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Sensitive turn-on fluorescence assay of methimazole based on the fluorescence resonance energy transfer between acridine orange and silver nanoparticles

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ABSTRACT

A new method for highly sensitive detection of methimazole was developed based on the fluorescence resonance energy transfer (FRET) between acridine orange (AO) and silver nanoparticles (AgNPs), in which AO acts as a donor and AgNP as an acceptor. The fluorescence of AO molecules was severely quenched when they were attached to the surface of AgNP by electrostatic attraction. Upon the addition of methimazole, AO molecules were detached from NPs because of strong adsorption of methimazole on the surface of AgNPs. Under optimum conditions, the enhanced fluorescence intensity displayed a linear relationship with the concentration of methimazole in the range of 8.0×10^{-9}–3.75×10^{-7} M, with a limit of detection of 5.5 nM. The method was applied for the determination of therapeutic levels of methimazole in human plasma samples with satisfactory results.

1. Introduction

Methimazole (2-mercapto-1-methylimidazole, Fig. 1a) is a major thioamide drug for treatment of thyrotoxicosis (elevated level of thyroid hormone). It acts mainly by preventing the synthesis of thyroid hormone through inhibiting the thyroid peroxidase-catalyzed reactions and blocking iodide organification [1]. Following an oral dose of 60 mg, peak plasma concentration of methimazole has been found to be in the range of 4.4–22 μM (0.5–2.5 mg L^{-1}) [2]. It has been reported that methimazole may also cause dose-related side effects including cutaneous reactions, arthralgia, gastrointestinal upset and agranulocytosis [3]. In addition, methimazole has been applied illegally to cattle as a fattening agent to obtain a higher live weight gain for economic interests [4]. Therefore, it is important to determine methimazole in various biological and drug samples. Up to now, several analytical methods have been developed for this purpose including high performance liquid chromatography (HPLC) and HPLC–mass spectrometry [5–7], gas chromatography–mass spectrometry (GC–MS) [8], thin layer chromatography (TLC) [9], potentiometry and voltammetry [10–13], flow injection spectrophotometry [14], resonance light scattering spectroscopy [15], derivative spectrophotometry [16], chemiluminescence [17,18], capillary electrophoresis [19,20], electrochemiluminescence [21], fluorimetry [22] and spectrophotometry [23]. These methods have their respective disadvantages, such as being time consuming and laborious and requiring complicated instrumentation or having low sensitivity. Therefore, it is necessary to develop simple, accurate and sensitive methods for the determination of methimazole.

Fluorescence resonance energy transfer (FRET) is a powerful and sensitive spectroscopic technique in which the excitation energy of a 'donor' fluorophore (D) is transferred to the 'acceptor' molecule (A) via a through-space dipole–dipole interaction. Energy conservation requires that the energy gap between the ground state and excited states of participating donor and acceptor molecules are nearly the same. This in turn implies that the fluorescence emission spectrum of donor must overlap with the absorption spectrum of acceptor and they should be within a minimal spatial range for donor to transfer its excitation energy to acceptor [24–26]. Traditional FRET pairs (e.g. organic dyes, fluorescent proteins and fluorescent polymers) suffer from inherent limitations of low quantum yields, narrow excitations and broad emission band and chemical and photo-degradation, which reduce the efficiency of FRET process. The introduction of nanomaterials like metal nanoparticles has greatly promoted the development of new FRET systems, which do not have most of the previously mentioned limitations [27–31].

Silver and gold nanoparticles (Ag and AuNPs) exhibit intense electronic absorption bands in the visible region, so they can be employed as either the acceptor or as both donor and the acceptor [32,33]. AgNPs have been widely investigated because they exhibit unique optical, electronic and chemical properties, depending on

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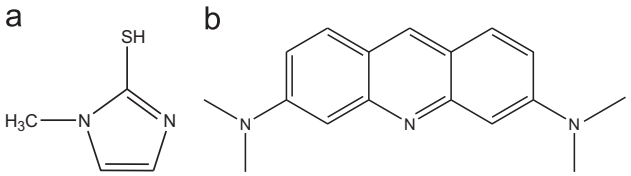

Fig. 1. Molecular structure of (a) methimazole, and (b) acridine orange.

their size and shape. But they have rarely been used as acceptors in FRET systems in place of organic quenchers [34,35].

The aim of this study is to develop a highly sensitive and selective AgNP-based nanosensor for the determination of methimazole. We prepared acridine orange (AO)–AgNP via AO noncovalent adsorption onto the surface of AgNPs which induces fluorescence quenching of AO as a result of FRET between AO and AgNPs. Upon addition of methimazole, they compete with AO molecules to adsorb on the surface of the AgNPs, and as a consequence, the fluorescence of AO switched to turn-on. We applied this probe for the determination of micromolar levels of methimazole in human plasma samples.

2. Experimental

2.1. Materials and reagents

Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), acridine orange and trisodium citrate were purchased from Merck (Darmstadt, Germany). A standard stock solution of methimazole (1.0×10^{-3} M) was prepared by dissolving an appropriate amount of methimazole (Loghman, Tehran, Iran) in distilled water and stored at 4 °C in dark place. Doubly distilled deionized water (obtained from Ghazi Serum Co., Tabriz, Iran) was used throughout. Human plasma, used as a real sample, was taken from Iranian Blood Transfusion Organization (IBTO).

2.2. Instrumentation

The fluorescence spectra were recorded using a Shimadzu RF-540 fluorescence spectrophotometer (Japan) equipped with a quartz cell (1 cm × 1 cm). UV–vis absorption spectra were obtained by a Cary-100 spectrophotometer (Varian, Australia). Transmission electron microscopy (TEM) images of the AgNPs were obtained by a Leo 906 transmission electron microscope (Zeiss, Germany).

2.3. Synthesis and characterization of AgNPs

Silver nanoparticles were prepared by a chemical reduction method described in the literature [36]. Briefly, 2 mL of 1% sodium citrate aqueous solution was added to an aqueous solution containing 50 mL of 2×10^{-4} M silver nitrate with vigorous stirring. After 10 min, 1 μL of 10 mM freshly prepared ice-cold $NaBH_4$ solution was added to the solution. The colloid was stirred for another 30 min and aged for 2 days at room temperature before being used. The absorption spectrum of AgNPs (Fig. 2a) shows a plasmonic peak around 410 nm. The size and morphology of the particles are determined using TEM image shown in Figs. 3a and S1 (Electronic Supplementary Material, ESM). The particles are spherical and have an average size of 20 ± 3 nm. The concentration of AgNP solution was calculated to be 5.1×10^{-11} M [37].

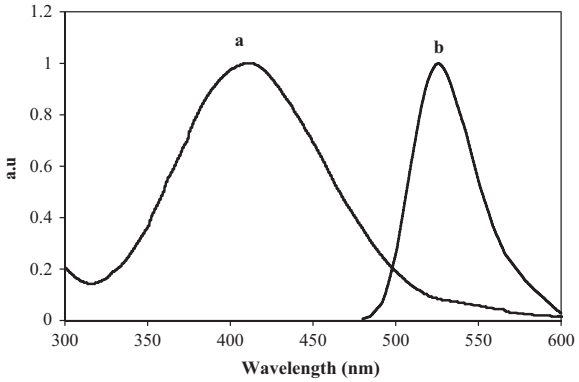

Fig. 2. (a) Absorption spectrum of AgNPs, and (b) fluorescence emission spectrum of AO at an excitation wavelength of 450 nm.

2.4. Procedures for the determination of methimazole

250 μL of 0.1 M acetate buffer solution (pH 5.0) was added to 1.0 mL of synthesized 5.1×10^{-11} M AgNPs. Then 175 μL of 1.0×10^{-5} M AO was added and the final volume of mixture was adjusted to 3.5 mL with deionized water. Afterwards, an appropriate amount of standard or sample solution of methimazole was added into the mixture and its fluorescence was measured at 525 nm with an excitation wavelength of 450 nm (F). A similar solution without adding methimazole was also prepared and its fluorescence intensity was recorded at the same wavelengths (F_0). The difference between two signals was used for calibration and analysis.

2.5. Sample preparation

Human plasma samples were spiked by adding different volumes of methimazole standard solution. A 500-μL aliquot of the spiked plasma was placed in a centrifuge tube and 2.0 mL of acetonitrile was added to precipitate proteins. The mixture was centrifuged for 15 min at 4000 rpm and its supernatant was diluted to 5.0 mL with deionized water in a volumetric flask. An appropriate aliquot of this solution was taken for analysis according to the general procedure.

3. Results and discussion

3.1. Fluorescence quenching of AO by AgNPs

One of the necessary factors for FRET to occur is the spectral overlap between the absorption spectrum of acceptor and emission spectrum of donor. As shown in Fig. 2, the AO emission spectrum (λ_{em}=525 nm) overlaps partially with the extinction spectrum of 20 ± 3 nm AgNPs (λ_{max}=410 nm). The as-prepared AgNPs are capped with citrate ions and negatively charged, while AO is a cationic dye (Fig. 1b) [38]. So, the AO and AgNPs coalesce to form larger clusters, due to charge neutralization, resulting in the formation of an AO–AgNPs assembly. TEM image of AgNPs in the presence of AO (Fig. 3b) confirms this interaction. The emission spectra of AO in the presence of varying concentration of AgNPs were also recorded. From Fig. 4, it is found that the fluorescence intensity of AO decreases gradually with the increase of AgNP concentration. The fluorescence quenching data are analyzed by the Stern–Volmer equation

$$F/F_0 = 1 + K_{sv}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively, K_{sv} is the Stern–Volmer constant (M^{-1}) and [Q] is the molar concentration of quencher [39].