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Ultrasensitive turn-on fluorescent detection of trace thiocyanate based on fluorescence resonance energy transfer

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ABSTRACT

Thiocyanate (SCN^-) is a small anion byproduct of cyanide metabolism. Several methods have been reported to measure SCN^- above the micromolar level. However, SCN^- is derived from many sources such as cigarettes, waste water, food and even car exhaust and its effect is cumulative, which makes it necessary to develop methods for the detection of trace SCN^-. In this paper, a simple and ultrasensitive turn-on fluorescence assay of trace SCN^- is established based on the fluorescence resonance energy transfer (FRET) between gold nanoparticles (AuNPs) and fluorescein. The detection limit is 0.09 nM, to the best of our knowledge, which has been the lowest detection LOD ever without the aid of costly instrumentation. The fluorescence of fluorescein is significantly quenched when it is attached to the surface of AuNPs. Upon the addition of SCN^-, the fluorescence is turned on due to the competition action between SCN^- and fluorescein towards the surface of AuNPs. Under an optimum pH, AuNPs size and concentration, incubation time, the fluorescence enhancement efficiency $[(I_F-I_0)/I_0]$ displays a linear relationship with the concentration of SCN^- in the range of 1.0 nM to 40.0 nM. The fluorescein–AuNP sensor shows absolutely high selectivity toward SCN^- than other 16 anions. The common metal ions, amino acids and sugars have no obvious interference effects. The accuracy and precision were evaluated based on the recovery experiments. The cost effective sensing system is successfully applied for the determination of SCN^- in milk products and saliva samples.

1. Introduction

As an important chemical raw material, thiocyanate (SCN^-) is widely employed in many industrial processes such as pesticide production, textile dyeing, electroplating, printing, photofinishing and hydrometallurgy. Compared with the lethal cyanide ion, SCN^- is a less toxic anion that can be found at μM in human bodies through the digestion of glucosinolate-containing brassica vegetables (broccoli, cauliflower, cabbage, etc.), or by ingesting cheese and milk, which contain SCN^- [1]. SCN^- ranks after perchlorate as a potent inhibitor of iodide uptake by the thyroid but may be more concentrated in some food items such as milk products [2]. And it is more serious for pregnant woman, infant or population in iodine deficiency area [3]. Besides, another significant source of SCN^- in the body is from smoker. Cyanide in tobacco, once absorbed, is eliminated through its conversion to SCN^- by the enzyme rhodanese, which is found in the mitochondria of liver and kidney cells [4]. Therefore, the concentration levels of SCN^- in human body liquids are considered to be an important indicator for assessing habitual smoking behavior as well as distinguishing nonsmokers from smokers [1]. For the general population, low levels of serum SCN^- may predispose them to inflammatory or inflammation-mediated diseases [5]. All of these aspects underscore the importance of SCN^- determination.

Several methods for the SCN^- detection have been developed over the recent years, including ion chromatography [6], gas chromatography/mass spectrometry [7], colorimetry [4,8,9], electrophoresis [10], electrochemistry [11], fluorimetry [12], and surface-enhanced Raman scattering (SERS) [13,14]. However, some of these methods lack sufficient sensitivity to detect SCN^- at low levels (5–8.5 mg L^{-1}) [15], which correspond to the SCN^- levels typically found in milk samples. And most of them are time-consuming and costly or require sophisticated instrumentation and professional staffs. Hence, exploring an accurate, rapid, sensitive and low-cost method to detect SCN^- in milk and human body liquids still remains a challenge.

Fluorescence resonance energy transfer (FRET) has been widely exploited as an extremely useful tool in analytical and sensing applications [16,17]. FRET is a kind of non-radiation energy transfer form, and the energy of the donors is transferred to the acceptors by the interaction of electric dipoles. The efficiency of FRET is determined by many factors including the overlap extent between the emission spectrum of the donors and the absorption

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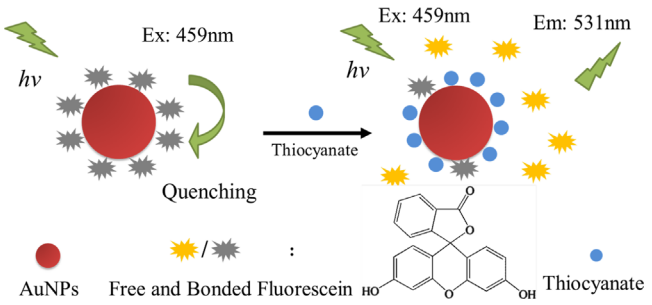
spectrum of the acceptors, the distance between the donors and acceptors, the relative orientation of the electric dipoles of the donors and acceptors and so on. Gold nanoparticles (AuNPs) have been widely exploited for chemical and biochemical sensing [18–21] because of their high surface area-to-volume ratios, size-dependent optical properties, and easy to be modified or functionalized chemically. Particularly, AuNPs have been explored as the efficient energy acceptors to substitute for organic acceptors [22] and selected for establishing a FRET system as a fluorescence quencher [23], which have been developed for detection of DNA [24] in the presence of Rhodamine B, protein [25] with aptamer as part of the biosensor, iodide and iodate [26] based on fluorescein-5-isothiocyannate-modified AuNPs, thiols [27] based on Nile Red-Absorbed AuNPs, medicine captopril [28], glyphosate [29] based on the FRET between charged CdTe quantum dots and AuNPs, metallic ions [30] using quantum dots and AuNPs co-sensing system. It was reported that fluorescein can be absorbed on the surface of AuNPs, resulting in fluorescence quenching due to FRET between fluorescein and the AuNPs to detect acetylthiocholine with the aid of enzyme [31] and melamine [32]. Inspired by these works, we have examined the potential of FRET between fluorescein and AuNPs as a novel and sensitive probe for the determination of SCN^- in milk products and saliva. Upon addition of SCN^- that competed with fluorescein molecules to absorb on the surface of the AuNPs, fluorescein molecules were freed from the surface of AuNPs and the fluorescence was recovered (Scheme 1). We have tested another 16 different kinds of anions and only the presence of SCN^- was able to restore fluorescence, allowing the ultrasensitive and selective determination of SCN^- concentration within 5 min. The aim of present work is to propose an alternative methodology with economic aspects, on site, real time and operating simplicity to conventional techniques for SCN^- detection in water, milk products and saliva samples of smokers and nonsmokers.

2. Experimental

2.1. Materials

Chemicals were of analytical grade and used without further purification unless otherwise stated. Hydrogen tetrachloroaurate hydrate ($HAuCl_4 \cdot 4H_2O$) was bought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Amino acids including L-arginine (Arg), L-histidine (His), L-lysine (Lys), L-tyrosine (Tyr), L-valine (Val) and L-threonine (Thr) were purchased from Shanghai Jingchun Technology Co. Ltd. (Shanghai, China). Na_2SO_4, Na_2SO_3, $Na_2S_2O_3$, Na_2S, Na_2CO_3, $Na_2C_2O_4$, CH_3CO_2Na, $NaNO_3$, $NaNO_2$, $KClO_4$, NaF, NaCl, kBr, KI, $FeCl_3$, $MgCl_2$, $CaCl_2$, $ZnCl_2$, and NH_4Cl were purchased from Shanghai Qingxi Technology Co. Ltd. (Shanghai, China). All the carbohydrates including D-fructose, D-glucose and sucrose were purchased from Shanghai Lanji Technology Co. Ltd. (Shanghai, China). The pH value of the solution was adjusted by mixing different volume ratio of NaH_2PO_4 and Na_2HPO_4.



Scheme 1. Schematic illustration for the sensing of SCN^- based on FRET.

2.2. Apparatus

All fluorescence measurements were carried out on F-4600 spectrofluorimeter (Hitachi, Japan) equipped with a xenon lamp source and a 1.0 cm quartz cell, and the scan speed was 12,000 nm min^{-1}. UV–vis absorption spectra were recorded using UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan) with a 1.0 cm quartz cell at room temperature. Transmission electron microscopy (TEM) was carried out with a JEM-2010 transmission electron microscope (JEOL Ltd. Japan). The samples were prepared by drop-coating the AuNPs solution onto the carbon-coated copper grid and were loaded onto a specimen holder for the purpose of TEM.

2.3. Preparation of colloidal Au NPs

All pieces of the experimental glassware used were cleaned in a bath of freshly prepared aqua regia solution (HCl:HNO_3, 3:1) and then rinsed thoroughly with H_2O and finally dried in the oven at 100 °C prior to use. Citrate capped gold nanoparticles with different sizes was synthesized based on the well-documented Frens' method [33] (trisodium citrate was used as reducing agent). In this method, it was possible to control the size of the particles by varying [Au(III)]/[citrate] ratio during the reduction step as listed in Table S1. A standard procedure for the preparation of AuNPs with size 13 nm was summarized as follows. A 50 mL aqueous solution of $HAuCl_4$ (0.25 mM) was heated to boiling point and 1.3 mL of trisodium citrate (1%) was added. The end point of the reaction was right after the solution reached a wine red color. The obtained wine-red solution was stored at 4 °C for further use. The UV–vis spectral characteristics and sizes for different sets of gold nanoparticles are summarized in Table S1 and results are in agreement with Frens' method. The molar concentration of 13 nm sized AuNPs was calculated according to Beer's law [34] (the molar extinction coefficient for 13 nm AuNPs is 2.78×10^8 $(mol\ L^{-1})^{-1}$ cm^{-1} at 520 nm).

2.4. Preparation of real samples

The pretreatment of liquid milk samples was carried out following the general procedure [35]. Briefly, 2 g milk product was added into 1.5 mL of 10% trichloroacetic acid and 5.0 mL of acetonitrile mixture to remove proteins in milk samples. The mixture solution was transferred to centrifugal tube to undergo sonication for 10 min and then centrifuged at 12,000 rpm min^{-1} for 15 min. The supernatant was filtered through a 0.22 μm membrane filter to remove lipids. The pH of filtrate was adjusted to 6.8, and the filtrate was filtered through 0.22 μm membrane filter again after centrifugation. The filtered liquid was diluted with water to 10 mL for further analysis.

In the case of infant formula, about 0.6 g sample was used for the determination. First, sample was dissolved with 2.0 mL water in a centrifuge tube, 1.5 mL of 10% trichloroacetic acid and 5.0 mL acetonitrile mixture were added to undergo 15 min ultrasonic treatment. Then, the mixture was centrifuged at 12,000 rmp min^{-1} for 15 min. The rest of operation was the same as the pretreatment of liquid milk.

Saliva is a cleaner matrix. Saliva samples from healthy smoking and non-smoking volunteers were collected and refrigerated for 30 min at 4 °C, then centrifuged at 3000 rpm for 10 min. After centrifugation, 100 μL of the supernatant solution was transferred into 10.0 mL volumetric flask and diluted to the mark with deionized water.

2.5. Detection of SCN^-

Typically, a stock solution of 10 μM fluorescein was prepared with Milli-Q water for the further use. 30 μL of 10 μM fluorescein was added into 200 μL AuNPs (15 nM), the resulted mixture was equilibrated at the room temperature for 10 min. Then, 770 μL of