Chapter 2

Analytical Chemistry of Glutamate: A Literature Review

2.1 Introduction

Glutamic acid (MSG) was isolated first from wheat gluten through the acidic hydrolysis process as a pure form by the German chemist Ritthausen [27]. Later, in 1908, Professor Kikunae Ikeda, department of chemistry, the Imperial University of Tokyo, isolated it. While he was replicating the savory taste of kombu, which is edible seaweed used as the base for Japanese soups [28]. MSG is identified as the taste enhancer in kombu. The first time, 30 grams of glutamic acid was obtained by extracting 40 kilograms of seaweed. Prof. Ikeda immediately patented a process for the isolation of MSG. In 1909 the first glutamate acid was produced commercially under Ajinomoto's trade name. It is used to pump up food flavour in many Asian and European countries. Now it is commercially mass-produced by fermentation of starch and sugar [29]. L-Glutamate is considered a vital analyte to decide MSG in food and clinical examples [30]. Food having MSG is labeled as the code E-621. L-glutamic acid and D-glutamic acid are the two optical isomeric forms of glutamic acid. Studies have shown the influential role of L-glutamate enantiomer as a flavor enhancer in food products [31]. Cagan [32] has examined the mechanism by which MSG stimulates taste receptors on taste buds. This is due to MSG's stimulation of specific receptors (T1R1/T1R3) found in taste buds or other glutamate receptors like the metabotropic receptors, which results in the induction of a new taste called UMAMI, also known as the fifth taste, which is distinct from the basic tastes of bitter, salty, sweet, and sour [33]. Umami is a basic taste evoking spicy, full-bodied, and meaty flavor sensations. Glutamate plays a vital role in many

metabolic processes [34] and acts as a donor of amino and methyl groups to synthesize non-essential amino acids. It is used for the transfer of energy, the transport of amino acids across cell membranes, and the removal of ammonia via the urea cycle. Glutamate also acts as a neurotransmitter and is a brain metabolite. Evidence suggests that it is responsible for 75% of the excitatory transmission in the brain [35]. Glutamate-induced neuronal injury is accountable for specific behavioral patterns such as retarded learning [36] and aggressive behavior [37]. MSG is traded as a white crystal substance like sugar. The Indian food processing industry uses MSG, and the consumption is estimated to be 10500 tons per year. MSG's utilization has turned out doubtful, and queries have been raised since the mid-1980s about its well-being. According to the Food and Drug Administration (FDA) report in 1959, MSG was considered generally safe. However, in 1990, the FDA received a complaint from consumers about nausea, vomiting, headache, etc., after consuming food with MSG. After that FDA requested an independent scientific group, the Federation of American Society for Experimental Biology (FASEB), to study the adverse effect of MSG [38]. The study outcomes are summarized in **Table 2.1.** The open deliberation was concerned about the side effects of MSG on the human body as Parkinson's disease, Chinese Restaurant Syndrome (CRS), Alzheimer's disease, Depression, Schizophrenia, and Birth deformity in posterity among pregnant women [39], which led to setting a standard for MSG concentration. In 1995, FASEB declared MSG safe in the report. This report also concluded a portion of the population who is sensitive to consuming 1 gram or more MSG without food at one time might face the mild adverse effect of MSG. However, most-off packed food with MSG contains a concentration of less than 0.5 gm. Intake of more than 1 gm of MSG without food on a single day looks impossible.

***Table 2.1**: Chronology of U.S. FDA, concerns about MSG.*

Year	**Concerns about MSG**
1959	FDA declares MSG as a safe component in packed foods.
1970	FDA supporters a complete assessment of the safety of MSG.
1980	A review by FASEB concludes that MSG is safe at the levels generally used in processed food.
1986	FDA issued an advisory that MSG is not a threat to the public, but overuse may lead to adverse reactions in some people.
1987	A joint committee of the FDA and the WHO places MSG in the safest category of food ingredients.
1995	Based on the report from FASEB, FDA is planning to propose that food containing free MSG declare on the label and set an upper limit for different types of foods.

The World Health Organization (WHO) requirement for MSG daily consumption is 0.12 gram. A concentration of 0.2% to 0.8% of MSG in packed food was designated as the upper limit in China and Japan. India's Prevention of Food Adulteration Act defined the permissible MSG content in food as 1%. Many times reports appear that the food industry uses MSG beyond standard limits to enhance the taste of its product.

A few years back, in India, a controversy flared up when the Food Safety and Drug Administration, Uttar Pradesh, found high levels of MSG in Maggi® noodles collected from the Barabanki district and tested in the state laboratory Gorakhpur. Later in April 2015, the Central Food Laboratory (CFL), Kolkata, that Maggi has a dangerous level of MSG and a high lead content, confirmed these results. Maggi's reported lead (Pb) content is 17.2 ppm,

which is 1000 times more than the company claimed and 18 times more than the permissible limit (1 to 2.5 ppm). The Indian government notified Nestlé to recall this product from the state market. The two other states, Gujarat and Maharashtra also adopted this recall. 38000 tons of Maggi were discarded from 24 June to 1 September 2015.

As per the FSSAI guidelines, MSG is not permitted in pasta and noodles (dried products). However, there are no guidelines for the maximum limit of naturally occurring MSG in noodles. The FSSAI, on 31 March 2016, issued an order that clarified the use of MSG as a flavor enhancer in seasoning noodles and pasta. The order states, "Specific enforcement/prosecution cannot be launched against manufacturers of noodles/pasta on account of the presence of MSG/glutamic acid unless the relevant government authority ascertains that MSG was deliberately added during the course of manufacture without the required declaration on the label." FSSAI labs used analytical methods for the determination and quantification of MSG. However, there is little information available about the usability of these methods to identify MSG, whether it is occurring naturally or has been added as a flavor enhancer. Thus, the FSSAI is still seeking an attentive way to quantify MSG. Since several analytical methods have been developed for determining glutamate, these existing methods can be broadly classified as enzymatic and non-enzymatic.

2.2 Non-enzymatic Methods for MSG determination

There are many non-enzymatic techniques, including high-performance liquid chromatography (HPLC), gas chromatography (GC), GC with mass spectrometry (GC-MS), thin-layer chromatography (TLC), paper chromatography (PC), ion-exchange

chromatography (IC), coulometry, amino acid analyzer, and capillary electrophoresis (CE). Here details of a few techniques, including HPLC, GC-MS, and CE, are briefly discussed.

2.2.1 High-Performance Liquid Chromatography (HPLC)

HPLC has been described as the most commonly used technique for determining glutamate due to its reliability and precision, even with a small amount of samples. Samples were run on HPLC equipped with a manual injector, a loop, and a detector. Samples were separated with mobile phase, with a constant flow rate at an ambient temperature of 25 °C, and the peak was detected at a particular wavelength by obtaining an absorption peak. Most cases involve pre-column derivatization followed by separation with reversed-phase liquid chromatography. These methods involve fluorescence and ultraviolet absorption.

For fluorescence detection, reagents that show the fluorescent properties after complex formation with amino acid are not fluorescent alone, which diminishes interference and reduces the need to separate the derivative from the reagent [40]. There are many reagents used for fluorescence detection. The most commonly used reagent is o-phthaldialdehyde (OPA) and its derivatives [41]. The methanol/acetate mobile phase is used for o-phthaldialdehyde with N-isobutyl-L-cysteine making a linear gradient system to reduce the retention time, stabilize the derivatives, and act as gradient elution [42]. l-alkylthio- 2-alkyl substituted isoindoles are highly fluorescent complexes formed via the reaction of OPA with primary amino acids [43].

Some other examples of derivatizing agents that are commonly used are naphthalene 2-3-di-carboxaldehyde [44], 9-fluoro methyl chloroformate [45], and dialkylamino naphthalene sulphonyl chlorides. Due to the formation of stable adducts of 9-fluoro, methyl chloroformate has advantages over the 2- mercaptoethanol or 2-methyl- 2-propanethiol noxious thiol derivatizing reagents. The mobile phase is acetic acid-methanol-acetonitrile used by isocratic

elution, and the ~5 pM of the adduct is the minimum detectable quantity [46]. Strongly fluorescent 1-cyanobenz (f) isoindole generates after the reaction of Naphthalene 2-3-di-carboxaldehyde with amino acids in the presence of cyanide ions [47].

In ultraviolet detection with gradient elution, phenyl isothiocyanate (PITC) is most commonly used [37,38]. Free amino acids and hydrolyzed samples are dissolved in a methanol:water:triethylamine mixture and are dried. In the dried specimen, the derivatizing agent is added and dried under a vacuum for 15 to 20 minutes. This method is stable, has adequate sensitivity, and requires short run times [50]. 6 aminoquinolyl-N-hydroxy succinimidyl carbamate is another example of the derivatizing agent [51]. O-phthaldialdehyde (OPA) is used for electrochemical detection, and isocratic elution occurs in the presence of sulfite ions [41,42]. Usually, mobile phases contain buffered acetonitrile/methanol with controlled pH. Glutamic acid retention time ranged from 5-25 min. The HPLC systems have sensible detection limits. The determination and separation of different amino acids concurrently. The disadvantage of this method such as the time-consuming processes and before derivatization is required.

2.2.2 Gas Chromatography (GC)

Gas chromatography (GC) and GC-mass spectrometry (GC-MS) are two methods used to analyze amino acids. GC-MS offers a robust system for determining amino acids with high sensitivity and inherent specificity of selected ions. The sample needed pre-treatment in the GC and GC-MS methods, and only pure amino acids can be determined. Precipitation, centrifugation, dialysis, or ultrafiltration are commonly used to remove proteins [54]. Ion-exchange chromatography is frequently used to purify amino acids [55], and before the separation, the compounds used for derivatization are volatile.

GC-MS methods had also been explored for the use of derivatization processes using pentafluoro propionic anhydride with hexafluoro isopropanol [56], N (0)-tert-butyl methanol [57], N-acyl alcohol esters, and trimethylsilyl derivatives. The minimum quantity can be detectable in the range of 0.01 to 0.001 f moles; derivatized N (0, S)-ethoxycarbonyl esters are responsible for fast-determined with good resolution [58]. The study described the use of a tert-butyl dimethylsilyl derivative formed by single-step derivatization of amino acids in tandem with MS allows rapid separation based on retention capacity. It can be measured at nanomoles and picomoles levels [59].

Various types of columns and detectors are available for Gas chromatography. For the amino acid, the best columns are capillary columns having a layer of barium carbonate coated with either methyl siloxane or methyl phenyl siloxane [60]. The disadvantage of this method needed purification and derivatization of amino acids. Some other examples of chromatographic techniques such as ion-exchange chromatography, thin-layer chromatography, and ascending paper chromatography are also used.

2.2.3 Capillary Electrophoresis (CE)

The working principle of CE is the separation of amino acids depending on the charge-to-mass ratio in a capillary field with a proper buffer under high voltage with a detection system. Capillary zone electrophoresis is a frequently used method in CE. It provides a fast and efficient separation. The separation mechanism mainly depends on the solute size and charge changes at a given pH [61]. Before separation and quantification of amino acids can be determined directly or indirectly by derivatizing [62].

Laser-induced fluorescence detection is used to determine fluorescein isothiocyanate derivatives [63]. It is susceptible to the presence of excess reagents that hampers the determination. Another derivatizing agent like fluorescein thiohydantion derivatives is used

with laser-induced detection [64]. In chemiluminescence emission, a post-column derivatization reaction takes place. For example, Zhao et al. [65] isoluminol thiocarbomyl derivatives determination of amino acid can be measured at the level of 30 fmoles. Amino acids can also be determined as dansylated derivatives [66], phenylthiohydantoin derivatives [67], or OPA derivatives [68]. Another approach is to use a background electrolyte containing a UV-absorbing species that is displaced by the analyte ion in the sample zone. Oefner [69] determined amino acids within 60 s by using chromate ions in the carrier. Here, 5, 5 diethyl barbiturate was added to avoid a gradual pH shift. This technique has better selectivity, sensitivity, and speed than the other methods (capillary electrophoresis technique). Zhou [70] used copper microelectrode zwitterionic buffers with amperometric detection. The buffer reduces the osmotic flow, resulting in a better separation of amino acids and an increase in the S/N ratio by reducing the noise due to the high current used in the partition. Though the method is sensitive, and in some cases, separation can be done within 60 s, there can be limitations due to the smaller diameters of the capillaries used and their capacity to dissipate heat. The selection of stationary and mobile phases available is less than the conventional separation techniques. Although it is efficient for the determination of DNA fragments, it is not superior to HPLC in its quantitative capabilities [71].

2.3 Enzymatic Methods for MSG determination

Many non-enzymatic analytical techniques discussed above have constraints that these analytical techniques require skilled personnel, sophisticated instrumentation, sample preparation, and time consumption. Thus, a cost-effective point-of-care (POC) diagnostic sensing system currently needs to test the MSG limit in food samples. Enzymes are a

significant and universal class of protein molecules that act as biocatalysts. They are distinguishable by their active sites, into which only particular substrates can fit. The primary structures of enzymes are due to the sequence of different amino acids linked to each other by peptide bonds. The number of amino acids, their position, and their interaction in forming bonds affects the enzyme's size, shape, and function. Enzymes affect the reaction rate without undergoing any permanent change and can often catalyze reactions 108 - 1011 times more rapidly than the corresponding non-enzymatic catalysts. Even though the enzyme-catalyzed reaction requires milder conditions than some non-enzymatic methods**.** The range of responses by enzymes is wide-ranging, and these reactions are usually precise or highly selective, and they are particularly ideal when low substrate concentrations are quantified. Even though enzymes have been used for diagnostic purposes for many years, their primary use has been restricted due to their instability**.** Electrochemical amperometry can be one approach to developing such diagnostic strategies as it holds several advantages like trustworthiness, low price, ease of management, and rapid detection method. In particular, the nanocomposite material having a large surface area and high electrical conductivity can be a potential candidate for developing these sensing platforms. Various L-glutamate electrochemical biosensors based on L-glutamate oxidase and L-glutamate dehydrogenase have recently been reported. Enzymes in aqueous solutions cannot find their catalytic ability relatively fast, and neither can the enzymes be recuperated, nor has their activity regrown [72]. This difficulty can be overcome by protein engineering or immobilization on an inert matrix or membrane. Protein engineering generally results in enzyme deactivation. The structural morphology of an enzyme can be retained by design. Immobilization is the process of entirely or significantly restricting the movement of enzymes, cells, etc., in a particular

region, typically leading to water-insoluble enzymes. The methods available for immobilization can be broadly classified and sub-classified as:

2.3.1 Glutamate Oxidase-based Biosensors

The integration of an enzyme within a polymer moiety while maintaining its protein structure is referred to as the entrapment of the enzyme [73]. By incorporating glutamate oxidase from Streptomyces sp. with various levels of purification drop cast into a poly (carbamoyl) sulfonate (PCS) hydrogel, a biosensor for detecting MSG in food flavoring was developed. The platinum electrode was drop-cast with the glutamate oxidase-PCS combination [74]. A cyclic voltammetry study characterizes the response to MSG under different conditions and for their inhibition, specificity, and storing assets. Liquid samples of commercial purified and crude extract were diluted to 10mL with PBS buffer. The biosensor was then applied to quantify the MSG in soy sauce and then correlated with other MSG-determining methods. At first glance, commercial purified shows its superiority over the stabilized L-glutamate oxidase in every aspect. However, the cost-effectiveness of the crude extract is a good option for mass production. A recent study of monosodium glutamate biosensors is probing artificial cerebrospinal fluid (CSF) under hypoxic conditions [75]. The fabrication technique is complex, whereby glutamate oxidase is immobilized with ceria-titania nanoparticles and chitosan. The ceria-titania NP stores oxygen in its crystalline lattice; it can help enzyme to generate H_2O_2 in the absence of oxygen. The fabricated biosensor operates over the concentration of interest under anaerobic conditions. A biosensor for the quantification of glutamate in brain extracellular fluid using a simple fabrication step has been reported. The fabrication involved dip coating of a 60 μm radius platinum wire electrode into a PBS solution with glutamate oxidase, *o*-phenylenediamine (PPD) [76]. After drying, the modified electrode is coated with phosphatidylethanolamine (PEA) and bovine serum albumin (BSA).

The glutamate oxidase was immobilized by the electro-polymerization of PPD on the surface of the platinum wire electrode. The PPD and PEA were used to block out interferences. The sensor has a high substrate sensitivity and enough selectivity to serve as a basis for in vivo neurochemical applications. An interesting entrapment approach of GluOx onto a gold disc electrode in 3-mercaptopropionic acid (MPA) solution, poly-L-lysine, and poly(4-styrene sulfonate) are described [77]. After drying the modified gold electrode, a mixture of glutamate oxidase and glutaraldehyde was drop-cast on the electrode to form a bilayer. As per the conclusion of the study, electrostatic interaction between carboxyl groups of the MPA and the amino groups of poly-L-lysine increases the adhesion of the poly-ion complex to the gold surface.

A linear response was observed between the 20μM and 200μM concentrations of glutamate. The response time is significantly less as compared to other biosensors. The study concludes that the rapid response may be due to the close packing of the enzyme and its oxidation reaction to the surface of the gold electrode. Pinnacle Technology Inc. lunch its glutamate biosensor after the better interest in MSG measurement. This company commercialized an *in vivo* MSG biosensor [78]; this biosensor is successfully tested in the rodent brain for real-time monitoring of MSG concentrations. An "inner-selective" membrane, GluOx, and an unknown substance that removes interferences are used in the biosensor. Using a platinum-iridium electrode, the oxidative reaction of hydrogen peroxide produced by enzymes is measured. Up to 50 μM, the biosensor exhibits linearity. According to the manufacturers, the miniaturized biosensor needs to be calibrated once an experiment is finished to maintain the sensors' integrity and selectivity.

The biosensor is based on the glutamate oxidase used to measure MSG in the serum of healthy patients [79]. The fabrication process consists of electrodepositing gold

nanoparticles, chitosan polymer, and MWCNT, and nanocomposite is drop cast onto the gold electrode. With PBS, the serum sample was diluted. The concentration of glutamate in the sample with PBS was calculated using a standard calibration curve created from the amperometric responses. The results were compared with a colorimetric test kit. A low operating potential of +0.135 V compared to the reference electrode was found for the oxidative reaction of enzymatically produced hydrogen peroxide. This potential was significantly lower than other biosensors [76], [80]. The operating time of the biosensor is two seconds which was also considerably lower than other biosensors

The protein structure of the enzyme can be immobilized by intermolecular cross-linking to other protein molecules or inside an insoluble support matrix. Jamal et al. [80] suggest an entrapment method consisting of drop-coating of a mixture of glutamate oxidase, Nafion (0.5%), glutaraldehyde, and BSA onto a platinum nanoparticle decorated with gold nanowire. The fabricated biosensor is allowed to dry overnight. The analytical response results from the oxidation of enzymatically generated H_2O_2 at the gold nanowire electrode [81]. The high sensitivity was obtained due to the nanoparticles on the surface of the gold electrode. The NPs act as conduction centers and increase the transfer of electrons and ions toward the surface of the gold electrode. Additionally, a high enzyme loading was used, which in amalgamation with the nanoparticles, resulted in amplified enzyme immobilization to the surface. Glutamate Oxidase was encored to the surface of a palladium-electrodeposited screen-printed carbon strip by a simple crosslinking technique using a cross-linked polymer (PVA-SbQ) [81]. The fabricated biosensor showed a stable, steady state response for six hours. This state indicates that the enzyme was retained within the polymer membrane.

A biosensor [82] was successfully fabricated and applied to measure MSG in various food samples. MSG levels were compared very favorably with a spectrophotometric kit. The

biosensor was fabricated using the mixture of glutaraldehyde, glutamate oxidase, and BSA, and then this mixture spreads onto the surface poly-carbonate membrane. The membrane was then attached to an oxygen probe using a push cap system, and oxygen intake was determined at an applied potential of -0.7 V. In this case, the response depends upon the reduction reaction shown in Equation 1.

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2 \quad (1)$$

The lowest detection limit was obtained with covalently immobilizing glutamate oxidase onto polypyrrole nanoparticles and polyaniline composite film [83]. The use of cyclic voltammetry achieved co-electro-polymerization of compounds onto the surface of the electrode. The PPy NPs are the conductive polymer and hence increase the electron transfer. Due to this, a low potential of +85 mVs is used for operating. The authors concluded that this is the reason behind the higher sensitivity of the biosensor. The biosensor was successfully applied to the quantification of MSG in food samples. High recoveries of 95% - 97% were achieved.

2.3.2 Glutamate Dehydrogenase-based Biosensors

These equations describe the electrochemical response of glutamate dehydrogenase:

$$\text{Glutamate} + NAD^+ \xrightarrow{\text{GLDH}} NADH + \alpha\text{-ketoglutarate} \quad (2)$$

$$NADH + \text{Mediator}_{ox} \longrightarrow NAD^+ + \text{Mediator}_{red} \quad (3)$$

$$\text{Mediator}_{red} \longrightarrow ne^- + mH^+ + \text{Mediator}_{ox} \quad (4)$$

Equation 2 demonstrates the enzymatic degradation of glutamate to -ketoglutarate and the reduction of the cofactor NAD^+ to NADH. The electrochemical reduction of the oxidized mediator to the reduced form is represented by Equation 3 (mediator_{red}). Equation 4 explains how mediator_{red} is electrochemically oxidized at the base transducer to provide the analytical response, after which the mediator_{ox} can engage in further reactions with NADH. Equations

3 and 4 depict the direct electrochemical oxidation of NADH at an unmodified electrode, which takes place at higher applied potentials than obtained by the electro-catalytic oxidation of NADH at lower applied potentials.

To design a novel MSG sensor using a modest fabrication technique, incorporating carbon paste with beef liver mitochondria containing glutamate dehydrogenase. The beef liver mitochondrial with carbon paste was used to quantify L–Glutamic acid in chicken bouillon cubes [84]. The utilization of mitochondrial fraction to reduce the cost. However, the sensitivity of biosensors is compromised than purified glutamate dehydrogenase. Ferricyanide is generated by the enzymatical oxidation of NADH, which works as an electrochemical mediator. Prominent retrievals of MSG were attained. Many different amino acids present in food did not affect the quantification procedure. Before analysis, the food items were required a pre-treatment process which included; thawing, vacuum filtering, washing, and further diluting with buffer.

A unique biosensor construction method was developed [85]. It involved the entrapment of GLDH flanked by layers of alternating poly (amidoamine) dendrimer Pt-PAMAM using multiwall carbon nanotubes. PAMAM has excellent biocompatibility and adhesive properties; it is used to alter the exterior of the glassy carbon electrode. The process was frequently used negatively charged GLDH and positively charged Pt-PAMAM, which was adsorbed layer-by-layer alternatively onto the CNTs. The construction method is multifaceted, and the biosensor holds more sensitivity than the earlier-mentioned biosensors.

Design a glutamate biosensor with a less time-consuming process [86]. This complex integrates GLDH and NAD^+ into carbon paste. A mixture was introduced in a holder, placed into a solution with O-phenylenediamine, and then electro-polymerized. An o-phenylenediamine film can concurrently avoid interfering, assist the NADH determination

at low potentials, and work as an electron mediator. The biosensor was used to determine glutamate in chicken bouillon cubes. The outcomes were equated to those developed via a spectrophotometric technique (12.6 ± 0.3% (n = 5) and 12.3% respectively). While the more straightforward construction method, the biosensor's linear range, and sensitivity were lesser than the sensor described by Tang et al. [85].

Biopolymer such as chitosan has been explored in various research, for example, the entrapping enzymes for biosensor design [87]. CHIT enhances enzyme strength and is simple to immobilize onto several materials. Glutamate biosensor [88] was designed by depositing CNTs, CHIT, and MB mixture onto a glassy carbon electrode and then drying. The surface was treated with an aliquot of GLDH in PBS and dried at 4ºC. In a solution concentration of a co-factor was found at four mM. Biosensor selectivity was measured by adding interferences (AA, UA), with no apparent amperometric responses being produced. Though, in this paper, the application of the biosensor in the field of clinical and food samples did not elaborated.

Glutamate biosensors based on screen printing technology are convenient for large-scale production at a low cost. Effectively useful to quantify glutamate in serum and food samples [89]. As screen-printed devices are low-cost to fabrication, it is accessible to disposable, compared to glassy carbon electrodes, which are very costly and not regarded as disposable devices. Therefore, there are many appropriate devices for designing biosensors, which have become a widespread commercialization method. The design method involved drop coating CHIT (0.05%) onto the Meldola's Blue (MB) SPCE (MB-SPCE) surface, followed by an aliquot of glutamate dehydrogenase (3U/μL). This device is fabricated GLDH-CHIT-MB-SPCE. The biosensor was dried at 4º C under a vacuum overnight. The solution is contained NAD^+ at a concentration of 4mM. The operating potential of +100 mV is only acceptable

for the electrocatalyst Meldola's Blue. The response was generated due to the electrocatalytic enzymatically oxidation which produced nicotinamide adenine dinucleotide hydrogen (NADH). The biosensor was effectively useful for determining monosodium glutamate (MSG) in Beef OXO cubes and in serum. By sonication process, the beef OXO cube was dissolved in phosphate buffer solution (PBS), and the endogenous content of both the OXO cube and serum were determined. Retrievals of 91% were attained for the spiked OXO cube (n = 6) and 96% for the spiked serum test (n = 6). These outcomes compare to those reported by Alvarez-Crespo et al. [86] and Basu et al. [82]. This device increases the linear range of earlier discussed biosensors [86], [88]. Further, develop this biosensor for potential commercial improvement, all the constituents required to be immobilized onto the transducer surface. The layer-by-layer assembly procedure is explained here [90].

An amperometric strategy using the reagent-free biosensor was designed. The biosensor was successfully used for the quantification of glutamate in spiked serum. A retrieval of 104% (n = 5, CoV: 2.91%) was determined, which compares to earlier discussed biosensors [82], [86], [89]. An interfering study was conducted in serum and food samples (stock cubes), and no interference signals were produced. Such reagent-free biosensors have the benefit; of low cost, being easy to use, and no requirement for adding other co-factor into the sample. These properties are essential for commercial devices.

Different pulse voltammetry is used for electrochemical measurement. A novel assay-based glutamate biosensor was developed to determine glutamate in naturally occurring biomolecules mixture usually present in biological samples [91]. The biosensor design process comprises vertically aligned carbon nanotubes (VACNTs), which are treated to convert the tips of the CNTs into carboxylic acid groups to bind covalently with the enzyme. GLDH was tied to the CNTs using 1-(3-dimethylamino propyl)-3-ethyl carbodiimide

hydrochloride (EDC) and hydroxyl sulfosuccinimide sodium salt (sulfo-NHS) to stimulate the formation of amide linkages between the carboxylic tips of the CNTs and the lysine residue of the enzyme. This was achieved by immersing the electrode in a solution containing EDC/sulfo-NHS and mixing. Once dried, the enzyme was drop-coated onto the electrode, dried for 2 hours, and washed with PBS/BSA mixture. The DPV found with the synthetic samples showed that no significant interferences should be expected over the concentration range studied. However, the biosensor was not valuable to an actual sample.

CNTs are effectively applied to generate cross-linking matrixes. Single-wall CNTs have been treated with thionine (Th) to make a Th-SWCNT nanocomposite on the glassy carbon electrode surface [92]. The nanocomposite acts as an electron mediator and an enzyme immobilization matrix. The GLDH was mixed with BSA, crossed-linked with glutaraldehyde, and coated onto the Th-SWCNT layer. The potential of +190mV was consumed for amperometric measurements. The linear range was more significant than that achieved by Gholiazadeh et al. [91] and Tang et al. [85]. Ascorbic acid, uric acid, and 4-acetamidophenol were possible interferents; no marked current responses were seen.

2.4 Antibodies-based Methods for MSG determination

A novel antibody-based amperometric immunosensor is developed by using a glassy carbon electrode modified with gold nanoparticles decorated on a molybdenum disulfide/chitosan (Au@MoS_2/Ch) nanocomposite. The pH value of the sensor is optimized and found to be at 7.4. Monoclonal anti-monosodium glutamate antibodies are immobilized onto the immunosensor with the amine functionalization and covered by the BSA. Immunosensor is tested against the MSG in a buffer solution and in tomato sauce. A linear relationship was

perceived between the current change and MSG concentration. The association was found to be consistent in the detection range of 0.05–200 μM. Statistical validation of the assay showed a limit of detection and limit of quantification values as 0.03 and 0.1 μM, respectively (R^2 = 0.99) [93].

2.5 Miscellaneous Methods

Glutamic acid has been determined using a series electrode piezoelectric quartz crystal sensor [71]. The amino acid is treated with formaldehyde, and condensation occurs between the amino and aldehyde groups. This reaction results in the amino group being masked, and its basicity disappears; hence, the solution shows acidic properties [94]. Frequencimetric titration with NaOH as titrant is used to determine glutamate with this sensor. The lowest titratable concentration of glutamic acid is 0.070 m.M. Though this sensor is sensitive, it is not selective for glutamate. Another approach using a chemical sensor for glutamate determination is near-infrared spectroscopy [95]. Titrimetry can also determine glutamate with a base and indirect atomic absorption spectrophotometer. Most of these techniques need separation before determination. In some cases, derivatization is a must before the quantification of MSG, and all these processes are invariably time-consuming.