Electrochemical detection of monosodium glutamate in foodstuffs based on Au@MoS2/chitosan modified glassy carbon electrode

Rashmita Devi, Satyabrata Gogoi, Shaswat Barua, Hemant Sankar Dutta, Manobjyoti Bordoloia, Raju Khan

Abstract

We report an amperometric immunosensor for the detection of monosodium glutamate (MSG) using a glassy carbon electrode modified with gold nanoparticle decorated on a molybdenum disulfide/chitosan (Au@MoS2/Ch) nanocomposite. In the present detection technique, Au@MoS2/Ch was used as a conductive matrix and anti-glutamate antibody was immobilized on its surface via carbodiimide coupling method. Chemical and morphological attributes of the various components of the immunosensor were confirmed by UV–vis spectroscopy, SEM, TEM and XRD analysis. Electrochemical characterizations were carried out by CV, DPV and EIS. Overall results showed the effective fabrication of highly conductive Au@MoS2/Ch nanocomposite for sensitive electrochemical detection of MSG. A linear relationship was perceived between the change in current and concentration of MSG. The relationship was found to be consistent in the detection range of 0.05–200 µM. Statistical validation of the assay showed limit of detection and limit of quantification values as 0.03 and 0.1 µM, respectively (R² = 0.99).

Keywords: Electrochemical Immunosensor Monosodium glutamate Molybdenum disulfide Gold nanoparticles

1. Introduction

Monosodium glutamate (MSG) is a sodium salt of a non-essential amino acid, which is widely used as a flavoring ingredient in a variety of food preparations. It is considered generally safe, yet there is a probability of developing monosodium glutamate symptom complex that can lead to uneasiness and nausea (U. S. Department of Health and Human Services, 1995). A concentration of 0.2–0.8% is acceptable in foodstuffs and the highest palatable dose for humans is approximately 60 mg/kg body weight (Prescott & Young, 2002). It plays a significant role in vital brain functions like formation and stabilization of synapse, cognition, learning, memory, etc. and also in cellular metabolism (Fontnum, 1984). However, ingestion of foodstuffs that are high in MSG content can result in the manifestation of psychiatric disorders like Parkinson’s disease (Gubellini, Pisani, Centonze, Bernardi, & Calabresi, 2004), Alzheimer’s disease (Fayed, Modrego, Rojas-Salinas, & Aguilar, 2011), schizophrenia (Boison, Singer, Shen, Feldon, & Yee, 2012) and depression (Paul & Skolnick, 2003). Considering the importance of MSG limits in foodstuffs, quantifying its levels with reliability and accuracy is of interest for researchers of many disciplines.

Different analytical techniques have been utilized to determine the concentration of MSG, including chromatography (Monosik, Stredansky, & Sturdič, 2013), fluorescence (Chapman & Zhou, 1999), capillary electrophoresis (Lada, Vickroy, & Kennedy, 1998), spectrophotometry (Khampha, Meevootisom, & Wiyakrutta, 2004), chemiluminescence (Blankenstein, Preuschhoff, Spohn, Mohr, & Kula, 1993), surface plasmon resonance (Cao & Sim, 2007) etc. The downside of these methods is that these can be expensive, labor intensive, time-consuming and utilize sophisticated instruments. In this regard, electrochemical sensors hold significant attention because of their reasonable limit of detection (LOD), reliability, affordability and ease of handling. Besides, combining the principles of immunological analysis in detecting analytes electrochemically, it provides the added advantage of high selectivity and specificity (Zhu, Cao, Sun, & Wang, 2013; Sun et al., 2012). The applicability of detecting minute concentration analytes can further be proliferated by incorporating nanomaterials, which dramatically improves the surface area and electrical conductivity of the electrodes, henceforth the performance. In recent years, nanomaterial-based electrochemical sensors have gained...
substantial interest. Amongst the various promising materials in nanotechnology, MoS₂ nanosheets have achieved a particular interest because of their unique physical and electrical properties and ease of synthesis (Barua, Dutta, Gogoi, Devi, & Khan, 2018; Gogoi & Khan, 2018). The ultrathin 2D MoS₂ layered structure provides high surface area and forms a promising supporting material to stabilize metal nanoparticles, forming metallic nanocomposites (Scharf, Goekte, Kotula, & Prasad, 2013). The synergistic effect of metal nanoparticles such as gold nanoparticles (AuNPs) and MoS₂ nanosheets helps in enhancing the charge transfer properties of the composite (Solanki, Soni, Pandey, Biradar, & Sumana, 2018). However, such composites suffer from challenges like poor adhesion and structural stability over the electrode surface. In this context, Chitosan (Ch) can be included in the metallic nanocomposite for boosting the biosensing characteristics. Ch gets chained into the plane of MoS₂ which doesn’t allow the readassembling of exfoliated MoS₂ sheets. Ch possesses remarkable structural and functional properties and possesses the ability to form uniform coatings on the electrode surface with good adhesion and permeability. Besides, Ch has abundant amino groups that provide active sites for immobilization (Huang, Liu, Liu, & Wang, 2014). Henceforth, the combination of such nanocomposites with electrochemical based label-free immunosensing technique is anticipated to widen the prospect of detecting MSG in foodstuffs with high sensitivity and selectivity.

Considering these facts, the present study reports an amperometric immunosensor platform for sensitive detection of MSG using Au@MoS₂/Ch nanocomposite. The study includes structural investigation and performance estimation of the sensor along with the statistical validation of the detection technique. The detection technique has also been demonstrated for analysis of glutamate in a real sample.

2. Experimental

2.1. Materials

L-glutamic acid monosodium salt hydrate 99% (MSG), molybdenum disulfide (MoS₂), chitosan (Ch), sodium citrate tribasic dehydrate 99%, gold(III) chloride (HAuCl₄), N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), bovine serum albumin (BSA), sodium phosphate monobasic (NaH₂PO₄), sodium hydrogen phosphate (Na₂HPO₄), potassium chloride (KCl), potassium hydroxide (NaOH), potassium ferrocyanide ([K₃Fe(CN)₆]₄), potassium ferricyanide ([K₄Fe(CN)₆]₃⁻) were procured from Sigma Aldrich, USA and used as received. Rabbit anti glutamate antibody (anti-Glu) was obtained from Merck Millipore, USA. Deionized (DI) water from a Millipore system (~18.2 MΩ cm) was used throughout the experiment.

2.2. Characterization techniques

UV–vis spectra were recorded in a spectrophotometer (SPECTORD-200, Germany). XRD patterns of MoS₂ were obtained by using a Rigaku (Rigaku, Japan) X-ray diffractometer (at scanning range 2θ = 10–60° and a scanning speed of 2.0° min⁻¹). The morphological study was conducted by electron microscopic analysis. A Field Emission Scanning Electron Microscope (Zeiss Sigma VP FE-SEM, Germany) was used to obtain low resolution morphological parameters. On the other hand, shape, size and high resolution morphology of Au and MoS₂ were visualized by a High-Resolution Transmission Electron Microscope (HRTEM JEOL, JEMCXII, Japan) at an operating voltage of 200 kV after coating the sample over a carbon-coated copper grid with 400 mesh (Ted-Pella Inc.). Electrochemical experiments were carried out in GAMRY Reference 3000, Potentiotstat/galvanostat/ZRA, USA. A typical three-electrode set up containing Au@MoS₂/Ch modified glassy carbon electrode (GCE) as working electrode, a platinum wire as auxiliary electrode and Ag/AgCl as reference electrode were used. Different electrochemical experiments, viz. cyclic voltammetry (CV), differential pulse voltammetry (DPV), electrochemical impedance spectroscopy (EIS) of Au@MoS₂/Ch-GCE were performed in 100 mM PBS containing 5 mM [Fe(CN)₆]₃⁻/₄⁻ solution at room temperature (25°C).

2.3. Preparation of MoS₂/Ch nanocomposite

MoS₂ nanosheets were prepared by exfoliation method (Liu et al., 2014; Wenelska & Mijowska, 2017). Bulk MoS₂ powder (5 mg) was added in 5 mL of water and ultra-sonicated for 10 h at room temperature. It was purified by the centrifuge method to obtain a stable dispersion. Then, Ch solution was prepared by dissolving 5 mg of Ch in 5 mL of doubly distilled water followed by the addition of 1% of acetic acid. This solution was subjected to constant mechanical stirring until a transparent solution was obtained. The exfoliated MoS₂ nanosheets (1 mg/mL) were then added to the Ch solution and allowed to stir for 1 h. The mixture was further sonicated for another 3 h to obtain MoS₂/Ch nanocomposite.

2.4. Preparation of Au nanoparticles (AuNP)

The Au nanoparticles (AuNP) were prepared by citrate reduction method (Storhoff, Elghanian, Musc, Mirkin, & Letsinger, 1998). An aqueous solution of HAuCl₄ (0.5 mM, 100 mL) was brought to a reflux condition with constant stirring. After that, 50 mL of 50 mM citrate solution was added immediately with a dropper. This results in a change in color of the solution from pale yellow to deep red. The solution was allowed to reflux for another 20 min and then cooled to room temperature.

2.5. Preparation of Au@MoS₂/Ch nanocomposite

The as-prepared MoS₂/Ch nanocomposite and AuNP were mixed in equal proportion. Then the mixture was sonicated by using ultrasound energy for 30 min. It was then stored in glass vials for different characterizations.

2.6. Preparation of modified glassy carbon electrode

Before modification, GCE was cleaned with 0.5 µm and 0.3 µm micro polish alumina (Buehler, USA) to give a mirror like finish (Mphuthi, Adekunle, Fayemi, Olasunkanmi, & Ebenso, 2017). It was washed several times in a sonication bath by using ethanol and water. After sonication, the electrode was allowed to dry thoroughly at room temperature. Typically, 10 µL of Au@MoS₂/Ch nanocomposite was drop cast onto the surface of a cleaned GC electrode with a micro-injector. The electrode was dried at room temperature for 48 h. As-prepared Au@MoS₂/Ch modified GC electrode was encoded as Au@MoS₂/Ch-GCE.

2.7. Fabrication of the immunosensor and detection of MSG

For selective detection of MSG, anti-glutamate antibody was immobilized onto the Au@MoS₂/Ch-GCE via EDC/NHS coupling. The modified GCE (Au@MoS₂/Ch-GCE) was soaked in EDC (0.4 M) and NHS (0.1 M) for 1 h to activate the nanocomposite system (Krittayavathananon & Sawangphruk, 2017; Hu, Zhao, & Wan, 2011). Subsequently, a stock solution of anti-Glu was prepared by adding 1 µL of anti-Glu in 100 µL of phosphate buffer solution (Khan, Gorski, & Garcia, 2011). In order to immobilize the antibody on the surface of the electrode, 10 µL of the solution of anti-Glu was drop cast onto the Au@MoS₂/Ch-GCE. It was incubated at 4°C for 12 h to obtain anti-Glu/Au@MoS₂/Ch modified GCE. After rinsing with PBS, 10 µL of 5 mg/mL BSA solution was deposited on the anti-Glu/Au@MoS₂/Ch-GCE to prevent non-specific adsorption. Finally, thus formed BSA immobilized anti-Glu/Au@MoS₂/Ch-GCE was rinsed with PBS in order to remove the unbound molecules and stored at 4°C. The prepared immunosensor was used in the detection of MSG. DPV experiment of modified GCE was
conducted by using GAMRY potentiostat/galvanostat (potential range of −0.3 to 0.7 V) for the analysis of MSG (Radhapary, Kotoky, Das, & Khan, 2013; Bhardwaj, Devarakonda, Kumar, & Jang, 2017). A series of MSG samples were prepared in buffer in the concentration range of 0.05–200 µM. The current variation of the immunosensor was recorded with the change in MSG concentration. A linear curve fitting procedure was adopted between the change in current and concentration of MSG by using Origin 8.5 analytical software. Statistical validation of the detection technique was carried out in Microsoft Excel 2010. Different statistical parameters such as sensitivity, lower detection limit (LOD), lower quantification limit (LOQ), linearity range etc. were determined. The same analytical procedure was adopted to analyze MSG containing real samples using commonly available foodstuffs. Locally manufactured soup item (MSG free) was collected from the market and the soup was diluted using water. Known amounts of MSG were spiked to the soup. Then DPV measurements were carried out by following the same conditions as described above. By measuring the change in current, the amount of MSG was determined from the calibration curve and thereby, the recovery percentage is calculated.

3. Result and discussions

3.1. Synthesis and characterization

AuNP were prepared by chemical reduction method by using sodium citrate as the reducing agent. Generally, the formation of AuNP follows a sequence of reactions. Small nuclei formed initially are coalescence to produce bigger particles that fall within the nanoscale region. Slow growth rate and subsequent reduction of HAuCl4 favor the formation of AuNPs with controlled morphology. Moreover, the morphological parameters viz. shape and size depend on the reaction condition and concentration of the precursors. Therefore, we followed a high temperature condition along with optimized reactant concentration to obtain the nano-dimensional AuNP.

On the other hand, MoS2 nanosheets were prepared by exfoliation method. The layers from bulk MoS2 were exfoliated by using the acoustic power of sound energy. High intensive ultrasound waves can peel off the layer nanosheets from the bulk sample. The exfoliated MoS2 were dispersed in low molecular weight Ch that was later grafting on the GCE. Ch chains bind to the successive layers of MoS2 nanosheets non-covalently, which prevent the restack of exfoliated MoS2 nanosheets so that they cannot collapse back to the bulk form (Ma, Xu, Xu, & Wang, 2017). Such dispersion of MoS2 in Ch was found stable for a long period of time. On the other hand, as-prepared AuNP were decorated on the surface of MoS2 by following a sonochemical method. The basic idea of such decoration was to obtain a nanocomposite system with high electrical conductivity. Generally, 0D/2D nanocomposite systems have gained tremendous interest in different domains in recent years. They possess high compatibility and often offer superior properties than the individual ones. MoS2 nanosheets as 2D nanomaterial are expected to hold 0D Au nanoparticles over their surface to provide a stable and conductive nanocomposite system. Thus, simple and one step methods were adopted to generate nanostructured Au, MoS2 and Au@MoS2 nanohybrid systems.

Individual nanomaterials and nanohybrid systems were characterized by using different spectroscopic techniques. UV–vis spectrum [Fig. 1(a)] of AuNP revealed an absorption near the wavelength 520 nm. This is the characteristic surface plasmon resonance peak of Au nanoparticles. On the other hand, MoS2 nanosheets showed two absorption peaks at wavelengths 615 nm and 673 nm [Fig. 1(a)]. (Chuang, Yang, & Chen, 2015) These peaks are assigned to the absorptions originated from the direct gap transitions at the K-point of the Brillouin zone. These peaks are also visible in the UV–vis spectrum of Au@MoS2 nanocomposite. This gives a hint about the decoration of AuNP over MoS2 nanosheets. XRD pattern confirmed the crystal phase of MoS2 nanosheets and is shown in Fig. 1 (b). It shows a space group P63/m. From the diffraction pattern, several peaks can be identified at diffraction angles (2θ) of 14.8°, 31.2°, 33.5°, 36.1°, 39.8°, 49.4°, 58.5° and 60.5°. They are assigned as (0 0 2), (1 0 0), (1 0 1), (1 0 2), (1 0 3), (1 0 5), (1 1 0) and (1 1 2) plane of nano MoS2 phase. The XRD peaks are confirmed by comparing with the data obtained from ICDD-PDF-2 (Card no-77-1716). Morphological attributes of the nanostuctures were studied by using electron microscopic studies. SEM images of MoS2, MoS2/Ch, Au@MoS2/Ch and anti-Glu/Au@MoS2/Ch were depicted in Fig. S1. Fig. S1(a) and (b) show that MoS2 possesses sheets like structure. Besides, Fig. S1(c) indicates the presence of AuNP over MoS2 nanosheets. However, the precise decoration of AuNP over MoS2 nanosheets is not very clear in the SEM images. It may be attributed to the fact that AuNPs are embedded within the 2D nanosheets, and in-depth electron scanning ability of the SEM technique is comparatively poor. Successful adsorption of the anti-Glu antibody was observed on the surface of Au@MoS2/Ch in Fig. S1(d). Thereafter, TEM analyses were carried out to obtain accurate morphological attributes of the nanostuctures. The low magnification TEM images showed the formation of exfoliated MoS2 nanosheets [Fig. 2(a)–(c)]. These images are imperative to confirm thin layered structures with lateral dimension distributions in the range of 100–200 nm. Fig. 2(d) shows HRTEM image of MoS2 nanosheets. A periodic arrangement of atoms on MoS2 nanosheets for selected area was also included in the inset of Fig. 2(d). The inner planar spacing was measured to be 0.27 nm which was attributed to the (1 0 0) facet of MoS2. EDX analysis showed the elemental composition of MoS2 nanosheets (Fig. S2). MoS2 contains Mo and S in a ratio of 37.49 : 62.51. Elemental analysis verified the phase purity of 2D MoS2 nanostucture. Similarly, Fig. 2(e) and (f) showed the morphological attributes of AuNP. They possess spherical shape. The average diameter was calculated as 14.4 nm with size distribution in the range of 12.7–18.4 nm. From the TEM images as depicted in Fig. 2(g) and (h), AuNP are clearly visible over MoS2 nanosheets. It can be seen from the figures that the AuNPs are distributed on the MoS2 nanosheets surface with high density. Thus, these images are conclusive about the successful generation of Au@MoS2/Ch nanohybrid system. The electrochemical properties of the nanocomposite system was evaluated and utilized in the modification of GCE for sensitive estimation of MSG. The fabrication process for electrochemical immunosensor (Au@MoS2/Ch) based on anti-Glu antibody immobilized on Au@MoS2/Ch-GCE is depicted in the Scheme 1(a) and (b).

3.2. Electrochemical characterization of Au@MoS2/Ch-GCE

CV and DPV are useful techniques to study the properties of surface modified electrodes (Su et al., 2016). The CV study of bare GCE, MoS2/Ch and Au@MoS2/Ch modified electrodes were carried out in 100 mM of PBS (pH 7.4) with 5 mM [Fe(CN)6]3−/4− and cycled between potential of −0.3 V to 0.7 V using Ag/AgCl as the reference electrode [Fig. 3(a)]. The figure reveals changes in the peak current and potential with every modified step. This provided the evidence for the structural and morphological changes occurred during the fabrication of the immunosensor. As shown in Fig. 3(a), a decreased redox peak was observed when MoS2/Ch nanosheets were only grafted on the GCE surface. This may happen due to the semiconducting behavior of MoS2 and non-conducting nature of Ch (Su, Sun, Xu, Yuwen, & Wang, 2013). But after the immobilization of Au@MoS2/Ch nanohybrid system, the peak current was increased. Such behaviors indicate improvement in surface area and conductive properties, which lead to the enhancement of the electron transfer between the electrolyte and the working electrode (Lin, Ni, & Kokot, 2016). Then, we were interested to immobilize anti-Glu antibody onto the electrode surface. Electrochemical behavior of anti-Glu immobilized Au@MoS2/Ch-GCE was initially investigated through CV. After immobilization of anti-Glu onto the Au@MoS2/Ch-GCE surface, a decreased redox peak current was observed. Here, it is pertinent to mention that anti-Glu was immobilized on to the working electrode by drop-casting process. It is therefore expected that the anti-

Glu will bind to the active groups available on the modified electrode surface. The bulky groups present in the antibody occupy the space over Au@MoS$_2$/Ch-GCE and thereby hinders the electron transfer between the electrode and solution, reducing the amperometric response. However, the reduction is much less as compared to the bare and MoS$_2$/Ch modified GCE, which confirmed the suitability of the modified electrode for immunosensing application.

The CV results were attributed to the 3D network structure of Au@MoS$_2$/Ch nanocomposite which could serve as a promising substrate to enhance the active loading of antibodies. To further confirm the effective immobilization of the antibody onto the electrode surface, we conducted the DPV study [Fig. 3(b)]. DPV is a prominent technique for measuring trace levels of organic and inorganic species where the measured current is directly proportional to the conductivity of the electrode and inversely to the concentration of the corresponding analyte (Pujol et al., 2014). Results indicate that the modification of GCE with MoS$_2$/Ch decreased the current response initially, but modification with Au@MoS$_2$/Ch increased the current corresponding to the peak potential as compared to MoS$_2$/Ch-GCE. But, immobilizing anti-Glu on the surface of Au@MoS$_2$/Ch-GCE lead to a reduction in the current response. Thus, all these independent studies confirmed the effective immobilization of anti-Glu onto the Au@MoS$_2$/Ch-GCE surface and also ensured good electrical conductivity of the immunosensor. These results are in accordance with the results obtained from the CV study.

3.3. Electrochemical impedance spectroscopy (EIS)

EIS is an effective tool for monitoring the interfacial properties of surface modified electrodes (Cheng et al., 2011). Here, electron transfer resistance ($R_{ct}$) was studied for the bare as well as modified GCE using EIS analysis. Fig. 3(c)–(e) depicted the Nyquist plot ($Z_{\text{Im}}$ vs $Z_{\text{Rea}}$) at different frequencies and the corresponding Bode plot ($Z$ vs Log ($\omega$)). In the Nyquist plot, the diameter of the semicircle portion (high frequency components) is proportional to the charge transfer resistance ($R_{ct}$), whereas the linear portion (low frequency components) can be correlated with the diffusion process. The bare GCE showed a small semicircle with the diameter of 560.5 $\Omega$. When MoS$_2$/Ch was immobilized on the surface of the GCE, the semicircle diameter (620.3 $\Omega$) increased distinctively, indicating the hindrance of electron transfer from [Fe (CN)$_6$]$^{3-}/^{4-}$ to electrode due to the poor conductivity of MoS$_2$/Ch composite. However, in the case of Au@MoS$_2$/Ch-GCE, the semicircle
was decreased dramatically (180 Ω). It suggests the faster electron transfer kinetics of $[\text{Fe(CN)}_6]^{3-/-4-}$ on the electrode surface, representing the excellent conducting property of Au@MoS$_2$/Ch-GCE. Nevertheless, immobilization of antibody increased the diameter of the semicircle (260.7 Ω) due to attachment of antibody molecules on the electrode surface as described previously. The calculated values of R$_{CT}$ are tabulated and shown in Table S1. The value of R$_{CT}$ for bare GCE is 788 Ω and was increased to 878 Ω after deposition of MoS$_2$/Ch on GCE.

Scheme 1. Schematic description of anti-Glu/Au@MoS$_2$/Ch-GCE immunosensor for detection of MSG.

Fig. 3. Comparison of the peak currents of GCE, MoS$_2$/Ch-GCE, Au@MoS$_2$/Ch-GCE, anti-Glu/Au@MoS$_2$/Ch-GCE: (a) in cyclic voltammograms and (b) in differential pulse voltammetry; EIS pattern of GCE, MoS$_2$/Ch-GCE, Au@MoS$_2$/Ch-GCE and anti-Glu/Au@MoS$_2$/Ch-GCE: (c) Nyquist plot (Z$_{\text{imag}}$ versus Z$_{\text{real}}$), (d) Bode plot (Z$_{\text{imag}}$ versus frequency) and (e) Phase shift.
Au@MoS$_2$/Ch-GCE showed $R_{CT}$ value of 258 $\Omega$. This value increased up to 405 $\Omega$ after immobilization of anti-Glu. Other relevant parameters like double-layer capacitance ($C_d$) and phase ($\Phi$) were also calculated from Fig. 3(d) and (e). The $C_d$ value is maximum for anti-Glu/Au@MoS$_2$/Ch-GCE and minimum for bare GCE. It implies that the accumulation of charges at the electrode-electrolyte interface is more in anti-Glu/Au@MoS$_2$/Ch-GCE, which reduces the efficient electron transfer between the electrode and the medium. This is in accordance with the results obtained from the Nyquist Plot and cyclic voltammetry analysis.

3.4. Analytical performance of the immunosensor

Analytical performance of anti-Glu/Au@MoS$_2$/Ch-GCE immunosensor was tested for the detection of MSG in buffer as well as in food samples. To assess the sensitivity and dynamic working range of the electrochemical immunosensor, DPV measurements were carried out in presence of varying concentration of MSG prepared in 100 mM PBS (pH 7.4) containing 5 mM $[\text{Fe(CN)}_6]^{3-/4-}$ as a redox probe. The variation of current with concentration was recorded in the concentration range of 0.05–200 $\mu$M. It was found that the redox peak current varies inversely with the concentration of MSG as shown in Fig. 4(a). The responses in Fig. 4(a) revealed that the recorded current gradually decreased with increasing MSG concentration from 0.05 $\mu$M to 200 $\mu$M. A specific non-covalent antibody-antigen interaction constitutes the keystone of the present immunoassay for selective detection of MSG. The components of the sensor also play a significant role to direct the detection process. The combination of MoS$_2$ and AuNPs enable the modified electrode with low background current, good conductivity and large electroactive surface area. Ch helps to obtain a film with high permeability, good adhesion and nontoxicity. It also facilitates the electron transfer after its swelling in the reaction mixture due to its hydrophilic nature. Thus, the combination of Au@MoS$_2$/Ch provides an excellent platform with enhanced electro-chemical properties. It facilitates an effective transfer of $[\text{Fe(CN)}_6]^{3-/4-}$ ions between the solution and the electrode surface. A consequent enhancement of peak current was observed for the modified electrode compared to the bare one. However, in the presence of MSG, surface captured antibodies interact with the glutamate molecules forming an anti-Glu/Glu immunocomplex. This causes the surface of the modified electrode overcrowded creating an inert electron and mass transfer blocking layer against the transportation of $[\text{Fe(CN)}_6]^{3-/4-}$ ions. Henceforth, the conductivity of the sensor decreases with the increase in MSG concentration due to the consequent reduction in the effective area and active sites for electron transfer. The decrease in current is correlated to the MSG concentration by using DPV measurements. A curve fitting procedure was adopted to establish the relationship between the changes in current with MSG concentration. As shown in Fig. 4(b), an excellent linear relationship was obtained. Statistical evaluation of the current detection technique was carried out. Regression analysis showed a correlation coefficient ($R^2$) of 0.99. The calibration curve in Fig. 4(b) shows a linear increase in the value of $\Delta I$ up to the concentration of 200 $\mu$M. However, beyond this range linearity was lost and a steady state was reached. The calculated value of LOD for the fabricated immunosensor was 0.03 $\mu$M and LOQ was 0.10 $\mu$M (estimated using the formula, LOD = $3\sigma/m$, LOQ = $10\sigma/m$; where $\sigma$ is the standard deviation of the response estimated by the standard error of y-intercept of regression line and $m$ is the slope of the regression line). The method holds an accuracy of $100.42 \pm 1.49$ over the detection range. The analytical parameters of the proposed immunosensor are
summarized in the supporting information section. A study was further undertaken to compare the performance characteristics of the present analytical technique with the contemporary literature, which shows that the results of the present study are well comparable with the reported ones (Table S2). In many occasions, sensitivity is even better. At this juncture, the authors wish to point out one important issue; i.e. the uncertainty in the permissible lower limit of MSG in many foodstuffs. Till date, there is no globally accepted permissible value available for MSG. However, most of the food regulatory agencies in many countries are exercising caution about MSG and imposed regulation on the indiscriminate use of MSG as a dietary flavor enhancer. For example, Europe’s Food Safety Authority (EFSA) has set a safe level for glutamate food additives which is 30 mg per kilo body weight per day. As per the Food Safety and Standards Authority of India (FSSAI) guidelines, MSG is not permitted in more than 50 food products including pasta and noodles. FSSAI has recently issued the guideline for maximum permissible limits of MSG to be added to canned crab meat (500 mg/kg maximum). However, in most of the foodstuffs, the lower permissible limit is not defined precisely. Therefore, on many occasions, it becomes difficult to set a target for lower detection value while developing an analytical technique. Thereby, a wide diversity in the detection limit can be observed in the reported literature, which is also evident from the comparison table (Table S2). Based on these points, we may conclude that the present technique possesses the potentiality to be developed as a tool to test real food samples.

3.5. Process optimization: pH, time and temperature

Different conditions such as pH of the medium, temperature, time etc. play a crucial role in a detection process. Therefore, we studied the influence of these parameters on the analytical performance of anti-Glu/Au@MoS2/Ch-GCE for the detection of MSG. The redox peak current of anti-Glu/Au@MoS2/Ch-GCE with and without MSG at different pH levels was measured and presented in Fig. 4(c). From the figure, it can be observed that the redox peak current of anti-Glu/Au@MoS2/Ch-GCE increased initially with increase of the solution pH from 6.2 to 7.4 and then it followed a decreasing trend with a further increase in pH. Therefore, it can be ascertained that anti-Glu/Au@MoS2/Ch-GCE possesses high conductivity in the mid pH region. On the other hand, in presence of a constant amount of MSG, maximum variation in peak current was observed at the pH of 7.4. In acidic and basic conditions, the variation of peak current is low. In acidic conditions, glutamate is protonated to glutamic acid thus prevent the ability of the antibody to interact with glutamate ion in the solution. However, in basic solutions, the changes in potential are due to the interference of hydroxyl ions for the cationic site at the functionalized electrode surface. Strongly acidic or alkaline environments would result in the denaturation of the antibody; hence mild pH condition was maintained to ensure the physiological activity of the antibody.

On the other hand, the analytical performance also varies with the temperature. The best analytical performance was achieved in the temperature range of 20–25 °C. With the increase in temperature, performance decreases drastically. This is obvious, because the antibody is denatured at high temperature (Fig. S3(a)). Incubation time is another important factor which may influence the detection process. The immunosensor was incubated at 25 °C for different intervals of time as shown in Fig. S3(b). It has been found that change in current increased with an increase in time and reached the maximum after 30 min. Thereafter, the change in current started decreasing. Therefore, 30 min was chosen as the optimal incubation time.

3.6. Selectivity, reproducibility and stability of the immunosensor

As we know, selectivity is another influential factor for evaluating the performance of an immunosensor. Therefore, specificity of anti-Glu/Au@MoS2/Ch-GCE towards MSG was tested in the presence of cysteine, arginine, aspartic acid, butylated hydroxytoluene and bisphenol-A as interfering species. The variation in current (ΔI) of the immunosensor in the presence of MSG was found larger than those with cysteine, arginine, aspartic acid, butylated hydroxytoluene and bisphenol-A at same analyte concentration (50 μM) and experimental conditions (Fig. 4(d)). Compared to MSG, the current changing ability of the other compounds is negligible, which indicates high selectivity of the current detection method. Such results are obvious, as anti-Glu specifically bind with MSG via antigen-antibody interaction out of all other entities that were present. Therefore, non-specific entities did not show any significant current changing ability. The minute response in the output signal could perhaps be due to from the physical interactions of the immunosensor surface with the non-specific entities. The results imply that the reported immunosensor offered high selectivity towards MSG. On the other hand, the reproducibility was examined by using five similarly fabricated immunosensors incubated with 50 μM of MSG in buffer (100 mM PBS, 0.1 M KCl, 5 mM [Fe(CN)6]3−/4−) under same conditions. All the electrodes displayed similar electrochemical response with the RSD of 0.05%, indicating that the as-prepared sensor was desirably reproducible. Additionally, the long-term stability of the immunosensor was also examined. A freshly fabricated immunosensor was used to detect 50 μM of MSG after which it was stored at 4 °C for future use. After one week, the same immunosensor was utilized to identify the same amount of MSG. The result showed only a small decrease of the peak current. The peak current of the immunosensor was about 98.7% of its initial response when stored at 4 °C and measured after 15–20 days. These results suggested that the immunosensor could be used for analytes detection with acceptable stability.

3.7. Detection of MSG in food sample

The fabricated anti-Glu/Au@MoS2/Ch-GCE was used to detect MSG in real food samples. Locally available vegetable soup product was used for the purpose. A known amount of MSG was spiked to the soup and a series of samples were prepared. Then, DPV measurements were carried out following the same experimental conditions as that of the buffer. From the DPV curves, the change in peak current was measured for each sample and corresponding concentration was calculated from the calibration curve. Table 1 shows the detail results. It was found that at almost all concentrations, the observed recovery was greater than 90%. Thus, the overall results suggest anti-Glu/Au@MoS2/Ch-GCE as a promising sensing platform for the detection of MSG with high sensitivity in food samples. It possesses feasibility and reliability for real food sample analysis.

4. Conclusions

In summary, it can be concluded that we have successfully developed an electrochemical immunosensor based on MoS2 nanosheets functionalized with chitosan and gold nanoparticles for the detection of monosodium glutamate. A nanocomposite comprising of Au@MoS2/Ch was used to modify the electrode surface in order to improve the rate of electron transfer and increase the surface area to capture substantial amounts of antibodies. The reported immunosensor exhibited a high sensitivity towards MSG concentration in a wide linear range from

<table>
<thead>
<tr>
<th>Spiked MSG (μM)</th>
<th>Measured MSG (μM)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.049</td>
<td>104.9</td>
</tr>
<tr>
<td>5</td>
<td>5.310</td>
<td>106.2</td>
</tr>
<tr>
<td>10</td>
<td>9.480</td>
<td>94.8</td>
</tr>
<tr>
<td>50</td>
<td>49.381</td>
<td>98.8</td>
</tr>
<tr>
<td>100</td>
<td>101.640</td>
<td>101.6</td>
</tr>
</tbody>
</table>
0.05 µM to 200 µM. Thus, the work explores the prospect of detecting MSG by using label-free immunosensing technique. The performance and effectiveness of the monosodium glutamate immunosensor were evaluated and found satisfactory in terms of selectivity, reproducibility and stability. The immunosensor was also successfully used for detection of monosodium glutamate in food sample with high recovery. Thus, the as-reported immunosensor can be useful for sensitive analysis of food samples.

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Appendix A. Supplementary data

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References