



Extraction Procedure and Cyclic Voltammetry Assay for Detection of Monosodium Glutamate from Different Processed Food Sources

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Abstract. *In this paper, the possibility of direct electrochemical detection of monosodium glutamate (MSG) from food products by the means of cyclic voltammetry is studied. There are four types of processed food included in this study: bologna sausage, frankfurter hot dogs, hot dogs with cheese, and vegetable soup concentrate cubes. The article describes the procedure of MSG extraction from these products. The MSG (as L-glutamic acid) content was precisely determined using an enzymatic spectrophotometry kit provided by Megazyme. Cyclic voltammetry (CV) experiments were performed using three types of screen printed electrodes having carbon, gold or platinum as working electrode. The results show signal appearance for MSG, so qualitative possibility of MSG presence identification; in the case of DRP-110 carbon electrode, the linear correlation between cathodic peak current and MSG concentration suggests the possibility of optimization and use of CV for MSG determination for food industry.*

Keywords: *monosodium glutamate, cyclic voltammetry, screen printed electrodes, food additives*

1. Introduction

There is a continuing and growing need for simple detection systems, new solutions and active materials in order to identify harmful elements that exceed the limit value in food and beverages. Sensors suitable for this purpose should be multifunctional, robust, have low cost, be precise and reliable. Until now, various types of methods have been used to detect food additives: for example spectrophotometry, chromatography, capillary electrophoresis, high performance liquid chromatography - HPLC and others [1-5]. These methods can be costly or can last for a long time. Also, electrochemical biosensors have been used for detection of MSG, based especially on enzymatic activity [6-11]. Screen printed electrodes with Pt, graphite or Ag recording site are often used in these sensors as microelectrodes.

Monosodium glutamate, MSG (additive number E-621) is a flavor and taste enhancer, first isolated in 1908 at Tokyo Imperial University, and can now be found in food such as chips, instant soups, fast food, fermentation products, salad dressings, Asian foods and many more besides [9]. Numerous studies have been carried out over the last decades to determine the side effects and safety concentration limit in which they should be administered. MSG toxicity is mainly, but not exclusively, in the central nervous system and is associated with a series of somatic disorders reunited under the generic name of *Chinese restaurant syndrome* [12]. MSG is an excitotoxin, ie a toxic element that causes the over-excitement of important receptors and neurotransmitters in the brain, resulting in neuronal death, as a factor in worsening, if not the main cause, of diseases such as Alzheimer's, Parkinson's, autism or various forms of sclerosis [13,14]. Thus, to ensure consumer's safety the levels of glutamate in commercial food products must be controlled.

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While enzyme-based sensing sensors for glutamate detection have been a research interest for years, non-enzymatic electrochemical sensors have received more attention during recent years because of their stability and low cost. Electrochemical techniques may be applied using metallic or carbon electrodes in place of using enzymes; several enzyme electrodes have been reported based on the enzymes glutamate oxidase and glutamate dehydrogenase [15].

Due to the difficulty in electrochemically detecting with simple electrodes, the term “non-electroactive” is commonly applied to glutamate ion. However, using electrodes which have appropriate catalytic properties the detection is possible. For example, the monosodium glutamate detection in food samples was successfully achieved by using a potentiometric method with modified multiwalled carbon nanotubes entrapped in polymer [16]. For many decades, cyclic voltammetry (CV) is a very popular and often used electroanalytical technique, because the peak current is usually proportional to the concentration of the analyte in the solution [17]. Sales et al. [18] studied the voltammetric response of glutamate at gold electrode and monitored its suitability at routine analytical applications. Jamal et al. [19, 20] obtained cyclic voltammograms using nickel nanowire array electrode, and a glassy carbon electrode modified with NiO nanoparticles. An increased anodic peak current upon the addition of glutamate in a NaOH solution was obtained in their works, exhibiting electrocatalytic activity towards the oxidation of glutamate, non-enzymatically.

Dorozhko et al. [21] used a simple, fast and free of enzymes CV method for the determination of L-glutamate; a carbon-containing electrode modified with gold was used as working electrode and a borate buffer with *pH* 9.18 was the supporting electrolyte. A cathodic peak was observed for the glutamate reduction with a peak potential dependent on *pH*. Although the process has irreversible nature, a linear relationship of peak current with the square root of the scan rate indicate that the electroreduction was mainly controlled by diffusion.

This study tries to determine if a method based on non-enzymatic CV is reliable for simple, fast and cheap analysis of MSG from processed food products, establishing an extraction protocol from these products and searching for electrochemical reduction signal from MSG, with applicability for food industry and quality assurance laboratories.

2. Materilas and methods

Reagents and solutions

The following food products purchased from the market were tested: chicken bologna sausage from Angst (PA), frankfurter hot dogs from Herta (CF), hot dogs with cheese from Aia-Wudy Formaggio (CB), and cubes of vegetable soup (SM) from Maggi. For the calibration of the MSG measurement, a commercial Megazyme detection kit (K-GLUT 04/18) was purchased and was followed the protocol indicated by the manufacturing company. Trichloroethylene used for MSG extraction was of analytical purity and provided by Fluka.

Procedure of extraction of food additive

The principle of the extraction method is based on the high solubility of MSG in aqueous solutions. Studies have shown that organic substances that can coexist in food and could dissolve in water do not affect electrochemical measurements. Only Ni, Cu, Fe metals can strongly affect the investigation, but the likelihood of meeting these elements in food is low. By the method described below, the MSG is separated with other non-interfering soluble compounds in water, and the fatty or hydrophobic substances are taken up by trichloroethylene and removed.

Protocol for MSG extraction from solid samples (sausages)

First, the solid samples were grounded with a small capacity blender, then 20 g of sausage were weighted and homogenized in 30 mL of hot distilled water and filtered. Then, distilled water was added to the filtered solution to obtain a total volume of 100 mL extract. Trichloroethylene was added in this clear solution, in a V / V ratio of 1: 4 trichloroethylene: aqueous extract, and stirred very well



by centrifugation at 4000 rpm for 10 min to separate the organic phase. Finally, the aqueous solution was taken and subsequently used for measurements.

Protocol for MSG extraction from Maggi soup

100 mg of concentrated soup cube were dissolved in 10 mL distilled water, the solution was brought to 70°C for 10 min and was filtered through standard filter paper, so that a clear, slightly yellowish liquid is obtained.

Methods, equipment and electrodes

For spectrophotometrical determinations, an UV-Vis Perkin Elmer spectrophotometer was used at 492 nm wavelength. The principle of spectrophotometrical determination consists in chemical oxidation of L-glutamic acid by nicotinamide-adenine dinucleotide (NAD⁺) in the presence of glutamate dehydrogenase (GIDH), leading to the formation of 2-oxoglutarate, nicotinamide adenine low-level dinucleotide (NADH), and ammonium ions (NH₄⁺). Since the balance of this deamination reaction is significantly directed in favor of the reactants, a further diaphorase-catalysed reaction is required, in which NADH reduces the idonitrotetrazolium chloride (INT) to an INT-formazan complex product, resulting in a rapid and quantitative conversion of L-glutamic acid. The amount of INT-formazan formed in this reaction is stoichiometric with the amount of L-glutamic acid. Actually, the INT-formazan formed is the chemical compound that is measured by increasing the absorbance at 492 nm wavelength [22]. The chemical reactions are:



The test is specific for L-glutamic acid. D-glutamic acid, while L-glutamine and L-aspartic acid do not react. The detection limit is 0.214 mg/L L-glutamic acid. The analysis in our conditions is linear in the range of 0.4 to 20 µg of L-glutamic acid on the assay.

The kit for analysis was K-GLUT 04/18 Megazyme kit which contained five bottles with different solutions: buffer (pH 8.6) + sodium azide (0.02% w/v) mixture, NAD + INT mixture, diaphorase suspension, glutamate dehydrogenase solution, and a mixture of L-glutamic acid standard solution (0.1 g/L) + 0.02% (w/v) sodium azide, respectively. First, a solution is prepared in cuvette by mixing of buffer + sodium azide, NAD + INT, and diaphorase, at which the absorbance (A1) is measured after approx. 2 min. Then, the reactions were started immediately by addition in each cuvette of either L-glutamic acid standard or sample extract in the presence of glutamate dehydrogenase (GIDH). The final absorbances (A2) of the solutions were read after approx. 8-10 min (the end of the reaction). Concentration of L-glutamate is calculated from $\Delta A = A_2 - A_1$ absorbance difference.

Cyclic voltammetry measurements for each food extract were performed at room temperature using a 0.12 V/s scan rate in a potential range of -1.0 - +1.0 V. A volume of 10 mL was introduced into the DRP electrochemical cell (from Metrohm DropSense) connected to an Autolab PGSTAT 302N potentiostat/galvanostat which runs with NOVA 2.0 software. Screen printed electrodes (SPEs) were provided by Metrohm DropSense and were by three categories, as shown in Table 1 [23]. They are strips having dimensions shown in Table 1, with electric contacts to counter electrode and reference electrode made of silver. The working electrodes (WEs), each having 4 mm diameter, were carbon (DRP-110), gold (DRP-220AT), and platinum (DRP-559).

Table 1. General characteristics of screen printed electrodes [23]

<u>SPE</u>	<u>DRP-110</u>	<u>DRP-220AT</u>	<u>DRP-550</u>
Image			
<u>Ceramic substrat dimensions</u>	L33 x W10 x H0,5 mm	L33 x W10 x H0,5 mm	L33 x W10 x H0,5 mm
<u>Electrical contacts material</u>	silver	silver	silver
<u>Working electrode diameter</u>	4 mm	4 mm	4 mm
<u>Working electrode material</u>	carbon	gold	platinum
<u>Reference electrode material</u>	silver	silver	silver
<u>Counterelectrode material</u>	carbon	gold	platinum

3. Results and discussions

The spectrophotometric results regarding absorbances obtained using Megazyme protocol for sample volumes of 0.1 mL are summarized in the Table 2. Also, a comparison between the MSG concentration in extract and the percentage in the whole solid sample for different types of food products is made in Table 2 and Figure 1. It can be seen a significant MSG concentration in Maggi soup and hot dogs with cheese (content decreases in this order), whereas bologna sausages have relative low MSG concentration and frankfurter hot dogs – the lowest MSG content.

Table 2 MSG concentrations obtained spectrophotometrically for food products

Sample	Absorbances			Acid L-glutamic	
	A1	A2	$\Delta A_{\text{acid L-glutamic}}$	g/L in extract	wt% in food sample
Standard L-glutamic acid	0.13	0.5692	0.4662	0.0999	-
CF	0.1448	0.2278	0.083	0.0178	0.0089
PA	0.1616	0.364	0.2024	0.0434	0.0217
CB	0.6589	1.4453	0.7864	0.1686	0.843
SM	0.12	1.4403	1.3203	0.283	2.83

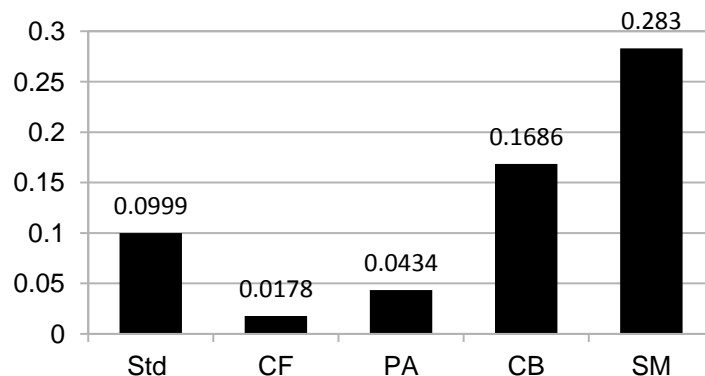


Figure 1. Comparison between MSG concentration (g/L) values from different processed food sources determined spectrophotometrically; Std = standard 0.10 g/mL, CF = frankfurter hot dogs, PA = bologna sausage, CB = hot dogs with cheese, SM = Maggi vegetable soup

Regarding the cyclic voltammetry results, the obtained CV curves using the DRP-550 electrodes having Pt material as WE are shown in the Figure 2. In the cathodic scan, for the extract from frankfurter hot dogs, which has the lowest monosodium glutamate concentration, we did not get any voltammetric signal using platinum electrodes, therefore this curve is not presented. It is found an irreversible (by electrochemical point of view) reduction process with a clear peak to the potential $E = -0.75\text{V}$ for Maggi soup (peak c). For the other two food extracts, rather a shoulder is obtained at more negative potentials, namely -0.85V for peak a and -0.90V for peak b.

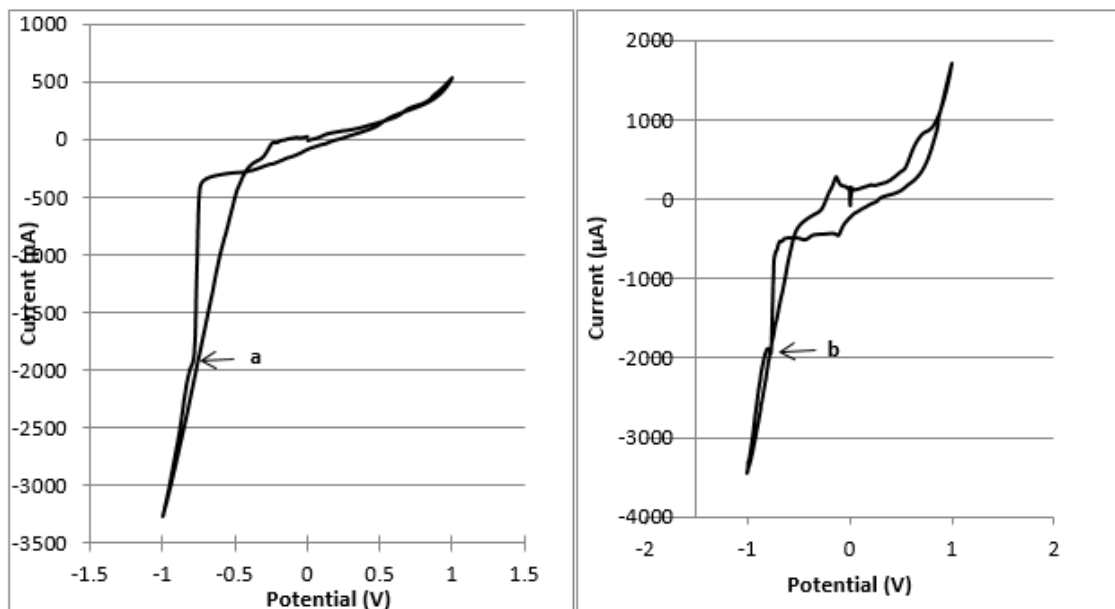


Figure 2. Cyclic voltammograms obtained for food extracts using DRP 550 (platinum WE) electrodes; the position of cathodic shoulder or peak is indicated: (a) bologna sausage Angst, (b) hot dogs with cheese

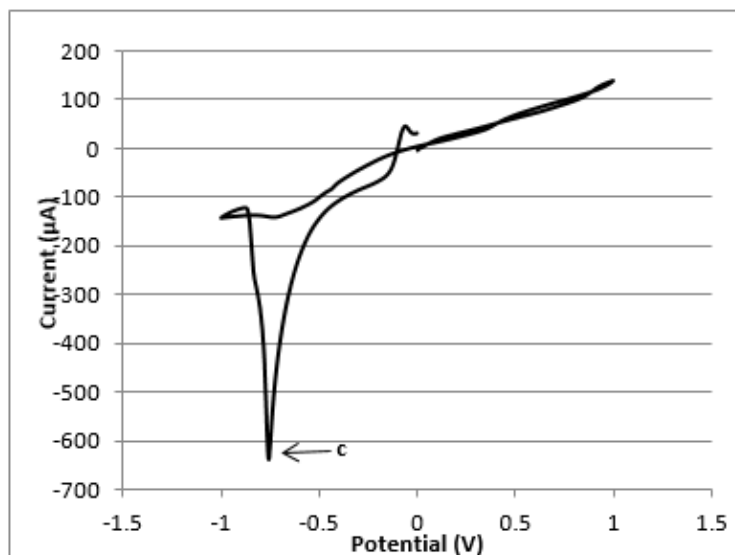


Figure 2. Cyclic voltammograms obtained for food extracts using DRP 550 (platinum WE) electrodes; the position of cathodic shoulder or peak is indicated: (c) Maggi vegetable soup

Voltammograms obtained using the DRP-220AT electrodes having gold material as WE are shown in the Figure 3. For the extract from frankfurter hot dogs, we only saw a vague increase of cathodic current at the reduction potential of approx. $-0.7 \div -0.8$ V, but the signal was too weak to be of analytical importance. We observed for the other food extracts (Figure 3) the appearance of small shoulders in the potential region around -0.8 V, corresponding to the MSG reduction potential, They increase clearly in current intensity with the increase in the MSG concentration, thus confirmed the order determined spectrophotometrically.

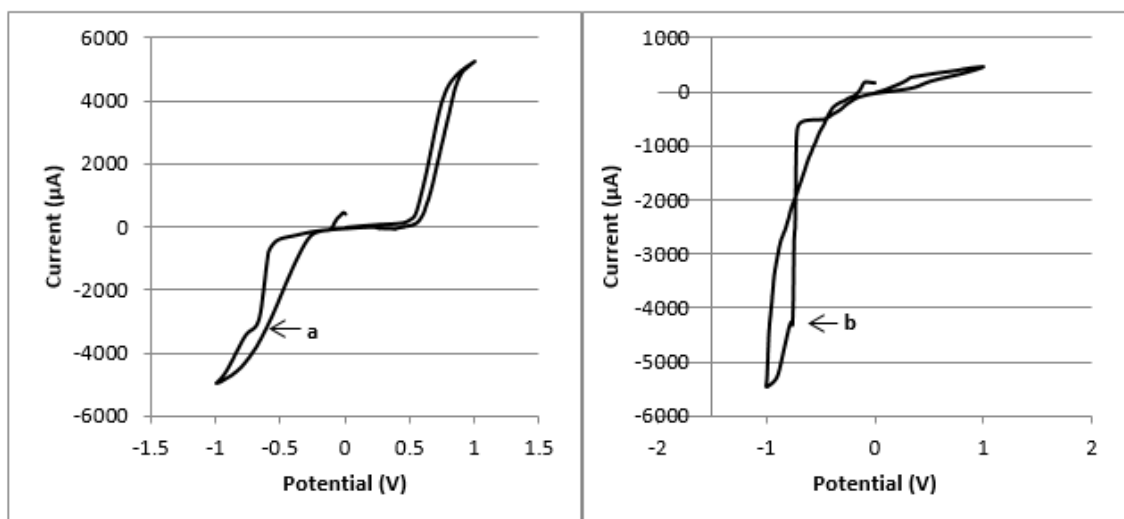


Figure 3. Cyclic voltammograms obtained for food extracts using DRP-220AT (gold WE) electrodes; the position of cathodic shoulder is indicated: (a) bologna sausage Angst, (b) hot dogs with cheese

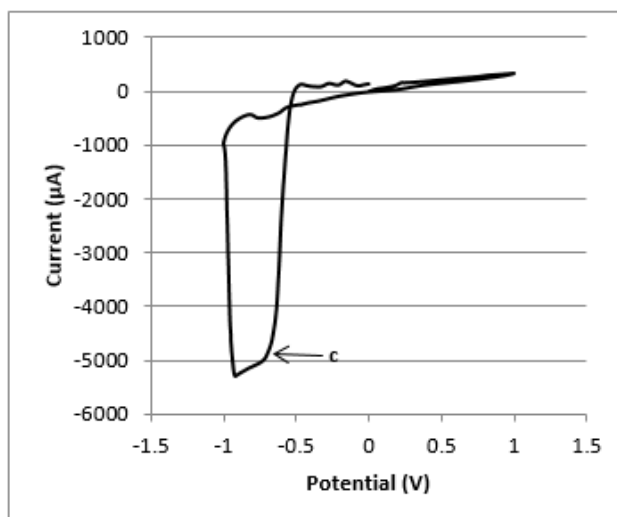


Figure 3. Cyclic voltammograms obtained for food extracts using DRP-220AT (gold WE) electrodes; the position of cathodic shoulder is indicated: (c) Maggi vegetable soup

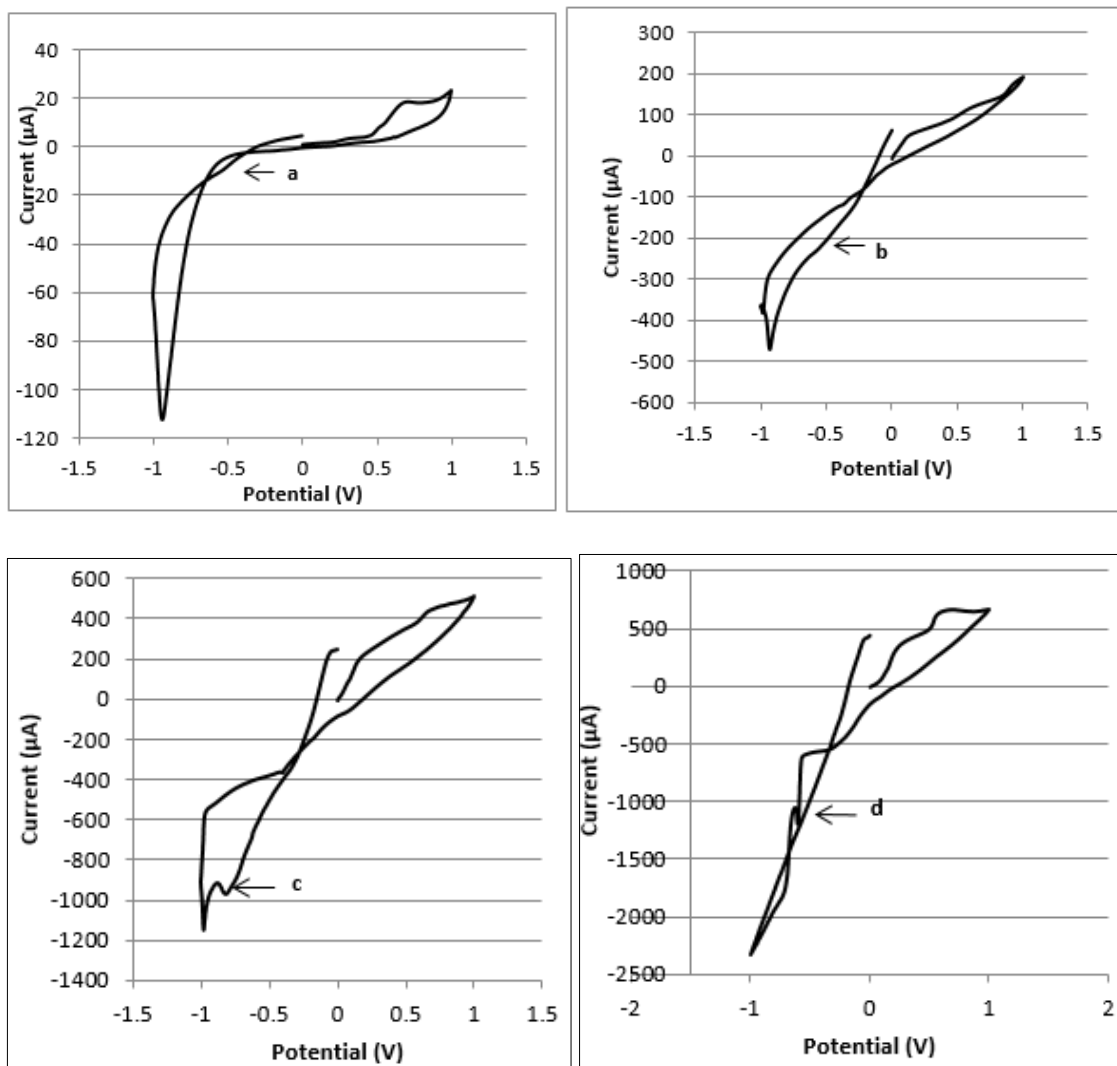


Figure 4. Cyclic voltammograms obtained for food extracts using DRP-110 (carbon WE) electrodes; the position of cathodic peak is indicated: (a) frankfurter hot dogs, (b) bologna sausage Angst, (c) hot dogs with cheese, and (d) Maggi vegetable soup

For DRP-110 electrodes having carbon as WE, all CV curves in Figure 4 show clearly a cathodic peak of MSG electroreduction for each sample. In this case, for frankfurter hot dogs and Angst bologna sausages the signal is very weak and moderately weak, respectively. For the other two foods, the peaks are strong and well defined and the increase in intensity is significant. It may be observed that intensity of peak current increases with increase in MSG concentration in foodstuff (Figure 5), in good agreement with the data obtained spectrophotometrically. Thus, the concentration order of $CF < PA < CB < SM$ is confirmed by voltammetric measurements. This behavior for MSG cathodic reduction on carbon electrodes suggests a diffusion controlled process.

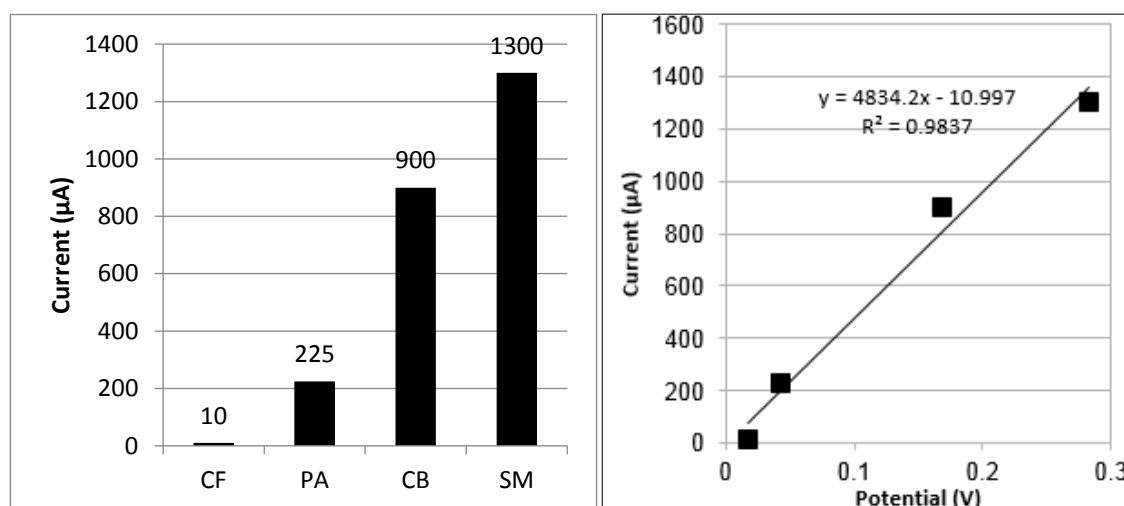


Figure 5. The quantitative correlation between MSG concentration and analytical signal (I_{peak}) obtained with DRP-110 (carbon WE); PA = bologna sausage Angst, CF = frankfurter hot dogs, CB = hot dogs with cheese, SM = Maggi vegetable soup

4. Conclusions

The extraction protocols applied here to obtain clear liquid samples with monosodium glutamate starting from four food products was simple, efficient and reliable. First, the L-glutamate concentrations were exactly determined using a spectrophotometrical method based on enzymatic oxidation of L-glutamate. These concentrations for four types of alimentary processed products were established and they correspond well with MSG concentrations.

The electrochemical behavior of glutamate on screen printed electrodes having platinum, gold or carbon as working electrodes has been investigated by cyclic voltammetry. Electrochemical reduction of glutamate is an electrochemically irreversible process and diffusion controlled process. The electrochemical reduction signals corresponding to monosodium glutamate were qualitative present for all the types of electrodes, but the best signal, which could be linearly correlated with MSG concentration was found to be given by the DRP-110 type of carbon electrode. The CV analytical procedure is an easy, fast and free of enzymes method for the determination of glutamate in solutions from food provenience. The method also offers advantages in terms long viability of application because it is not enzymatic-based and is inexpensive regarding reagent consumption and equipment involved. Further studies are required to establish and improve main analytical features (linear range, limit of detection, etc.) and interfering effect of some coexisting compounds and confirm application of the proposed method to real food product analysis and quality control applications.

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