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Molecularly imprinted Au-nanoparticle composite-functionalized EQCM sensor for L-serine

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ABSTRACT

Here, a piezoelectric sensor to assess L-serine (L-ser) through functionalized Au nanoparticles on EQCM electrode is fabricated via molecular imprinting. Molecular imprinting, an alternative to natural molecular recognition phenomena, is a robust methodology where polymerization of monomers in the presence of a target molecule imprints structural information into resulting network polymers. Molecular imprinting is being proposed for the development of novel biorecognition techniques for human health and bioterrorism protection technologies. Here, imprinting matrix was prepared by AuNPs functionalized with 4-ATP electropolymerized on self assembled monolayer (SAM) formed by 4-amino thiophenol (4-ATP) on the surface of gold layer coated on EQCM electrode in presence of L-ser as template. Various parameters were optimized for controlling the performance of molecularly imprinted polymer (MIP)-sensor such as the number of electropolymerization cycles, mass deposited in each cycle, pH, etc. The prepared electrochemical-MIP sensor showed good enantioselectivity and was highly specific towards L-serine. In the optimal condition, the response of the MIP sensor to L-ser was linearly proportional to its concentration with limit of detection (LOD) as 0.41 μM. Hence, a facile, specific and selective piezo-electrogravimmetric MIP sensor using surface-grafted specific molecular contours is developed for specific and selective uptake of L-ser in presence of various interferrants, in different kinds of matrices.

1. Introduction

Serine is a key component for the synthesis of neurotransmitters glycine and D-serine in brain. It is a non-essential amino acid, a polar one with a dipole moment of 12.9 [1], which plays central role in cellular proliferation as precursor of neurotransmitters and is essential for specific functions of the central nervous system [2,3]. Monitoring of L-serine (L-ser) is necessary for optimization of clinical therapy in patients suffering from psychiatric disorders. Molecular imprinting, a technique for creating artificial receptors is apposite for such monitoring in a facile manner [4]. MIPs often named as 'artificial enzymes' and/or 'artificial antibodies' are one of the most promising technique in sensor designing [5–8]. MIPs, offer high and almost unlimited-stability, their easy way of preparation at a large scale in lesser time scale - unquestionably outperform antibodies in many ways. In fact, a new era has begun by a synergistic merging of synthetic polymers (MIPs) with biomedicine replacing biosensors [9–11]. The versatility of this technology is proven by its applications in almost all arenas of life, comprising of separation, extraction, detection of either drugs or their metabolites [12–17], etc. The sensitivity and selectivity of MIP-sensing devices are augmented by incorporating nanoparticles in imprinting matrices to achieve the levels comparable to those of antibodies and enzymes. High surface area, better rates of electron transfer, easy tailoring for any transducing device and many more favourable features make nanoparticles a desired material among material scientist [18] as well as analytical scientists [19–24]. Keeping abreast of these developments, we are attempting here to incorporate functionalized Au nanoparticles for developing a piezoelectric sensing platform. Willner's group has introduced an imprinting matrix comprised of cross linked Au nanoparticles tethered on conductive surface; matrix created by electropolymerization of thioaniline functionalized Au nanoparticles on thioaniline-functionalized gold electrode for sensing a variety of analytes [25–35]. Ease of fabrication and their good sensitivity motivated us to fabricate a piezoelectric sensor for serine using thioaniline-functionalized Au nanoparticles with cysteine as a zwitterionic hook to enhance sensitivity.

Being a vital biomarker, L-serine level in cerebrospinal fluid is 18.6 μM, while it is raised to 21.5 μM in a schizophrenic patient, various analytical methods were developed for serine estimation [36–47]. Electroanalytical methods reported are an amperometric determination of L-serine with other amino acids on NiO nanoparticle-modified glassy carbon electrode [36]; an estimation of L-serine with L-phenylalanine also on bamboo charcoal derived carbon nanosphere electrode [37]. Other methods incorporating imprinted matrices are a self assembled imprinted monolayer of cysteine [38], a piezoelectric estimation using imprinted copolymer of ethylene glycol dimethacrylate and methacrylic

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acid casted on gold surface of quartz crystal [39], imprinted sol-gel [40], chiral separation of D,L-serine racemate by a MIP composite membrane [41], MIP separation membrane for estimation of D-serine [42], an amplified self-assembled cysteine modified electrode for the recognition of L-serine on porous gold film [43], MIP sensor for serine on carbon nanotube [44], sensor based on graphene sheet Congo-red MIP (GSCR-MIP) and organic thin film transistor (OTFT) [45], an electrochemical imprinted polycrystalline nickel-nickel oxide half nanotube-modified boron-doped diamond electrode was fabricated for the detection of L-ser in basic medium [46]. In spite of these sensing devices, various limitations such as cumbersome fabrication of electrode [37,46], pretreatment, matrix interference (either not tested [38] or could not be eliminated, or not verified in real samples [36]), large amount of chemical usage, high cost, etc., fail to deliver a universally accepted methodology. Hence, a sort of lacunae can be spotted regarding need of a facile, robust, specific, sensitive, economic method which would be able to avert such restraints and would be able to process a large number of samples. Here, a piezoelectric sensor to estimate L-ser through imprinting on functionalized Au nanoparticles on EQCM electrode is presented. In this work, an attempt is made to fabricate piezoelectric sensor to estimate L-ser through functionalized Au nanoparticles on EQCM electrode for the first time. High level of selectivity offered by MIP will be complemented here with extremely sensitive mass-measuring device (nano grams), quartz crystal microbalance (QCM) which allows dynamic monitoring of chemical interactions for fabricating a highly sensitive and selective piezoelectric sensor. QCM are well suited as transducer elements for chemical sensors, which dynamically monitors chemical interactions, using an oscillating crystal. As Speight and Cooper highlighted that 'Unlike the majority of biosensors that simply report the presence or absence of a molecule or analyte, QCM sensors can give information interaction with 'soft matter' in which changes in contact mechanics, interfacial dynamics, surface roughness, viscoelasticity, density and mass can be monitored in real time', QCM provide a higher level of information than other optical label-free techniques [47]. This work aims to prepare homogenous polymeric film surface with selective and specific cavities which is an indispensable requirement for a sensing matrix mimicking 'antibodies'.

2. Experimental section

2.1. Reagents and materials

L-Serine (99.0%), aryl chloride ($HAuCl_4$) and 4-aminothiophenol (4-ATP) (97%) were purchased from Sigma-Aldrich. L-Methionine (98.0%), L-alanine (97.0%), L-cysteine (97%) and L-aspartic acid (99.0%) were purchased from Loba Chemie Pvt. Ltd. Sodium dihydrogen orthophosphate, disodium hydrogen orthophosphate, sodium borohydride and potassium ferricyanide [$K_3Fe(CN)_6$] were purchased from Fischer Scientific Pvt. Ltd. Human blood plasma samples were collected from Institute of Medical Science (IMS), Banaras Hindu University (BHU) (Varanasi, India) as per approved protocol by the institutional ethical committee of the IMS, BHU after patients' written informed consent.

2.2. Instruments

2.2.1. Piezoelectrogravimmetric measurements

All electrochemical measurements were performed on a CHI 410B electrochemical workstation. For EQCM measurements, model 400B series of CHI 410B was used. EQCM contains a potentiostat/galvanostat (440B), an external box with oscillator circuitry and the EQCM cell. Two contact pins from the oscillator box is connected to the crystal holder of the EQCM cell. A gold electrode surface was coated on both faces of a 8 MHz AT-cut QCM chip. A seal is formed by two O-rings that are pressed by four screws. The diameter of the quartz crystal is 13.7 mm. The gold electrode coated on quartz crystal has a diameter of 5.11 mm. Gold coated quartz crystal was used as working electrode, a platinum wire and Ag/AgCl electrode were used as counter and reference electrodes respectively.

2.2.2. Electrochemical impedance spectroscopy (EIS) measurement

Electrochemical impedance spectroscopy (EIS) measurement was performed using an Autolab potentiostat instrument. For the EIS measurements, a sine wave potential with 5 mV amplitude superimposed on formal potential of the redox probe of 0.18 V was applied, a wide frequency range from 100 kHz to 1 Hz was scanned, and the impedances were recorded.

2.2.3. Scanning electron microscopy measurements

Scanning electron microscopy was performed by FESEM Quanta 200F at 5 KV in low vacuum. Al stub was covered with carbon tape and sample was put on carbon tape for imaging.

2.2.4. Atomic force microscopy measurements

Atomic force microscopy (AFM) was performed by instrument Solver Next model of NT-MDT Company which is used for visualization of evaluation of surface dominant feature. Contact mode with soft silicon nitride tip, covered with reflective gold coating on the back side, was used in order to obtain the topography images. The AFM imaging was performed in air. The scanned area of the sample 5 μm × 5 μm and scan rate of AFM was 0.5 Hz.

2.2.5. Contact angle measurements

Static contact angles of water were measured using the sessile drop method by contact angle goniometer equipped with CCD camera (model HO-IAD-CAM-01) from Holmarc Opto Mechatronics Pvt. Ltd., India.

2.3. Synthesis of functionalized gold nanoparticles (AuNPs)

For the synthesis of 4-aminothiophenol (4-ATP) and cysteine functionalized AuNPs, a 10 mL solution of $HAuCl_4$ (197 mg in 10 mL ethanol) was prepared and mixed with another solution containing L-cysteine (10 mg) and 4-ATP (24 mg) in 5 mL methanol and subsequently stirred in 2.5 mL of glacial acetic acid on ice bath for 2 h. To this resulting mixture, 7.5 mL aqueous solution of 1 M $NaBH_4$ was added drop wise resulting in a dark coloured solution associated with formation of AuNPs. This solution was stirred for an additional hour in ice bath, followed by 20 h at room temperature. The particles were successively washed and centrifuged with methanol, ethanol, and diethyl ether.

2.4. Modification of EQCM electrodes

EQCM electrode was immersed in piranha solution (H_2O_2/H_2SO_4 = 1/3) for 5 min, and rinsed exhaustively with deionized water. Thiol functionalized electrode was prepared by immersing the EQCM gold electrode in a 4-ATP solution (50 mM in ethanol) for 12 h. In order to prepare the *bis*-aniline-cross-linked AuNPs composite on the electrode, the surface-modified with 4-ATP were electropolymerized with 1 mg mL^{-1} solution of L-ser in a 0.1 M phosphate buffer solution (PBS) (pH 7.2) containing 1 mg mL^{-1} of 4-ATP-functionalized AuNPs. The polymerization was performed by application of 8 potential cycles between −0.35 and 0.90 V by cyclic voltammetry (CV) having Ag/AgCl electrode as reference and Pt wire as counter electrode, at a scan rate of 0.1 V/s. The resulting films were then washed with PBS to exclude any residual monomer from the electrode. Non-imprinted polymer (NIP)-coated EQCM electrodes was also prepared in similar manner without the template (L-ser) in polymerization solution.