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A Facile Technique of Sensing Adulteration in Emulsion: A Road to Safety

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Research Article

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Abstract

Urea is a regular contaminant used in raw milk. Excess intake of urea-contaminated milk produces various health hazards. The key experimental challenge is to develop a rapid and inexpensive urea detection technique. Despite multitude of analytical schemes, they fall short either in rapid sensing or in cost effectiveness. In the quest of solutions, we unravel the systematic and procedural colorimetric quantification of urea through pristine gold nanoparticles. The sensing provides a limit of detection of 0.03 mg/ml, which is well below the permissible range of the World Health Organization (WHO). Being endowed with an excellent linearity of~0.99 and cost effectiveness, the sensing unit possesses a good reproducibility with potential scope in real sample analysis.

Keywords: Safety; Milk; Food Industries; Urea

Introduction

Maintaining quality of food products has become a major challenge for producers as well as regulatory agencies. Several protocols are followed in keeping nutritional elements intact. Despite existence of stringent regulation conditions in food industries, the compositional analysis reveals the presence of some contaminating elements leading to a loss of nutritional order. The presence of adulterating species makes the whole thing unusable. Milk is a vital food product, which caters from infants to adults. It is an emulsion of fat in watery solution of sugars, mineral salts and proteins in colloidal solutions. It is the base of whole dairy industry.

It is known that the milk is one of the most frequently adulterated item in the country. In countries like India, Pakistan, China, Bangladesh etc., the demand of milk exceeds the supply, which is fuelled by rising human population and urbanization. Consequently, the producers and vendors are tempted to adulterate milk. Several reports of adulteration in milk have been reported recently [1-9]. The common chemicals adulterants**,** which have been reported in milk, are sodium hydroxide, formalin, hydrogen peroxide, cane sugar, starch, gelatine, synthetic dyes, soap detergents and urea [10-18]. Among them, urea is frequently used in raw milk as per reports [4-19].

The typical concentration of urea in milk is 18-40 mg/dl [1, 4, 9-13]. Urea is a normal constituent of milk and it is a major part (55%) of the non-protein nitrogen of milk. However, for commercial benefits chemicals like urea, caustic soda, refined oil and detergents are used to

adulterate the milk. These adulterations decrease the nutritive value of milk and pose a great threat to human health [1]. A cut off limit of urea in milk is normally accepted at 70 mg/dl. The presence of urea above the cut off limit in milk can cause health problems such as indigestion, acidity, ulcers, cancer etc. Hence, quantitative detection and estimation of urea play a significant role in dairy industries and food processing technology.

A variety of analytical techniques has been developed for the determination of urea, although no single technique is that versatile to provide satisfactory results in all areas [19-32]. Methods are often categorized as Direct or Indirect [13]. The term indirect refers to the enzymatic degradation of urea prior to detect. Direct procedures have been defined as those resulting in a coloured product, which indicates the presence of urea, but this method, cannot give quantitative measurements. Various analytical techniques available to detect urea are based on colorimetric or chromatographic methods, which consume much time to get the species analysed and are too tedious and expensive to be used. Although lacking the inherent selectivity of the UV absorbance measurement also offers a convenient method of detection when coupled with chromatographic or electro-phonetic separation [14]. Infrared spectrometry is another technique for the dairy industry research, where this technique is already employed for analysis of milk fat and proteins.

Recently several methods for melamine detection, including chromatographic methods [9-14], which consume much time to get the species analysed and are too tedious and expensive to be used. Also high-performance liquid chromatography and potentiometric and electrochemical methods are time consuming, are expensive and need trained people [4-8]. Some authors have also proposed nanotechnology based methods [9-11]. In the current study, we aim to synthesize gold nanoparticles (AuNPs) and exploit them for visual detection of urea in milk as a colorimetric probe. The citrate stabilized gold nanoparticles are wine red in colour in the absence of urea, whereas in the presence of urea they change to blue because urea causes the aggregation of nanoparticles.

Experimental Procedures

Materials

Chloroauric acid, Trisodium citrate were purchased from Sigma Aldrich (USA). All the chemicals are used without further purification. For controlling the pH of the solutions, 0.01M NaOH solution was used. Aqua regia was used to clean the all glass wear. Ultra pure Milipore water had been utilized in all analysis.

Synthesis of nanoparticles

All glassware used in preparation of AuNPs were dipped in freshly prepared aqua regia (HNO_3/HCl, 1:3) and rinsed thoroughly. AuNPs were prepared by the citrate reduction method. Chloroauric acid (100 ml, 1mM) was boiled in a 250 ml round-bottom flask. Then, 5ml of Trisodium citrate (38.8mM) was added rapidly into boiling Chloroauric acid solution with rapid stirring. The pale yellow colour of Chloroauric acid gradually changed to a wine red colour within 3 minutes. Stirring was continued for 15 minutes. The wine reddish AuNPs formed were then cooled to room temperature and stored at 4ºC for further use.

Colorimetric Sensing Protocol

Initially, 4.0 mL raw milk (real sample) has been taken in a 10 mL centrifuge tube and 1.2 mL of 300g/L Trichloroacetic acid (TCA) has been added. Subject to cetritrifugation for 5 minutes, the supernatants are then transferred into another centrifuge tube. Afterwards, the pH is adjusted to 7 with small amounts of 6 M NaOH. Finally, the filtrate is extracted. Subsequently, 100 µL of the obtained filtrate has been added to 400 µL GNPs solution and kept at room temperature. On addition of traceable amount of urea, there arises distinguishable colour change from the pristine conditions. Finally, absorption spectra of the reacted solution were recorded with 1cm path length cell. The schematic of the elaborated procedure is shown in Figure 1.

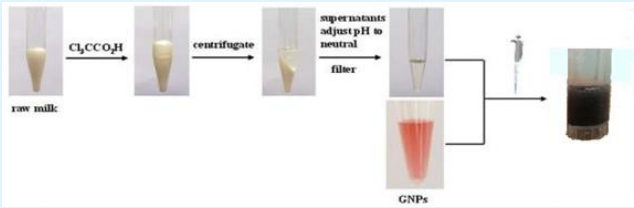


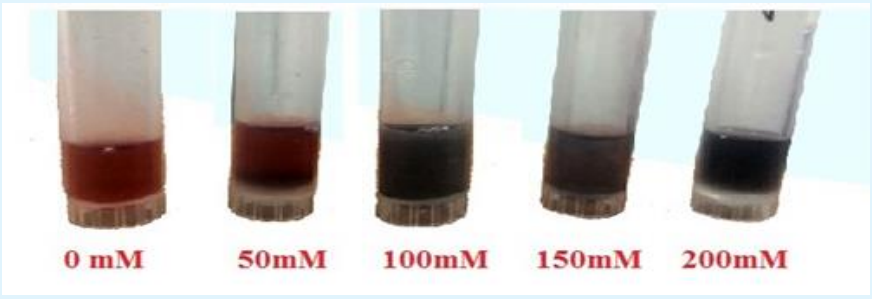
Figure 1: Schematic representation of the analytical process for determining urea in raw milk.

Results and Discussion

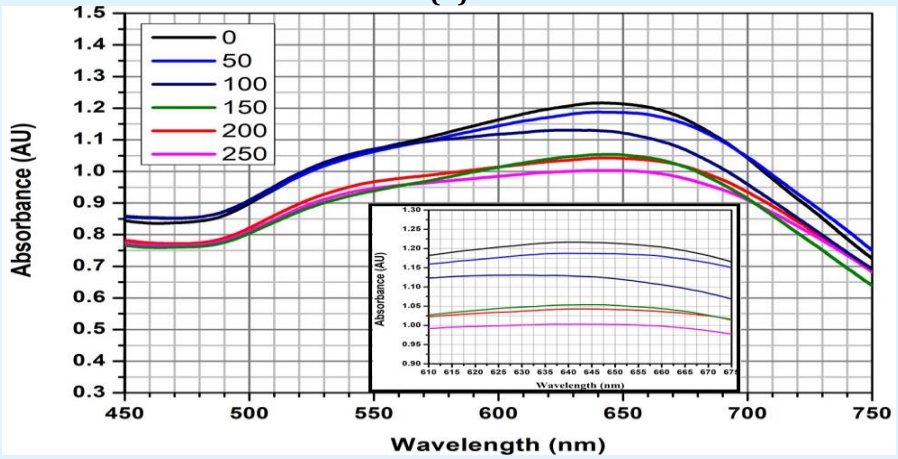
Principle of Urea Detection using AuNPs

The principle of urea detection is based on the surface stabilization of AuNPs. AuNPs are stabilized by negatively charged citrate ions. The negatively charged citrate ions

constitute an electrostatic layer on AuNPs, keep the nanoparticles separated, and stable in aqueous solution. However, in presence of urea, there arises destabilization and aggregation of nanoparticles, which further increases with concentration of urea. Urea possesses two amide groups ($-NH_2$) that interact with AuNPs through the legend exchange with negatively charged citrate ions. This aggregation results in a red-to-blue colour change, as depicted in Figure 2(a). We have taken the UV-vis spectra of coolorimetric responses with varying concentrations of urea whcih facilitate the ease quantification of urea contamination in milk. Corresponding spectra are shown in Figure 2(b).



(A)



(B)

Figure 2: A. Photographs of GNPs solutions in the presence of urea with different concentration. **B.** UV-Vis absorption spectra of AuNPs in the presence of different concentrations of urea. Expanded view of absorption values (inset image).

The colorimetric sensing produces a good linearity of the order~0.99. We further observe the reproducibility of the results and found identical color change in specified proportions. As the sensing is done in controlled condiotions, the chance of other adulterating species is close to nill, except urea.

Optimization of Colorimetric Response

The current colorimetric method is based on urea catalyzed aggregation of AuNPs. The aggregation of AuNPs can be impacted by factors such as media pH and reaction time. Therefore, we investigated these parameters for making this method highly stable and reproducible. The *pH* of media is an important factor influencing the stability of AuNPs. When AuNPs and milk sample are prepared, we utilized Trichloroacitic acid (TCA); the pH of colloidal AuNPs solution is approximately 2. AuNPs exhibit best stability near neutral. When *pH* of filtrate from milk sample was highly acidic or basic, it caused the abrupt colour change of AuNPs even in the absence of urea. When *pH* of filtrate was adjusted to 7 using 6M NaOH, no colour change was observed in absence of urea, whereas the presence of urea caused a significant colour change of AuNPs. Thus, the *pH* of media was kept at 7.0 for all experiments.

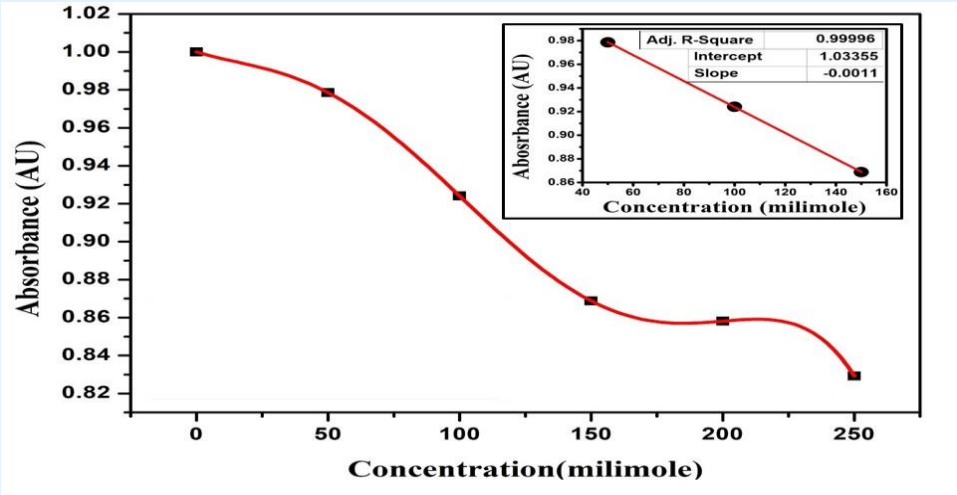


Figure 3: Absorbance vs. urea concentration of urea. (Inset shows the linearly fitted absorption spectra).

As shown in Figure 4, we performed a thorough analysis of absorbance with respect to two wavelengths From UV-Vis curves; we have taken two wavelengths 660 and 740. When the corresponding absorbance values of these two wavelengths are taken into account, we find a linear rise with increase in concentration of urea in raw milk. This profile validates the functioning of the colorimetric assessment of urea contamination in raw milk.

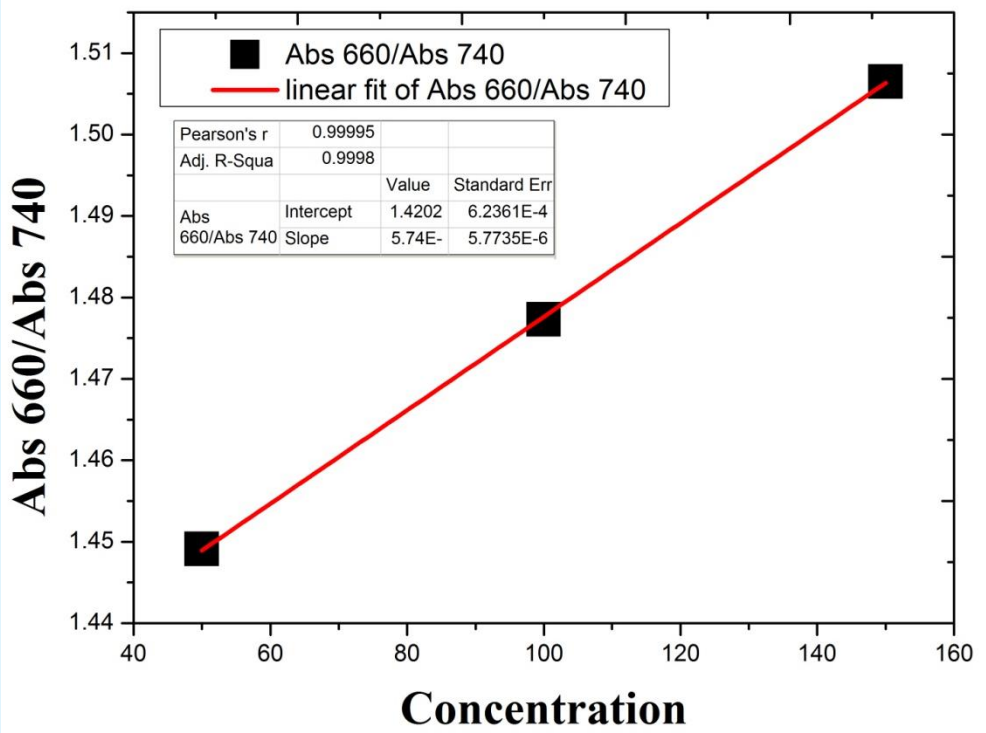


Figure 4: Calibration curve of urea detection Absorption ratio vs. Concentration of urea.

Performance Assessment

The limit of detection (LOD) of the urea was determined by spiking milk with increasing concentration of urea (0-250 mM). When the urea-spiking sample was added to AuNPs complex, concentration dependent aggregation was observed. The aggregation was monitored by change in the absorbance values of AuNPs. A linear correlation was obtained between absorbance and urea concentration ranging from 50 to 150 mM with regression value (R^2) of 0.99 (Figure 4). As per estimates, the detection limit of the present method for urea is 0.03mM and similarly, the limit of quantification has been determined to be 1.2 mM following the available techniques [33-41]. It is quite apparent that absorption values are accompanied by gradual decline with increasing concentration of urea. The scheme provides a well-defined linear range of 50mM to 150 mM of urea concentration. In the same note, the precision of the sensing scheme emerges to be 0.92 (normalised absorption value) +100mM. Apart from this, we find convergence of absorption estimates corresponding to increasing as well as decreasing concentrations of urea when the cycle is repeated over a long span of time with minimal deviation. This substantiates the stability of estimates done during the analysis, thereby validating the sensing approach. As the whole process is through visual eye detection with affirmation form UV-Vis spectroscopy, it is envisioned that it will help in fabrication of more portable arrays for other contaminants found in raw milk.

Conclusion

In this work, we have demonstrated an AuNP-based colorimetric method for the detection of urea contamination in raw milk. When urea contaminated milk interacts with AuNPs, there emerges colour change red/violet, detectable even without any aid of sophisticated instrument. Through varying concentrations of urea in milk, we have found considerable amplitude variation in absorption spectra. Overall, this colorimetric sensing scheme can detect traces of urea in milk down to a minimum value of 0.03 mg/ml. The reported experimental scheme could have enough potential to be applied for the impurity analysis in the case of other fodder products through tuning the shape and size of nanoparticles properly.

Conflict of Interest

The authors declare no conflict of interest.

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