Sri Lankan Journal of Technology
Research

SLJoT
2020 (01 & 02) 35-40
ISSN 2773-6970

Development and validation of a portable paper-based low-cost electrochemical sensor for the detection of monosodium L-glutamate in food

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Received:2020-11-05 * Accepted:2021-03-04 * Published Online:2021-03-30

***Abstract*—**Monosodium L-glutamate (MSG) is a common ingredient that is added to food to enhance its taste. However, due to the negative perception of consumers towards MSG, most food manufacturers do not inform customers if MSG has been added to food. This work describes the development and validation of a low-cost electrochemical microfluidic paper-based analytical device (μPAD) for the rapid, on-site detection of MSG in food samples. This device is a portable, low-cost, one-time sensor that can be used on the field even directly without sample preparation. This would greatly benefit groups of society such as Public Health Inspectors and individuals who regularly consume fast food. The μPAD was fabricated with a three-electrode system consisting of a working electrode modified with Co_3O_4 nanoparticles, a pseudo reference electrode, and a counter electrode. The device is tested using food samples spiked with MSG 5 g/L. The test results prove that the device can be used as a qualitative sensor to detect MSG in food with the limit of detection 0.82 g/L.

***Keywords*—**Monosodium L-glutamate, paper-based device, sensor, food analysis, low-cost

I. Introduction

Monosodium L-glutamate (MSG or E-621) (Figure 1), is the sodium salt of L-glutamate and is an ingredient that is commonly added to food to enhance flavour (Basu, *et al*., 2006).

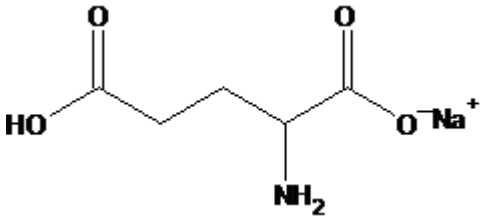

Figure 1: Farmers' educational level

MSG (E-621), is capable of delivering a meaty, savoury taste by binding to the amino-acid receptors located on the tongue (Ruiz-Capillas, *et al*., 2009). In 1959, the U.S. Food and Drug Administration (FDA) classified MSG as 'generally recognized as safe' (GRAS). However, a medical condition involving itching, nausea, vomiting, headache, rashes, chest pain, drowsiness, excessive thirst and many other symptoms have been reported after the consumption of food containing large amounts of MSG (Udomsopagit, *et al*., 1998). These symptoms were collectively termed as 'Chinese restaurant syndrome' or 'MSG symptoms complex' (Afraa, *et al*., 2013). Furthermore, in animal studies, large doses of MSG have been shown to cause brain damage and stunted skeletal development. In the light of these revelations, the FDA revised its regulations and designated that if MSG has been added to a food item, it must be disclosed on the label of the food (Olney, 1969).

Due to the negative perception of consumers towards MSG, a majority of food manufacturers attempt to conceal the fact that MSG has been added to food. Some manufacturers resort to strategies such as using other names for MSG that customers are not familiar with. Others ignore FDA regulation altogether. These actions have prompted government agencies to test food samples for the addition of MSG and take legal action against food manufacturers who add excess amounts of MSG to food without disclosure (Olney,1969). Therefore, the development of an efficient analytical method for the detection of MSG in food samples is an important requirement.

Various methods have been developed to detect the amount of MSG in food samples. These include the HPLC (Soyseven, *et al*.,2020), spectroscopic (Acebal, et al., 2008), gas chromatographic (Conacher, *et al*., 1979), biosensors based (Basu, *et al*., 2006) and electrochemical (Zhang, *et al*., 2006) detection methods. Sensors based on electrochemical methods can also be used for the detection of MSG in aqueous food samples. Electrochemical methods are capable of monitoring ion transport, ion distribution, and electron-

transfer reactions at the electrode-solution interface and are based on techniques such as potentiometry, amperometry, or impedometry (Urban, 2013).

Microfluidic Paper-based Analytical Devices (μPADs) are ideal miniaturized sensors because they are low-cost, user-friendly, robust, durable, small, and disposable after one-time use. μPADs can be used as sensitive and rapid on-site detection tools whilst requiring only a small volume of sample and do not require the use of analytical instruments that require trained personnel with the scientific skill to operate them. Electrochemical detection is considered the most favourable detection method for μPADs due to its compatibility with miniaturized sensors, low cost, portability, rapid response time, easy operation (Xue, *et al*., 2014), ability to detect low concentrations, and tunability for selective detection (Santhiago, *et al*., 2013).

The present work describes the development of a simple, low-cost, non-enzymatic μPAD for the qualitative determination of MSG in real samples using voltammetry. This enables rapid-on site analysis of food samples suspected of containing MSG. Selectivity was achieved by the modification of the working electrode with Co_3O_4 nanoparticles.

II. Methodology

A. Construction of the μPAD

Whatman No. 1 filter paper was selected as the base for the construction of the μPADs. The hydrophobic barriers were initially drawn using the hydrophobic ink of a CD/DVD marker pen. A 9B Staedtler pencil was used to fabricate the counter electrode whose length inside the hydrophilic region was 4 mm. The application of the pencil was carried out by repeatedly drawing over the same spot a total of 20 times, to obtain a well-conducting counter electrode. A silver (Ag) conductive ink pen was used to fabricate the (pseudo) reference electrode whose length inside the hydrophilic region was 2 mm. The resistance of this electrode was measured and found to be close to zero. All electrochemical studies were performed using ZIVE LAB, SP5 model.

It was necessary to confirm that the behaviour of the reference electrode drawn using silver (Ag) conductive ink was analogous to that of a standard Ag/AgCl reference electrode. The voltammogram of the device using the standard Ag/AgCl reference electrode was obtained by dipping the device in a bulk solution of potassium ferrocyanide (10 mM) and dipping the PAD developed using Ag pseudo reference in the same solution.

Graphite powder and varnish were weighed in the ratio of 2:3 and thoroughly mixed using a mortar and pestle to make the conducting paste for the construction of the unmodified (bare) working electrode. To construct the modified working electrode, Co_3O_4 nanoparticles were synthesized in the laboratory. The characterization of the prepared Co_3O_4 particles was carried out using FTIR (Varian FT-IR, Model: 660-IR), UV-Visible Spectrophotometry (Thermo Science, GENESYS 10S) and the morphology of the particles was studied using scanning electron microscopy (SEM), Carl Zeiss, EVO 18). Graphite powder and Co_3O_4 nanoparticles were weighed into a mortar in the ratio of 15:1 by weight. These two powders were thoroughly mixed using a pestle. Varnish was added to this mixture in the ratio of 3:2 by weight. The resulting mixture was well mixed until a homogeneous paste with a soft consistency was obtained.

Mixed electrode pastes were transferred into separate plastic bottles that contained a fine tip for the convenient and efficient application of the paste onto the μPAD. The completed μPAD is shown in Figure 2. The completed μPAD was cut into a suitable shape and used to analyze simulated solutions of MSG as well as real food samples spiked with MSG by voltammetry.

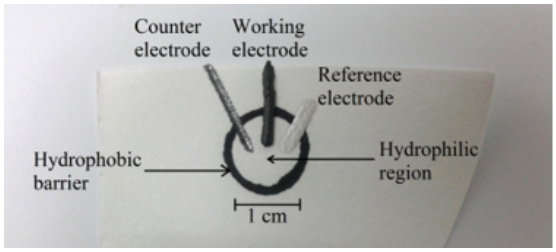

Figure 2: The developed μPAD

B. Voltammetric characterization of the basic μPAD

Electrochemical measurements were performed by introducing 20 μL of the test solution onto the center of the hydrophilic zone of each μPAD using a micro-pipette. A waiting time of 30 seconds was allowed and all voltammetry experiments were performed at a scan rate of 50 mV/s. The repeatability of the μPAD was tested for two redox systems; potassium ferrocyanide in 0.1 M KCl and ruthenium hexamine in 0.1 M in KCl. For the potassium ferrocyanide redox system, cyclic voltammetry was carried out from -1.0 to +1.3 V. For the ruthenium hexamine redox system, cyclic voltammetry was carried out from -0.5 to +0.5 V.

C. Voltammetric characterization of the μPAD with working electrode modified with Co_3O_4 nanoparticles

The comparison of the electrochemical behaviour of the unmodified and modified working electrodes was investigated by performing voltammetry for MSG in 0.05 M $Na_2B_4O_7$ pH 9.2 buffer and 0.05 M KCl. Kinetic studies of the electrode surface and MSG were carried out by registering the voltammograms of 2 g/L MSG in 0.05 M $Na_2B_4O_7$ pH 9.2 buffer and 0.05 M KCl at scan rates between 25 and 150 mV/s.

D. Analyzing MSG containing solutions

Initially, 20 L of the blank solution that contained 0.05 M $Na_2B_4O_7$ pH 9.2 buffer and 0.05 M KCl were introduced and after an initial delay of 30 s, the voltammogram was registered at a scan rate of 50 mV/s. Secondly, 20 L of an MSG-containing solution in 0.05 M $Na_2B_4O_7$ and 0.05 M KCl were introduced and after an initial delay of 30 seconds, the voltammogram was registered at a scan rate of 50 mV/s.

E. Establishing the Limit of Detection (LoD)

The response was measured for a series of concentrations with six replications using a new μPAD for each replicate. A plot of positive response percentage vs. MSG concentration was constructed. A response was considered to be positive if the current generated at -1.10 V from the MSG-containing solution exceeded the current generated at -1.10 V from the blank solution. The MSG concentration at which a positive response percentage of 50% had been achieved was designated to be the LoD.

F. Determination of the locations of electrodes

When deciding the locations of the three electrodes, consideration was given to the fact that the working and counter electrodes should be next to each other to eliminate the effect of uncompensated solution resistance. A 2 mm gap was introduced between the bottom-ends of the electrodes and a 3 mm gap was introduced between the top-ends of the electrodes to prevent the electrodes from short-circuiting due to the considerable thickness of the alligator-type connectors used to connect the μPAD to the potentiostat.

G. Real sample analysis

A laboratory prepared vegetable soup sample is used as the negative sample. The same soup sample is spiked with MSG to use as the positive test sample. A volume of 20 μL of the blank solution that contained 0.05 M $Na_2B_4O_7$ pH 9.2 buffer and 0.05 M KCl 20 L was initially introduced. Then after a 30 s delay, the negative/ positive soup samples were introduced onto the μPAD and the voltammogram was collected at a scan rate of 50 mV/s.

III. Results and Discussion

Electrochemical properties of the developed μPADs were tested using a series of potassium ferrocyanide solutions and the voltammograms obtained are shown in Figure 3.

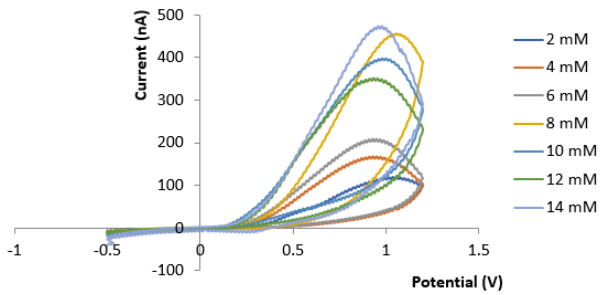

Figure 3: Voltammograms obtained using the developed μPAD for different concentrations of potassium ferrocyanide in 0.1 M KCl

From the cyclic voltammograms, it is apparent that although the anodic peak was obtained in the forward scan, the cathodic peak was not obtained in the reverse scan. In the case of a typical cyclic voltammetry experiment carried out for a bulk solution of potassium ferrocyanide, both the anodic and cathodic peaks should be observed. This highlights the fact that there is higher resistance for ion migration through the cellulose fibers of the filter paper when compared to the ion migration across a physically unhindered bulk solution.

Responses produced at +0.91 V by the sensor for the potassium ferrocyanide solutions are shown in Figure 4.

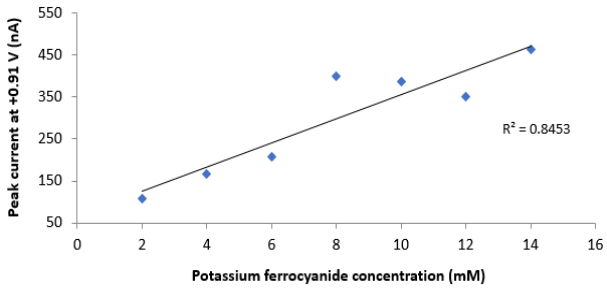

Figure 4: Calibration graph developed using voltammogram peak current at +0.91 V for the potassium ferrocyanide series in 0.1 M KCl.

Since the correlation coefficient (R^2) obtained for the graphs is 0.845, it can be inferred that the electrochemical response of the μPAD is approximately linear. Therefore, the developed μPAD can be regarded as a tool that can be used for the quantitative determination of analytes.

The FT-IR spectrum obtained for the Co_3O_4 nanoparticles is shown in Figure 5.

In Figure 5, the bands centered at 562.8 and 662.4 cm^{-1} correspond to the Co-O bond vibrations in Co_3O_4. The strong band at 1383.6 cm^{-1} corresponds to the stretching vibrations of NO_3^-, indicating that a significant amount of the starting material $Co(NO_3)_2$ was still present in the sample.

The SEM images obtained for the synthesized Co_3O_4 particles are shown in Figure 6.

SEM images were obtained to evaluate the morphology of the synthesized particles. It can be inferred from Figure 6 that the shapes and sizes of the synthesized particles were non-uniform. The smallest particles observed were approximately 150 nm in diameter. However, the majority of the particles were observed as agglomerates.

The heterogeneous shape and size distribution of the particles can be attributed to the non-uniform heating of the sample. The use of microwave radiation to heat the sample is proposed. Microwaves have been demonstrated to heat the sample uniformly and rapidly due to its high depth of penetration, thus reducing the reaction time and suppressing side reactions giving rise to particles with narrow size distribution and increased purity (Vijayakumar *et al.*, 2013).

Voltammograms collected for the MSG test solutions using the working electrode modified with the Co_3O_4 nanoparticles are shown in Figure 7. The response for MSG test solutions produced a significant change in the response compared to the response of the blank solution.

The response change was determined using the developed μPAD at various scan rates. The collected voltammograms changing the scan rate from 25 to 150 mV/s are shown in

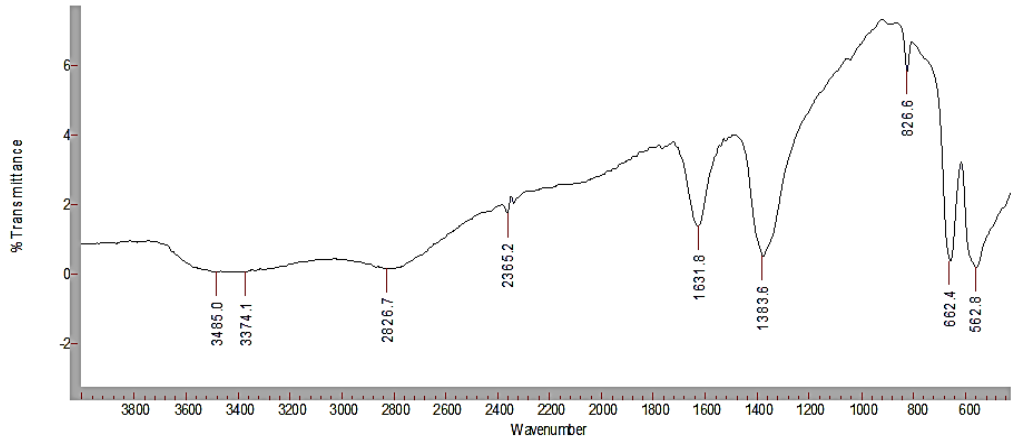

Figure 5: FT-IR spectrum of the synthesized Co_3O_4 particles

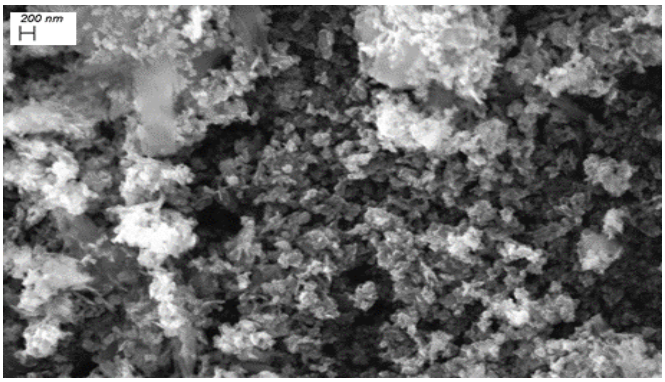

Figure 6: SEM images of the synthesized Co_3O_4 particles collected using parameters of I Probe = 20 pA, EHT = 20.00 kV, beam current = 100 µA and WD = 10.5 mm.

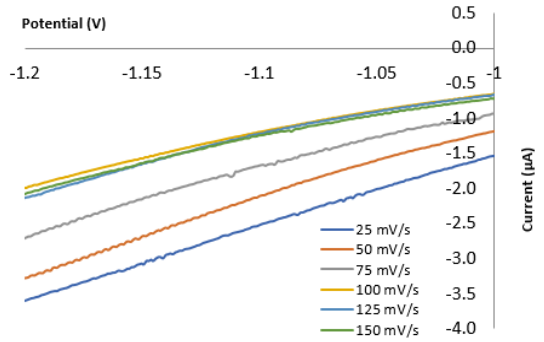

Figure 8: Variation of the cathodic current collected for MSG in 0.05 M KCl and 0.05 M sodium tetraborate buffer solution using the developed μPAD fabricated with graphite working electrode modified with Co_3O_4 nanoparticles in the ratio of 1:15 by weight at the scan rates of 25, 50, 75, 100, 125 and 150 mV/s.

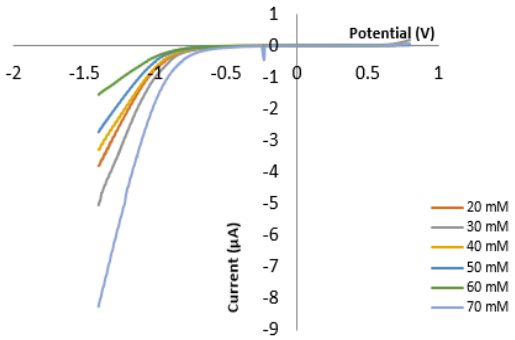

Figure 7: Responses collected for 20, 30, 40, 50, 60 and 70 mM MSG using the working electrode modified with Co_3O_4 particles

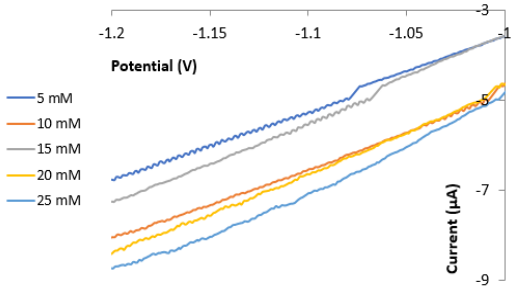

Figure 9: Response collected using μPAD fabricated with graphite working electrode modified with Co_3O_4 nanoparticles in the ratio of 1:15 by weight, for MSG concentrations of 5, 10, 15, 20 and 25 mM in 0.05 M KCl and 0.05 M sodium tetraborate buffer solution.

Figure 8. The largest negative current was observed for the scan rate of 25 mV/s. Based on these observations, the scan rate was selected as 25 mV/s.

The response variation with the MSG concentration for the sensor at a 25 mV/s scan rate is shown in Figures 9 and 10. The negative current at –1.10 V was selected to study the linear relation between the MSG concentration and the current.

Based on the weak linear correlation of R^2=0.574, it was inferred that the μPAD could not be utilized for the quantitative determination of MSG. Instead, the μPAD could be used for the qualitative detection of MSG.

The LoD, 0.82 g/L was calculated using the midpoint concentration of the 075 – 0.88 g/L (concentration at the 50% response). A comparison of this method LoD with other

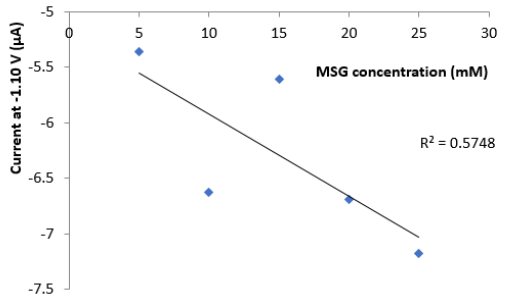

Figure 10: Graph of current at -1.10 V vs. MSG concentrations of 5, 10, 15, 20 and 25 mM in 0.05 M KCl and 0.05 M sodium tetraborate buffer solution collected using μPAD fabricated with graphite working electrode modified with Co_3O_4 nanoparticles in the ratio of 1:15 by weight at the scan rate of 50 mV/s

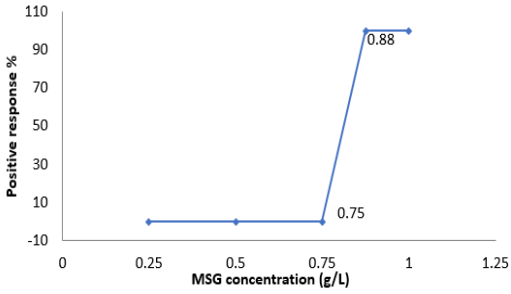

Figure 11: Graph of positive response vs MSG concentration developed for the analysis of 0.25, 0.50, 0.75, 0.88 and 1.00 g/L MSG samples using μPAD fabricated with graphite working electrode modified with Co_3O_4 nanoparticles in the ratio of 1:15

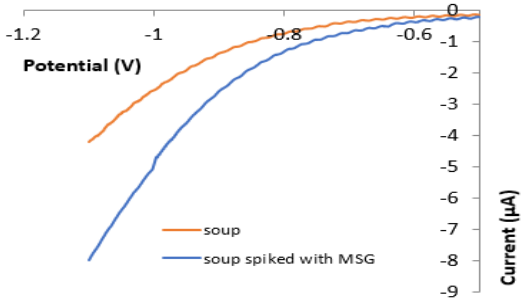

Figure 12: Cathodic current produced for the analysis of soup sample and soup samples spiked with 5 g/L MSG using μPAD fabricated with graphite working electrode modified with Co_3O_4 nanoparticles in the ratio of 1:15 at a scan rate of 50 mV/s

similar methods is listed in Table 1. However, these methods cannot be used for on-site detection. Hence, even with a higher LoD, the novel μPAD is advantageous over the other reported methods. Also, this μPAD based method for the detection of MSG is economical, faster and can be performed with μL volumes.

Method	LoD
HPTLC ((Soyseven, *et al*., 2020))	0.015 mg/L
Spectroscopic (Acebal, *et al*., 2008)	16.1 mg/L
Electrochemical (Zhang, *et al*., 2006)	0.016 mg/L

A. Analysis of real samples

The effect of interferents present in the matrix of a real food sample (vegetable soup) was investigated using real food samples spiked with MSG. A blank sample of soup and a sample of soup that had been spiked with MSG (5 g/L) were analyzed. The results of the blank sample and the sample spiked with MSG are shown in Figure 12.

The difference in negative current between the soup sample and the sample spiked with MSG was approximately 4 μA. Since this value is greater than 2 μA, this can be identified as a positive response and indicates the presence of MSG in the sample at a concentration greater than 0.82 g/L.

IV. Conclusions

A novel method was introduced to fabricate the hydrophobic barrier in μPADs using the hydrophobic ink of a CD/DVD permanent marker. This is an inexpensive, quick, and easy fabrication method to develop μPADs for one-time-use devices. Further, a low-cost qualitative electrochemical μPAD sensor was developed for the detection of MSG in food samples. The working electrode was modified with Co_3O_4 particles. The developed μPAD is capable of qualitatively detect MSG with an LoD of 0.82 g/L in real samples.

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