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Silver nanoparticles ensemble with Zn(II) complex of terpyridine as a highly sensitive colorimetric assay for the detection of Arginine

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ABSTRACT

Arginine play numerous roles in several biological processes, and also act as a precursor for many biomolecules. Arginine derived biomolecules were found to involve in many of the events that lead to diseases and therefore selective detection of Arg in biological fluids has an enormous impact on disease diagnosis and in the drug development. Although number of methods were developed for the selective detection of Arg, the colorimetric method has an advantage over these methods due to their operational simplicity, high selectivity, and speediness. Herein, silver nanoparticles ensemble with Zn(II) complex of α-liopoic acid conjugated terpyridine (ZnLATP-AgNPs) were developed for the selective colorimetric detection of Arg. The nanoparticle ensemble exhibited selectivity towards Arg by showing distinguishable colour change from yellow to orange, among all the other naturally occurring amino acids studied. The new ZnLATP-AgNPs assay allows detection of Arg down to 200 ± 15 nM, provides an easy and sensitive method to detect Arg visually. The current approach was further validated by the quantification of Arg in supplement tablets.

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1. Introduction

Arginine (Arg) is a semi-essential amino acid, crucial constituent of animal and human diet and plays a significant role in the urea cycle and the energy metabolism (Barbul, 1986). Arginine promotes wound healing and stimulates the release of various hormones and enzymes (Stechmiller et al., 2005; Tapiero et al., 2002; Vermeulen et al., 2007). It acts as a precursor for several biomolecules and in the biosynthesis of nitric oxide (NO), a signal transduction molecule (Wu and Morris Jr., 1998) and also found to play a significant role in the setting of several diseases and in many of the events that lead to cancer (Stechmiller et al., 2004; Morris Jr., 2004; Lind, 2004; Gokce, 2004; Loscalzo, 2004). Hence the development of a sensitive method to quantify Arg in biological fluids has an enormous impact on disease diagnosis and in the drug development.

For the past several years, various methods such as high performance liquid chromatography (HPLC), liquid chromatography tandem mass spectrometry (LC-MS), enzymatic end point analysis and electrochemical, fluorescence and colorimetric sensors have been developed for the selective detection of Arg (Gopalakrishnan et al., 1996; Chen et al., 2011; Williams, 1993; de Orduña, 2001; Komaba et al., 1998; Ren and Yan, 2012; Zhou et al., 2011; Nasomphan et al., 2012; Cao et al., 2014). While some of these approaches made great contributions to the sensing of Arg in biological fluids, most of them require expensive instrumentation techniques and complicated analysis procedures. Colorimetric sensing method has an advantage over the methods mentioned above. This method opens the scope for an easy, inexpensive, rapid and sensitive method for real-time quantification of amino acids in biological fluids. Functionalized silver nanoparticles were widely used in the colorimetric sensing and imaging of various biomolecules due to their extraordinary photophysical, photochemical and bioactive properties (Doria et al., 2012). For example, cysteine modified silver nanoparticles have been used as a selective colorimetric sensor for histidine in the presence of Hg^{2+} (Li and Bian, 2009). *p*-Sulfonatocalix[4]arene and β-cyclodextrin (CD) modified Ag NPs were used for colorimetric sensing of histidine and chiral recognition of tryptophan respectively (Xiong et al., 2008; Li et al., 2009). Recently, picric acid capped silver nanoparticles were used for sensing creatinine in blood and cerebrospinal fluid samples (Parmar et al., 2016). Although nanoparticles clusters formed by the combination of zinc tetraphenyl porphyrin (ZnTPPS) modified silver nanoparticles and L-arginine was used for the chiral recognition of L-Histidine (Sun et al., 2012), quantification and real-time application of these silver nanoparticles clusters for the selective and sensitive detection of Arg have not been reported. Therefore, in this article, a simple and rapid colorimetric assay based on the silver nanoparticles for the selective and sensitive detection of Arg have been demonstrated. The method developed

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has also been successfully used for the quantification of Arg in supplement tablets.

The current assay constitutes of silver nanoparticle ensemble with zinc complex of lipoic acid conjugated terpyridine. In this assay, zinc complex of lipoic acid conjugated terpyridine (ZnLATP) was used as a recognition element that mediates the sensing of Arg to silver nanoparticles due to the known coordination ability of zinc with the groups present in Arg (Aoki et al., 2002; Alagha et al., 2011). For example, Zn(II) complex of benzo crown appended terpyridine has been used for the selective detection of Arg (Zhou et al., 2011). Initially, the silver nanoparticle ensemble is well dispersed in water and the colour of the solution was yellow. In the presence of Arg, the coordinating groups (guanidine, carboxylate and amine) present in the Arg interact with zinc complex on the silver nanoparticles which induces the crosslinking of neighboring nanoparticles. As a result aggregation of silver nanoparticle ensemble takes place that brings change in colour of the solution from yellow to orange. This sensing process was demonstrated by absorption and Transmission electron microscopy (TEM) and dynamic light scattering (DLS) studies. The principle involved in the detection of Arg by the present assay has been illustrated in the cartoon diagram shown below (Fig. 1).

2. Experimental details

2.1. Materials

All the solvents used were procured from local source, dried and distilled before use. Silver Nitrate and 28–30% ammonia solution were purchased from Merck (India). Sodium borohydride was procured from (s.d. fine-CHEM limited, Mumbai). 2-acetyl pyridine, 1-ethyl-(3-dimethylaminopropyl)-3-carbodiimide hydrochloride salt (EDCI.HCl) and α-Lipoic acid were purchased from Sigma-Aldrich. $SnCl_2 \cdot 2H_2O$ was purchased from Spectrochem Pvt. Ltd. Hydroxybenzotriazole (HOBT), 4-nitro benzaldehyde and L-amino acids were supplied by SRL. D-Arginine was purchased from MP Biomedicals. 4′-*p*-aminophenyl-2,2′:6′,2″-terpyridine was synthesized following literature reported procedure (Das et al., 2006). All chemicals were of analytical grade and were used without further purification. Arginine supplement tablets containing 1000 mg each were purchased from Corona Remedies Pvt Ltd.

2.2. Characterization

^1H and ^{13}C NMR spectra were measured on a Bruker Avance II 500 FT NMR spectrometer working at 500 MHz. The mass spectra were recorded on a Micromass Q-ToF micro™ using the electrospray ionization method and elemental analyses were performed on Elementar Vario MICRO CUBE analyser. FT IR spectra were measured on a Perkin-Elmer Spectra GX 2000 spectrometer using KBr pellets. Absorption measurements were recorded on a Varian CARY 500 spectrophotometer. Transmission electron microscopy (TEM) measurements were conducted on a JEOL, model JEM 2100 transmission electron microscope. DLS measurements were performed using Spectro Size™ 300 (NaBiTec, Germany).

2.3. Synthesis of Lipoic acid conjugated terpyridine (LATP)

Alpha-lipoic acid (0.38 g, 1.84 mmol) was dissolved in dry CH_2Cl_2 and the solution cooled to 0 °C. 1-ethyl-(3-dimethylaminopropyl)-3-carbodiimide hydrochloride salt (EDCI.HCl) (0.375 g, 1.95 mmol) and hydroxybenzotriazole (HOBT) (0.248 g, 1.84 mmol) were added and stirred for 30 min. To this solution, amino terpyridine (**1**) (0.6 g, 1.84 mmol) was added and the stirring continued for another 30 min at 0 °C. The reaction mixture was allowed to come to room temperature and stirred overnight. Solvent was evaporated under reduced pressure and residue was re-dissolved in dichloromethane. The reaction mixture was

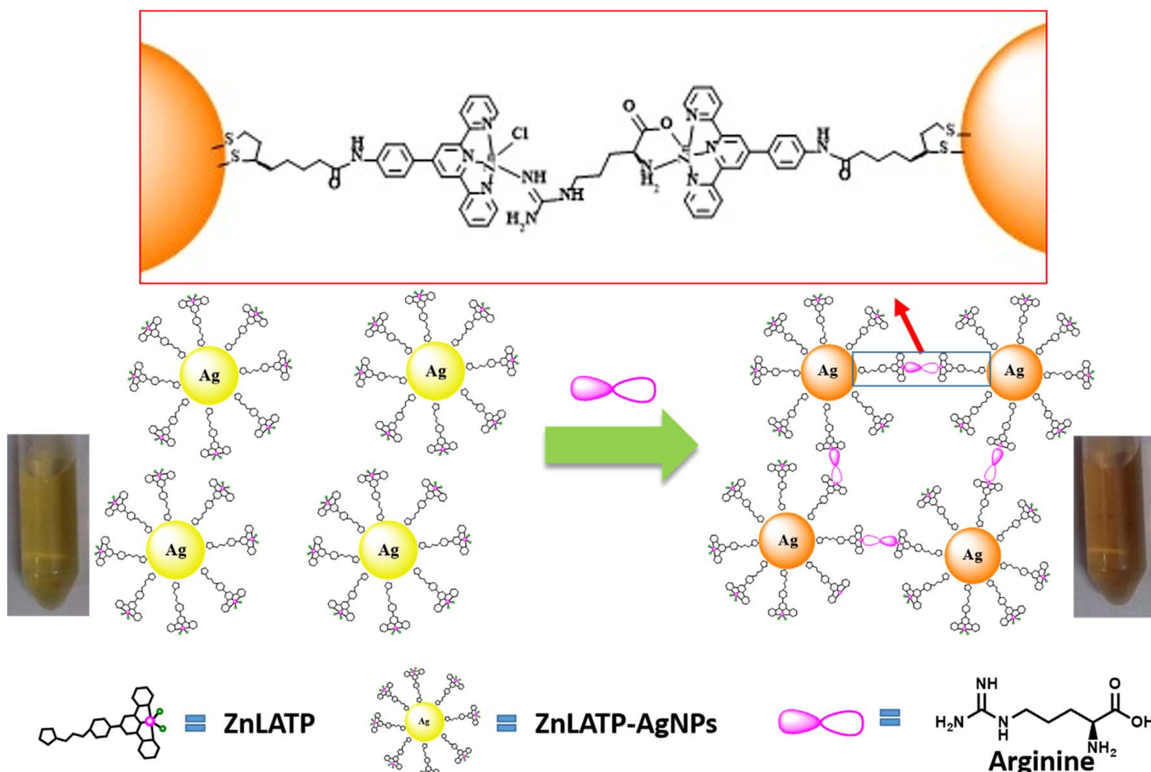

Fig. 1. Schematic representation of the detection principle involved in the assay.

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