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Original Research Article

Detection of Melamine in Milk Using Nanotechnology

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ABSTRACT:
Melamine is a poisonous substance which causes formation of kidney stone, nephrolithiasis, kidney injury in infant as well as in adult human beings by the ingestion of melamine contaminated food items, powder formula, powder milk. Nobel metal may be used to shape a type of nano-particles, known as NMNPs, which are inert (oxidation-resistant/corrosion resistant) and have special physical and optical properties. NMNPs are extremely detailed and responsive visual bio sensors for the analysis of a broad variety of inorganic and organic components and are particularly gold and silver nano-particles (AuNPs and AgNPs). Colorimetric changes result in a specific and sensitive identification of contaminants, heavy metals, nucleic acids, lipeid, protein, antibody, and other molecules through interactions between noble metal nanoparticles (NMNPs) and inorganic/organic molecules. Capping agents can react or transfer, causing cross-connection and non-cross binding, broadening, or modifying local surface plasmone resonance absorption, to the hydrogen bondage, electrostatic interaction, and steric effects of inorganic and organic molecules with surface NMNPs. A collection of independent, fast and low-cost diagnostic products with colorimetric or clear visual reading have been extensively applied to NMNPs-based bio sensors.

Keywords: Noble Metal Nano Particles, Colorimetric, Biosensor, Critical Coagulation Concentration, Salt Titration, Melamine

INTRODUCTION

Melamine (C3H6N6; molecular weight: 126.12) is an organic compound with a 1, 3, 5-triazine and 2, 4, 6-triamine skeleton and the aqueous solution of melamine is weakly alkaline (Chang et al., 2017). Melamine is also a simple chemical and can be used, for example, to render fire-resistant, water-reducing compounds and cleaner with formaldehyde (Popescu and Pfriem, 2019). Fresh milk, powdered formula, and other ilk related bakery products are well known for melamine scandals (Xin and Stone, 2008). The unnecessary consumption of melamine is causing great health damage and contributing to the disordered kidney or urinary system owing to its biotoxicity (Hua et al. 2009; Haque 2009).

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Melamine tainted milk consumption has created renel stones and hematuria in Children and finally acute renal failure (Yang and Battle, 2008).

As a food substitute in livestock feed or human food, melamine is also not authorized by governments. However, often melamine has been applied to protein-rich food through ethical processing to raise the amount of nitrogen (66 percent nitrogen by mass). In China in 2008, the most popular food safety melamine incidents happened. The dairy corporation, which caused nearly 40,000 children with diseases, introduced melamine illegally into baby milk powder (Gossner et al. 2009). As recorded in 2012, melamine levels in foods at concentrations lower than 2.5 mg/kg have been restricted by foreign experts in Europe and America (Zhu and Kannan 2019).

Melamine was added to milk to raise insincerely assay results for protein content. A variety of toxic effects from melamine, comprising chronic kidney inflammation, nephrolithiasis, bladder carcinoma all have been studied in animals including human beings. The baseline concentrations of melamine existing in the environment and in the food chain as a result of extensive use of materials that contain melamine. Many base line levels are expected to be <1 mg/Kg (WHO, 2009).

Melamine concentration in food and animal feed above baseline level are considered to be the result of exploitation or adulteration. Previously, the detection and estimation of melamine were done by utilizing different types of analytical techniques. Some important literature reports are melamine detection using wet strength paper Ultraviolet spectrophotometry (Hirt et al., 1954), detection of urea, melamine, isocyanate, and urethane resins (Swann et al., 1958), determination of melamine in muscle tissue by liquid chromatography and emmass spectrometry (Filigenzi et al., 2007), high- performance liquid chromatographic method for the simultaneous detection of the adulteration of cereal flours with melamine and related triazine by products ammeline, ammelide, and cyanuric acid (Ehling et al., 2007), validation of melamine and its derivatives in rice concentrates using liquid chromatography and its application to animal samples (Valenica et al., 2008). There is also a rising need for efficient and accurate methods for the identification of food melamine concentrations.

METHODOLOGY

Reagents

Sinopharm Chemical Reagent Co., Ltd. has acquired chloroauric acid ($HAuCl_4$), sodium citrate and sodium carbonate (Na_2CO_3) (Shanghai, China). Tianjin Chemical Co., Ltd. was acquired with glucose, fructose and lactose. Tianjin Reagent Chemical Reagent Co., Ltd. has been collected with melamine (Tianjin, China). The nearby store bought liquid milk. Both solvents and reagents were diagnostic and used without further cleansing. During the whole experiment, ultrapure water was used. The glassware used during the procedure has been thoroughly rinsed in water and drained up in air before using nitrohydrochloric acid.

Pretreatment of liquid milk samples

The first is the inserted 4 mL of condensed milk with 1.2 mL of 10% trichloroacitic acid and 1.5 mL of Chloroform (Edward et al., 2013).The mixture was put inside the centrifugal tube and stirred with the vibrator 2 minutes until it was placed in an ultrasound bath for 15 minutes to maintain balance. After that, the suspension of the condensed milk was homogenized and then centrifugated at 13000 rpm for 10 minutes (Haung et al., 2019). The transparent supernatant was put in another centrifugitive tube, and a pH change of 4.3~4.5 by applying a 1.0 mol/L Na_2CO_3 solution. Again, at a pace of 3000 to 3,000 minutes, this supernatant was centrifuged. The supernatant has finally been stored for further use in the refrigerator (Edward et al. 2013)

Principle of LSPR and the experimental setup

For the quantitative identification of chemical and organic targets, nanoparticular optic sensing techniques were found to be efficacious. This

technique is focused on the LSPR spectrum study of noble metal nanoparticles, which is linked to the shift in the refractive index induced by the atmosphere (see Fig 1a).

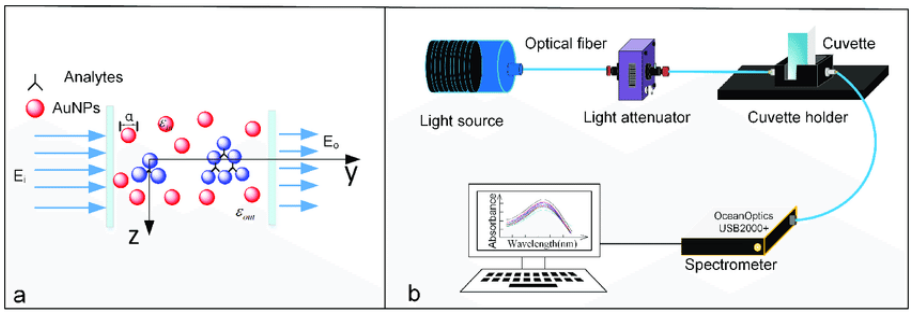

Figure 1: Schematic diagrams illustrating the principle of (a) localized surface plasmon resonance and (b) the optical fiber-based LSPR system.

When the LSPR resonance is known to have a sphern nanoparticle of radius a, irradiated with light at a wavelength λ (a is somewhat smaller than the rorotia), the strength of the electromagnetic wave happens when the frequency of the osscillative frequency of the electrons on the nanoparticle surface is coinciding. The gold-sphere extinction range can be estimated using the near-static approximation by solving Maxwell's equations.

$$E(\lambda) = \frac{24\pi^2 N \times a^3 \times \varepsilon_{out}^{3/2}}{\lambda \times \ln(10)} [\frac{\varepsilon_i(\lambda)}{(\varepsilon_i(\lambda) + \chi \times \varepsilon_{out})^2 + \varepsilon_i(\lambda)^2}]. \quad (1)$$

When the dielectric constant of βin and τoout are the dielectric gold nanoparticle constants and the exterior world, the dynamic dielectric constant depends on the period of incident light and can be represented as $\varepsilon(\lambda) = \varepsilon_r(\lambda) + \varepsilon_i(\lambda)$, where $\varepsilon_r(\lambda)$ and $\varepsilon_i(\lambda)$ reflect both the actual and the theoretical. N'est the mass of the photon. For the case of the sphere · for a standard 2 meaning is the metal nanoparticles form factor.

Incidence of LSPR depends on the material's scale, geometry, anti-particle space, dielectrical properties as well as dielectrical properties. The maximal wavelength (αmax), which can be calculated by using ultra-violet-visible extinction spectroscopy in the case of nanoparticles, is extremely susceptible to the n refractive index. μmax therefore shifts when the adsorbate is connected to AuNPs by changing the local environment's refractive index. The LSPR change is due primarily to the adsorbate and the maximum shift in LSPR extinction wavelength is given as follows:

$$\Delta\lambda_{max} = m \times \Delta n \times [1 - \exp(-\frac{2d}{l_d})]. \quad (2)$$

Where m is the bulk refractive indicinal reaction of the nanoparticle, oscillating the adsorbate refractive index is oscillated, d is the adsorbate thickness and l_d is the duration of the deteriorating electromagnetic field.

RESULTS AND DISCUSSION

In many countries different kinds of food products like powder milk and powder formula were contaminated by melamine poisonous substance. Due to this adulteration, many infants and adults were associated with renal damage, central nervous system, and placenta. Therefore this study is made to detect food industry, milk production industry, and disreputable preparation. In order to find out what way these toxic substance affect different organs of human body.

Melamine measurement based on colorimetric detection

The colorimetric detection process for melamine is seen in Figure 2 (a). Due to the repulsive force between citrate anions at the surface of AuNPs, the synthesized AuNPs are stable in an aqueous solution that prevents auNPs from aggregating (Sakellari et al. 2020). The AuNPs solution with its citrate consistency is wine-red. The electrostatic repulsion among AuNPs

is screened when the melamine solution is applied, resulting in the accumulation of AuNPs and the color of a solution transforms into bleu (Chang et al. 2017). The explanation is because of the association between three amine groups (-NH2) of melamine molecules and the dissociation of citrates ions from the surface of AuNPs, which makes them lose capacity to stabilize AuNPs (Chang et al. 2017)

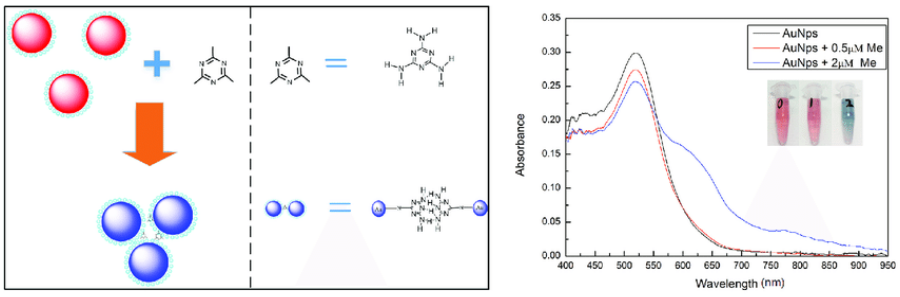

Figure 2: (a) Mechanism of the colorimetric detection of melamine based on the unmodified AuNPs; (b) UV-Visible absorption spectra of AuNPs (blank), AuNPs+0.5µM melamine and AuNPs+2µM melamine.

The colorimetric sensing process of melamine as seen in Figure 2. (a). Due to the repulsion force of citrate anions at the surface of AuNPs, the synthesized AuNPs are stable in an aqueous solution (Grys et al. 2020). The AuNPs solution with citrate stabilization is wine-red. The electrostatic discharge between auNPs is observed when melamine solution has been applied, resulting in an AuNP accumulation and blue coloring of the solution. The explanation is that three amine classes of melamine ($-NH_2$) can associate with AuNPs and their dissociation from the surface of AuNPs will trigger them to lose their capacity to stabilize AuNP (Grys et al., 2020) (Figure 3).

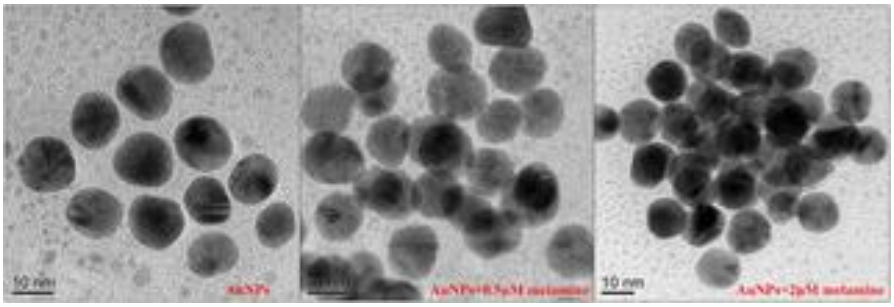

Figure 3: TEM images of the citrate-stabilized AuNPs, AuNPs+0.5µM melamine and AuNPs+2µM melamine.

Establishment of standard curve

In addition, the AuNP solutions are complemented with regular melamine solutions with varying amounts, 0,05, 0,2, 0,4, 0,6 and 0,9 µM. The rise in melamine concentration results in the decrease in the absorption of AuNPs with wave length 520nm, as can be seen in Figure 4. We are evaluating the absorption gap (To A) between the bare AuNP solution and the AuNPs solutions applied to melamine with different concentrations as the abscissa variable and the melamine variable as the standard variable for the quantitative examination of the interaction between melamine concentration and AuNPs absorbance. A strong linear curve between concentration and absorption is obtained with an R-square of 0.99. The fit-in equation for the linear curve is y = 0.026x + 0.01 and the sensor maximum

is 33 nM as determined by the formulation where α is the default variance and slope is the correct pitch. The linear measurement curve obtained is shown in Figure 4.

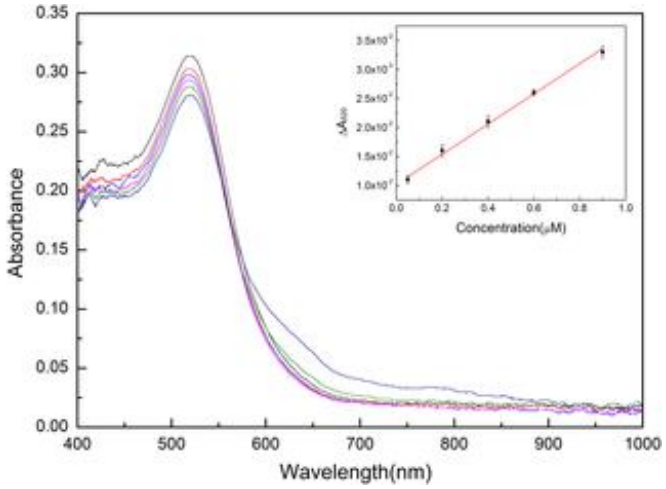

Figure 4: UV-Visible absorption spectra of AuNPs added with different concentrations of melamine (0, 0.05, 0.2, 0.4, 0.6, and 0.9 μM) and the calibration curve of melamine detection (inset).

Detection of melamine in liquid milk samples

This portion includes more tests to test the viability in actual milk samples combining varying concentrations of melamine of the suggested procedure for detecting melamine (Chen et al. 2015).

During the process mentioned above, milk samples with varying melamine levels are produced (Chen et al. 2015). With the Vortex-Qilinbeier5, melamine solutions with 0.5 μM, 1.5 μM and 2.5 μM concentrations were stirred for 0.5 min. It was exactly from above 0.5 μM, 1.5 μM and 2.5 μM of melamine solutions that 100 μL of melamine solutions were extracted, supplemented by 400 μL of AuNPs for a 5-fold decontamination. Afterwards, this optical fiber-based LSPR detector system was used to take 100 μL of the mixtures and measure their absorption range.

Results from the tests indicate that the reaction relationship between wavelength 520 nm absorbance values and liquid milk melamine concentrations is still linear in the 0.1μM to 0.9 μM range. Table 2 displays a measured recuperation rate of the colorimetric measurement identification of melamine samples. With detection limit of 33 nM a recovery rate of 99.2 percent was reached by ~111 percent. This retrieval study demonstrates that the AuNPs colorimetrical resonance of melamine in liquid milk is also suitable for the fast detection of melamines in practice optical fiber dependent Localized surface plasmon.

CONCLUSION

The optical fiber-based biosensing method for the colorimetric identification of melamine in liquid milk was explored using the optical fibre-based localised surface plasmone resonance AuNPs. It does not only have strong advantages over the traditional ultraviolet visible spectrophotometer, since it is fast and precise, but in reality it is often stable to detect melamine effectively in actual milk samples.

The experimental findings indicate a strong linear association between the absorption values at the wavelength of 520 nm and liquid milk concentrations in the range 0.1μM to 0.9μM and the detection maximum 33 nM. In comparison, in the liquid dairy samples there is a recovery rate of 99.2 percent ~111 percent. The technique suggested will have a tremendous opportunity for use and most molecules can only be

added by choosing the necessary ligands for the molecules to be identified. Altogether these types of defensive steps can decrease global disaster in health sectors as well as increase the trust issues in general people. So, it is high time to create awareness among the general people about the adulteration of food products through the melamine.

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