Extended Abstract

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Spectrofluorometric assay using gold nanoparticles and cationic dye Rhodamine B for selective and sensitive detection of L-Cysteine in aqueous environment

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L-Cysteine (abbreviated as L-Cys) is an important thiol containing amino acid which is found in human plasma and is known as the primary building block of protein. This amino acid is involved in many essential and important biological processes in our physiological system. Although the presence of L-Cys in our body has number of health benefits, but excess amounts of this amino acid in human plasma or urine causes several health problems such as neurotxicity1, urinary stones2 etc. So, it is of prime importance to detect L-Cys selectively and more accurately in order to prevent our body from various diseases. In this present study we address a mechanism for selective and sensitive sensing platform utilizing the interaction of colloidal gold nanoparticles and cationic dye Rhodamine B (RhB) towards the detection of L-Cys from the fluorometric change of the dye molecules in an aqueous environment. Initially the presence of Au NPs causes the drastic reduction of fluorescence signal of RhB molecules in their mixed solution due to some non-radiative energy transfer process. But the addition of L-Cys solution to Au/RhB mixed solution recovers the fluorescence signal and is found to be linear within the concentration range of 0.01 µL – 1000 µL of L-Cys. The experimental limit of detection (LOD) was 0.01 µL and may be comparable to that present in human blood plasma. Also the recovery of fluorescence of RhB due to the selective interaction of L-Cys with Au NPs is accompanied with a colour change from wine to bluish black. The interference of all other amino acids including some thiol (-SH) containing amino acids along with some neurotransmitters (Na+, K+ etc.) present in our body have been tested in the same aqueous environment. The proposed mechanism for sensing of L-Cys is also tested with human urine sample to confirm its applicability to the real biological sample in vitro. UV-vis absorption and Transmission electron microscopy have been employed to characterize the as synthesized Au NPs. Our proposed fluorometric assay method for L-Cys detection may have great potential for biomedical applications with high degree of accuracy.

Introduction: The thiol containing amino corrosive L-Cys for the most part found in human plasma is notable as the structure square of proteins and is engaged with verity of significant natural procedures. Ordinarily a limited quantity of L-Cys is available in human body as N-acetyl-L-cysteine (NAC) generally known as L-Cys and is normally gotten from the amino acids serine and methionine. It is broadly utilized in pharmaceutical industry as medication to treat diverse sort of physiological intricacies . The body readies the significant cell reinforcement from NAC by changing over it right off the bat into L-Cys and afterward into glutathione. Cancer prevention agents, for example, or ascorbic corrosive (nutrient C) battle against the free radicals in our body and lessen oxidative worry before harm of the indispensable particles. For sound grown-ups about 4.1 mg/kg/day of L-Cys is required as suggest by Joint FAO/WHO/UNO master conference . In spite of the fact that L-Cys has various medical advantages, the raised measures of such amino corrosive may cause neurotoxicity , advances urinary stone development and so on and it can likewise be viewed as the natural marker for different illnesses . Accordingly, it is of most extreme significance to identify the centralization of L-Cys by an exceptionally delicate, specific and helpful strategy with serious extent of precision so as to forestall its unfriendly impacts in our wellbeing. Different logical strategies, for example, immunoassay, electrochemistry, chromatography and so on are as of

late being investigated for the identification of LCys and are acted in conjugation with fluid chromatography, fluorimetry, colorimetry, differential heartbeat voltammetry and spectrofluorimetry and so on. Be that as it may, immunoassay and chromatography required costly organic reagents and entangled instrumentations. Likewise the electrochemical strategies demonstrate moderately low selectivity to L-Cys. A few strategies use the fluorometric recognition after direct naming of appropriate fluorescent test with target bio-examiner. Anyway immediate marking once in a while causes basic rotation of the organic species coming about mistaken data. Additionally these recognition techniques straightforwardly using the outright fluorescence power once in a while may incorporate undesirable clamor because of natural bother in the estimation. Lately the colorimetric strategies are pulled in incredible consideration however are as yet constrained as a result of their absence of adequacy at extremely low fixation. Hence, it is of urgent significance to structure the suitable detecting stage for the discovery of L-Cys both specifically and quantitatively with serious extent of exactness in the physiological range. Toward this path, the strategy dependent on the fluorometric examine may offer incredible focal points for the discovery of L-Cys as they empower an immediate and level free identification in a watery domain even at extremely low fixation. Likewise the proportion metric fluorescence recuperation from the completely extinguished condition of any appropriate fluorescent test in nearness of the expert biomolecule guarantees the end of any outer annoyance engaged with the estimations.

Materials: The amino corrosive of our advantage i.e., L-Cys (C3H7NO2, M.W: 121.15 gmol-1) alongside all other basic and unnecessary amino acids utilized in this current work, RhB (C28H31ClN2O3, M.W: 479.02 gmol-1), chlorauric corrosive (HAuCl4, 3H2O, M.W: 339.79 gmol-1), glutathione (M.W: 307.32 gmol-1), ascorbic corrosive (C6H8O6, M.W: 176.12 gmol-1), uric corrosive (C5H4N4O3, M.W:168.11 gmol-1) were bought from Sigma Aldrich substance organization, USA and utilized moving forward without any more filtration. The virtue of RhB was checked by means of spectroscopic technique before use in the trial. Trisodium citrate (Na3C6H5O7, M.W: 258.06 gmol-1), sodium chloride (NaCl, M.W: 58.44 gmol-1), potassium chloride (KCl, M. W: 74.5513 gmol-1) were bought from Merck Chemical Company, Germany. All the dishes were cleaned with newly arranged aquaregia arrangement (3:1 blend of hydrochloric corrosive (HCl) and nitric corrosive HNO3) trailed by ensuing flushing with triple refined deionized Milli-Q water (resistivity 18.2 Mω cm, pH~7, gathered from Synergy coordinated with an Elix®-Advantage set-up, Millipore SAS, France) and afterward were autoclaved for 24 h before use. Fluid arrangements of the synthetics were likewise arranged with a similar triple refined Milli-Q water.

Conclusion: We have effectively built up a detecting stage for specific and quantitative recognition of significant bio-thiol to be specific L-Cys by means of fluorometric test of RhB and Au NPs in fluid medium just as in genuine biosample (human pee). The LOD for L-Cys was seen as 0.01 µM in our trial scope of focus. The underlying fluorescence extinguishing of RhB in nearness of Au NPs without L-Cys in the blend was perhaps ruled by FRET component which relies on the relative separation among color and nanoparticles. As the abatement in the radiative pace of RhB/Au NPs complex frameworks contributed just 20% of the general fluorescence extinguishing, which likewise recommends that the energized state vitality move was predominant factor for the radical fluorescence extinguishing. The specific fluorescence reaction of RhB/Au NPs complex sub-atomic congregations in nearness of L-Cys in the blend was likewise went with a shading change from wine to somewhat blue dark which was not watched for all other amino acids including other thiol containing mixes, to be specific L-Methionine, L-Homocysteine, glutathione in the equivalent watery condition. Moreover, the

other pertinent biosamples, for example, uric corrosive, ascorbic corrosive, synapses have indicated irrelevant obstruction over the particular assurance of L-Cys. In any case, Fig. 9. Genuine biosample examination by utilizing the fluorometric test with unadulterated human pee and amino acids. Fig. 10. Fluorescence rot plots of unadulterated RhB fluid arrangement and of RhB/Au NPs blended arrangement in nonattendance and nearness of L-Cys. P. Maiti et al Materials Chemistry and Physics 234 (2019) 158–167 166 the examine with genuine biosample appears about 35% fluorescence recuperation happened from the blend. This is essentially because of the typical nearness of L-Cys in ordinary pee. In watery arrangement the thiol moiety in L-Cys shaped solid holding with the citrate caped Au NPs and might have uprooted RhB particles from the outside of Au coming about the conglomeration of individual NPs as affirmed by UV–Vis ingestion spectroscopy. The TEM study affirmed that our orchestrated Au NPs was about circular with normal breadth of 27.5 nm. DLS and Zeta potential investigations uncovered the homogeneity of Au NPs circulation in watery arrangement and didn't total preceding their utilization in the examinations. Fluorescence lifetime of RhB adsorbed onto Au NPs surface was diminished contrasted with that of unadulterated RhB in fluid arrangement as affirmed by TCSPC study. Expansion of L-Cys in the blend reestablished the fluorescence lifetime of unadulterated RhB coming about the lessening of fluorescence extinguishing by means of FRET. In this manner, the current investigation might be proposed as an effective detecting stage for particular and profoundly delicate discovery of L-Cys through fluorometric measure system utilizing Au NPs and RhB.