimating protein in food are unable to distinguish between nitrogen of protein and nonprotein sources and, hence, give false results. The U.S. Food and Drug Administration has issued strict guidelines for the level of melamine in food products (1 ppm in infant formula and 2.5 ppm in other food products). So, there is an urgent need to develop rapid and reliable methods for melamine detection and to validate existing methods.

Recently, several methods for melamine detection, including gas chromatography/mass spectrometry (MS)[1] [1–3], high-performance liquid chromatography/MS [4,5], capillary zone electrophoresis/MS [6–8], and potentiometric [9] and electrochemical [10–12] methods, have been reported. However, most of these methods are time-consuming, are expensive, and need trained people. Some authors have also proposed nanotechnology-based methods [13–15].

In the current study, we aimed to synthesize gold nanoparticles (AuNPs) and use them to detect melamine in milk. The citrate-stabilized gold nanoparticles are wine red in color in the absence of melamine, whereas in the presence of melamine they change to blue because melamine causes the aggregation of nanoparticles.

Materials and methods

Chemicals

Melamine (99%) and chloroauric acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium citrate was purchased from Glaxo Laboratories (India) (Mumbai, India). Sodium hydroxide was purchased from Thermo Fisher Scientific India (Mumbai,

* Corresponding author.
E-mail address: nkft87@gmail.com (N. Kumar).

[1] *Abbreviations used:* MS, mass spectrometry; AuNP, gold nanoparticle; UV, ultraviolet–visible; TEM, transmission electron microscopy; DLS, dynamic light scattering; RSD, relative standard deviation; LOD, limit of detection; LOQ, limit of quantitation.

http://dx.doi.org/10.1016/j.ab.2014.04.002
0003-2697/

India). Potassium hexacyanoferrate(II) trihydrate and zinc sulfate were purchased from Merck (Mumbai, India). Melamine stock solution was prepared by dissolving 10 mg of melamine in 1 L of water. All of the solvents and reagents were of analytical grade and were used without further purification. Millipore Milli-Q water (18 MΩ cm) was used in all of the experiments. The raw milk was obtained from the cattle yard of the university campus.

Preparation of citrate-stabilized AuNPs

All of the glassware used in preparation of AuNPs was dipped in freshly prepared aqua regia (HNO_3/HCl, 1:3) and rinsed thoroughly with water. Aqua regia is highly corrosive and must be handled with care; otherwise, an explosion, skin burns, or eye/respiratory tract irritation may result. One should work under a fume hood while dealing with aqua regia, and before disposal it should be neutralized. AuNPs were prepared by the citrate reduction method as described by Grabar and coworkers [16] with some modifications. Chloroauric acid (100 ml, 1 mM) was placed in a 250-ml round-bottom flask with three necks and boiled on a magnetic hot plate. Then 5 ml of trisodium citrate (38.8 mM) was added rapidly into boiling chloroauric acid solution with high-speed stirring. The pale yellow color of chloroauric acid changed to a wine red color within 3 min. Stirring was continued for 15 min. The wine red AuNPs that formed were then cooled to room temperature and stored at 4 °C for further use.

Sample extraction and detection of melamine

Milk proteins (e.g., casein) may interfere in melamine detection using AuNPs; therefore, samples were treated before analysis to precipitate the protein and remove fat. The sample extraction procedure is described here. A 10-ml raw milk sample was taken in a centrifuge tube. Then 2.5 ml of potassium hexacyanoferrate(II) trihydrate (3.6% aq.) solution was added into the sample and vortexed for 1 min. Next 2.5 ml of zinc sulfate (7.2% aq.) solution was added and vortexed for 1 min. The mixture was then centrifuged at 10,000 rpm for 5 min. The clear supernatant was taken

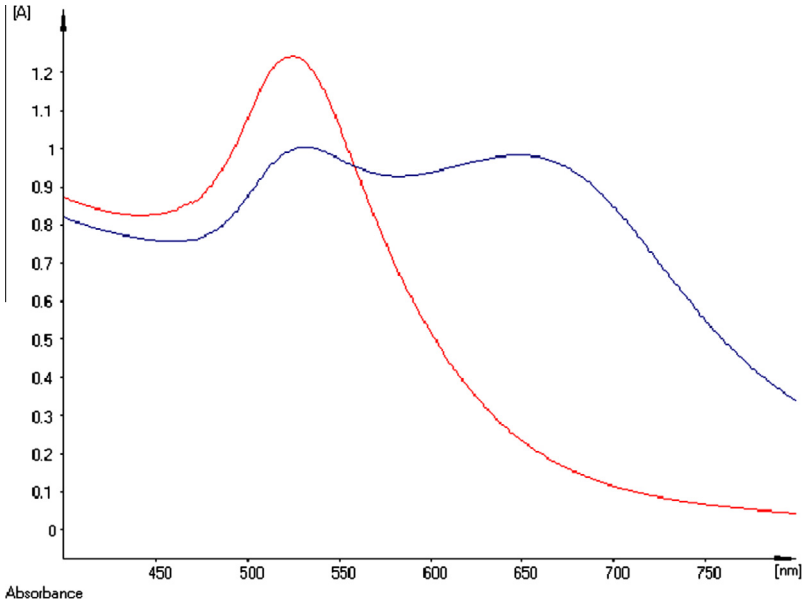

Fig.1. Absorption spectra of AuNPs in the absence of melamine (red line) and in the presence of melamine (blue line). Experimental conditions: 700 µl of AuNPs + 400 µl of H_2O (red line) and 700 µl of AuNPs + 400 µl of melamine (blue line). The level of the addition of melamine was 0.5 mg/L, and the incubation time was 15 min. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

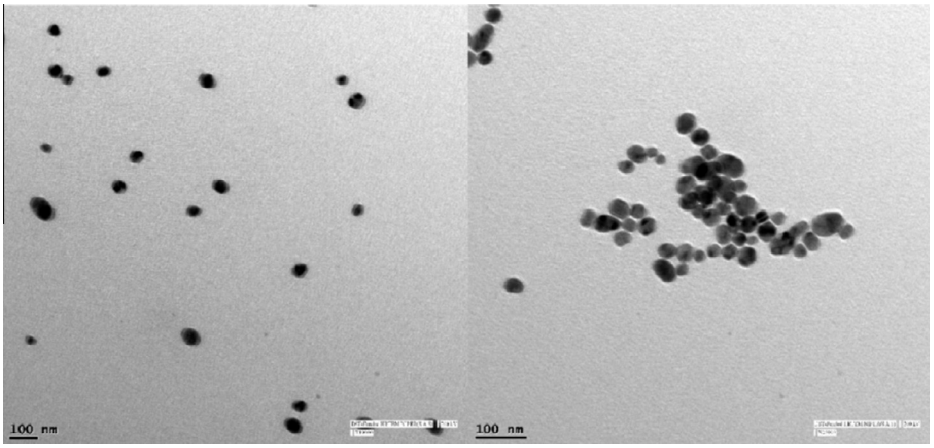

Fig.2. TEM images of AuNPs in the absence of melamine (dispersed) and in the presence of melamine (aggregated).