Simple Determination of Melamine Based on Self-assembly of Citrate-capped Gold Nanoparticles

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In the presence of melamine (2,4,6-triamino-*s*-triazine), citrate-capped gold nanoparticles (Au-NPs) can self-assemble and form a network structure through electrostatic interactions. A new absorption peak appears at approximately 700 nm, and the intensity is proportional to the concentration of melamine. Thus, a new simple method of detecting melamine is developed.

Melamine (also known as tripolycyanamide) is an industrial chemical, which is usually used in producing plastic foams, melamine–formaldehyde resins and gluewater. When added into dairy products, melamine can increase the concentration of nitrogen, and the dairy products will show a false increase in protein concentration. Melamine has low oral acute toxicity, but excessive exposure in animals causes renal stones. When consumed by human beings, babies and children are affected most because of their dependence for nutrition, compounded by immaturity of their organs, which renders them vulnerable to chemical damage.[1] Melamine can lead to human urinary system disease. So, it is forbidden to use in foodstuff. Up to now, some detecting methods of melamine have been reported, such as GC-MS, HPLC-UV, LC-ESIMS-MS,[2–4] and so on. However, expensive instruments and complex operations are needed.

Analyte-induced aggregation of gold nanoparticles (Au-NPs) results in the formation of a long-wavelength band and changes the color of Au-NPs solution from red to purple,[5,6] which has been applied to determination of many substances.[7,8] It is found that under appropriate conditions, citrate-capped Au-NPs can assemble to form a network structure by adding melamine, a new characteristic absorption appears at about 700 nm, and the intensity is enhanced with increasing melamine concentration. Thus, in this contribution, a new method is developed to determine melamine in milk powder.

In a 5-mL centrifugal tube, 300 μL of Britton–Robinson (B–R) buffer (pH 5.33), 400 μL (1 mmol) of Au-NPs (Average particle diameter is 14.7 nm, standard deviation is 1.5 nm. See Supporting Information), which were prepared by citrate reduction of $HAuCl_4$ according to the literature[9] with slight modification, and different concentrations of melamine were added, and then diluted with distilled water to 3 mL. After vortex mixing, the color of the solution changes gradually from red to purple then to blue as the melamine concentration increases. Figure 1 shows that the maximum absorption of individual Au-NPs locates at 520 nm (seen in curve 1), while a new characteristic absorption appears at about 700 nm when melamine is added (seen in curves 2, 3, and 4), and the intensity increases with melamine concentration.

The pK_b of melamine is 9.0 and the pK_{a1} and pK_{a2} of carboxyl groups in citric acid are 3.128 and 4.761, respectively.[3,10] Under weak acidity conditions, the amino group ($–NH_2$) in melamine charges electropositively (namely, $–NH_3^+$), and carboxyl (–COOH) of citric acid on the surface of citrate-capped Au-NPs charges electronegatively (namely, $–COO^-$). One melamine molecule has three $–NH_3^+$ groups, thus in the presence of melamine, Au-NPs can form a huge network structure through electrostatic interactions between $–COO^-$ groups on the Au-NPs and $–NH_3^+$ groups. Comparing TEM and SEM images of individual Au-NPs (Figures 2A and 2C) with assembled Au-NPs (Figures 2B and 2D), huge network structure can be confirmed. The new absorption peak at about 70 nm is brought by the network structure.

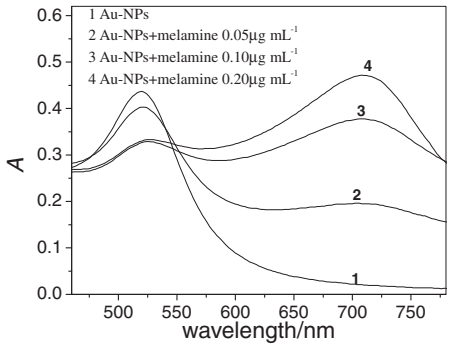

Figure 1. UV–vis absorption spectra of Au-NPs in the absence and presence of melamine. Concentrations: Au-NPs, 133 nmol mL^{-1}; melamine (from curve 1 to 4 μg mL^{-1}), 0.00, 0.05, 0.10, 0.20; pH 5.33.

The pH plays an important role in the interaction of melamine with Au-NPs. Experiments show the dependence of the absorption intensity at 700 nm on the pH, and the maximum of

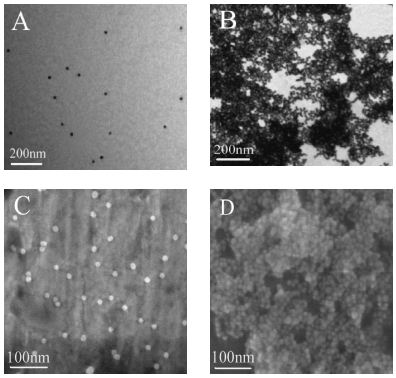

Figure 2. TEM and SEM images of individual (A) and (C) and assembled Au-NPs (B) and (D). Concentrations: Au-NPs, 133 nmol mL^{-1}; melamine, 0.00 μg mL^{-1} (A) and (C), 0.07 μg mL^{-1} (B) and (D); pH 5.33. Scale bars indicate 200 nm (A) and (B) and 100 nm (C) and (D).

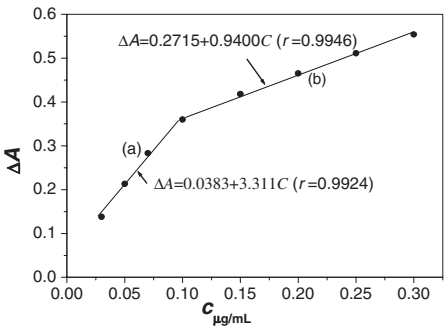

Figure 3. Linear relationship between the enhanced absorption intensity (ΔA) at 700 nm and the concentration of melamine. (a) A linear correlation exists over the range of 0.03–0.10 μg mL^{-1} ($r = 0.9924$), (b) A linear correlation exists over the range of 0.10–0.30 μg mL^{-1} ($r = 0.9946$). Concentration of Au-NPs, 133 nmol mL^{-1}, pH 5.33.

absorption intensity appears at pH 5.33 (see Supporting Information).[11] When the concentration of melamine is invariable (0.24 μg mL^{-1}), the absorption intensity enhances simultaneously at both 520 and 700 nm with increasing concentration of Au-NPs, and a ratio of A_{700nm}/A_{520nm} reaches the maximum until the concentration of Au-NPs is close to 133 nmol mL^{-1} (see Supporting Information).[11]

Under optimal conditions, the enhanced absorption intensity (ΔA) at 700 nm is linear with the concentration of melamine. Figure 3 shows that the concentration of melamine over the range of 0.03–0.3 μg mL^{-1} could be detected. For inflexion point at the melamine concentration of 0.1 μg mL^{-1}, we think that it is induced by two different aggregation stages. When the concentration of melamine is low (namely, 0.03–0.1 μg mL^{-1}), Au-NPs assemble into a smaller aggregation, which become large gradually as increasing the concentration of melamine, and the enhanced absorption intensity (ΔA) at 700 nm is linear with the concentration of melamine. When the concentration of melamine is over 0.1 μg mL^{-1}, Au-NPs assembles into a bigger aggregation and the enhanced absorption intensity (ΔA) at 700 nm is linear with the concentration of melamine, too. According to the difference of ΔA, the linear relationships can be respectively constructed of $\Delta A = 0.0383 + 3.311C$ ($r = 0.9924$, $n = 4$) and $\Delta A = 0.2715 + 0.9400C$ ($r = 0.9946$, $n = 5$).

According to the nutrition information for milk powder, some soluble salts, vitamins, and sugars are at a high concentration except protein, which will be precipitated out from the analytical sample. These substances in high concentration are studied. Table 1 lists the tolerance concentration of coexisting foreign substances with the tolerance level of ±10%. It can be seen that vitamin C, lactose, NaCl, KCl, $Fe(NO_3)_3$, and $CaCl_2$ can be present at very high concentrations, and only Cu^{2+}, Mg^{2+}, and glucose are tolerated at relatively low concentration level. The method can tolerate the concentrations which are typically added dairy products. This result indicates that our method has good selectivity.

To test the reliability of our method, reference samples containing known quantities melamine in milk powder were determined. The samples were treated in three steps. Concentrated HCl was used to adjust the acidity of the milk powder solution at approximately pH 4.7 to precipitate casein. Then ethanol is added to precipitate albumin, and the mixture is centrifuged.

Table 1. Tolerance levels of coexisting foreign substances[a]

Foreign substance	Concentration/mg g^{-1}	Change of ΔA_{700nm}/%
Vitamin C	100	−10.0
K^+ (Cl^-)	80	−9.4
Na^+ (Cl^-)	100	3.6
Ca^{2+} (Cl^-)	100	4.9
Cu^{2+} (SO_4^{2-})	10	10.0
Fe^{3+} (NO_3^-)	100	−0.3
Mg^{2+} (Cl^-)	51	−4.0
Zn^{2+} (Cl^-)	69	−7.9
Lactose	80	3.5
Glucose	50	−0.8

[a]Au-NPs, 133 nmol mL^{-1}; 4 mM B–R buffer (pH 5.33).

Table 2. Determination results for the reference samples[a,b]

Synthetic samples	Main additives	Added concentration /mg kg^{-1}	Detected concentration /mg kg^{-1}	Recovery rate /%
1	Melamine 0.1201 g + Milk powder 6.0 g	20.0	19.12	95.6
2	Melamine 0.0621 g + Milk powder 6.0 g	10.0	9.83	98.3

[a]Au-NPs, 133 nmol mL^{-1}; 4 nM B–R buffer (pH 5.33). [b]Before detecting the samples are dissolved in 60 mL of distilled water.

Table 2 shows that the determination results of these practical samples are satisfactory, and the recovery is 95.6–98.3%, indicating the method is reliable and practical.

In summary, at pH 5.33, melamine with positive charge interacts with $-COO^-$ groups on the surface of citrate-capped Au-NPs through electrostatic interactions, leading to the formation a network structure. Meanwhile, the new absorption peak at about 700 nm is brought by the huge network structure, and the intensity is enhanced gradually with increasing melamine concentration. Thus, we establish a new method to detect melamine using UV–vis spectrophotometery, which is economical, simple, rapid, and sensitive.

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11 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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