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Procedia Chemistry 15 (2015) 365 – 370

Procedia Chemistry

16th International Scientific Conference "Chemistry and Chemical Engineering in XXI century" dedicated to Professor L.P. Kulyov, CCE 2015

Electrochemical Determination of L-Glutamate on a Carbon-Containing Electrode Modified with Gold by Voltammetry

E.V. Dorozhko [a,*], E.I. Korotkova [a], A.A. Shabaeva [a], A.Y. Mosolkov [a]

[a] *National Research Tomsk Polytechnic University, Lenin avenue, 30,Tomsk, 634050, Russia*

Abstract

In this paper the L-glutamate electrochemical behavior on a carbon-containing electrode modified with gold in simulated solutions by cyclic voltammetry have been studied. The analytical signal of glutamate reduction at the potential of -0.8 V in pH 9.19 has been found. It has been determined that the cathodic reduction of glutamate is irreversible and mainly controlled by diffusion in the reduction electrode process of glutamate. The conditions for glutamate determination in simulated solutions by voltammetry have been found. The plots of current versus concentration present a linear behavior from $3.2 \cdot 10^{-4}$ to $2.5 \cdot 10^{-3}$ M. The detection limit is 4.4 10^{-5} M. The glutamate determination in simulated solutions has resulted in acceptable deviation from the stated concentration where relative errors are -2.7 to +2.6 %.

Peer-review under responsibility of Tomsk Polytechnic University

Keywords: L-glutamate, voltammetry, carbon-containing modified electrode, flavor enhancer, food.

1. Introduction

L-Glutamate, food additive E-621, known as monosodium glutamate, is found in many foods, such as sauces, instant soups, fast food, fermentation products[1,2]. Excess content of sodium glutamate in the human body causes a toxic effect of adverse reaction such as rash, itching, nausea, vomiting, and headaches[2]. Thus, to ensure food quality the levels of glutamate in commercial food products must be controlled.

There are several methods for the determination of glutamate in various foods and biological samples[3-6].

* Corresponding author. Tel.: +79631942232; fax 8(3822)56-38-32.
E-mail address: elena-dorozhko@yandex.ru

Peer-review under responsibility of Tomsk Polytechnic University
doi:10.1016/j.proche.2015.10.058

Commercially available and the most common method for glutamate determination is a colorimetric method[7]. However, the method is laborious and time consuming. Table 1 shows the other optical methods for determining different samples of glutamate.

Table 1. Optical methods for glutamate determination in food and biological samples

The experimental conditions	Linearity range (μM)	Limit of detection (μM)	Sample	Ref.
Technique: UV/Vis L-Glutamate dehydrogenase transforms glutamate in 2-oxoglutarate and NAD+. The latter is reduced to NADH while the former reacts with D-4 hydroxyphenylglycin to form 4-hydroxybenzoylformate after catalysis by Dphenylglycine aminotransferase. NADH and 4-hydroxybenzoylformate are quantified by monitoring absorbance readings at 340 nm.	0.2 – 20	0.14	Fish and soy sauces	3
Technique: Luminescent glutamate reacts with glutamate oxidase to form H_2O_2; this product reacts with luminol when peroxidase is present, producing a maximum emitted radiation at 280 nm; carried out in flow media.	0.020 – 50	0.010	Serum	5
Technique: Luminescent glutamate is oxidized by NAD+ thus producing the NADH. This reduced form is made to react with resazurin to obtain a fluorescent product (Iexc 530 nm and Iem 590 nm).	0.02 – 1.25	0.020	Food	6
Technique: Luminescent is similar to the previous one, but carried out in flow media. Fluorimetric detection is made at Iem of 460 nm after Iexc of 340 nm.	2.5 – 50	0.4	Fish and other sauces	4

The enzymatic reactions using in these methods allow determining glutamate with high selectivity. However, the main disadvantages of these methods are a short viability of enzymes and high cost of enzyme preparations. Another group of methods for the determination of glutamate in different objects are HPLC (see Table 2)[8-12].

Table 2. HPLC determination of glutamate in food and biological samples

Detection	Operating conditions	Linearity range (μM)	Limit of detection (μM)	Sample	Ref.
MS	Stationary phase: 5 μm inertsil ODS-2 (150x4.6 mm i.d.); Mobile phase: 10% of acetonitrile in ammonium acetate 100 mM Flow-rate: 0.9 mL/min. Retention time < 2 min.	-	40	Mice	8
FL	Stationary phase: 4 μm C18 (150x3.9 mm i.d.); Mobile phase: Gradient procedures of (A) 120 mM potassium phosphate and (B) 120 mM potassium phosphate/ acetonitrile/methanol (46:18:18) λexc 340 nm; λem 425 nm	40-200	80	Mice	9
	Stationary phase: 5 μm C18 (250x4.6 mm i.d); Mobile phase: sodium hydrogenophosphate 0.05 M, hexansulphonic acid 0.01M, triethylammine trichloroacetic acid 0.0072M Retention time < 5 min. λexc 340 nm; λem 425 nm.	40 – 200	8	Drugs	10

UV	Stationary phase: For free aminoacids (30x3.9 mm i.d.); Mobile phase: Gradient procedure of (A) sodium acetate 70 mM (pH 6.5) and acetonitrile and 0.025% EDTA, and (B acetonitrile/water/methanol (45:40:15) Retention time < 5 min. Chemical derivatization λ 254 nm.	25-2500	80	Stomach mucosa	11
	Stationary phase:5 µm LiChorospher 100 RP-18 (250x4 mm i.d.); Mobile phase: 100 mM ammonium acetate with 10% acetonitrile Flow-rate: 0.9 mL/min. Retention time < 2 min. Chemical derivatization λ 254 nm	-	2	Not applied	12

Chromatographic methods are precise, accurate and robust. However, they are expensive for a routine glutamate analysis in different objects.

The other methods published in literature over the past 10 years are electrochemical biosensors[13-15]. Biosensors allow selective determination of glutamate at the level of ppm in different objects. However, biosensors are not stable and expensive devices for the use in daily practice.

The authors offer a simple, fast and free of enzymes method for the determination of glutamate in simulated solutions that can be used to assess the content of glutamate in food. Thus, this work aims to study the voltammetric response of glutamate on a carbon-containing electrode modified with gold.

2. Experimental

Apparatus

All voltammetric measurements were performed using a voltametric analyzer (Tomanalyt, Tomsk, Russia) containing an electrochemical cell with a carbon-containing electrode modified with gold (CCEMG) as a working electrode (0.5 mm diammeter), a platinum electrode as a counter electrode and an Ag/AgCl (KCl 3.00 M) reference electrode.

CCEMG electrode was prepared by electrochemical reduction of gold from $HAuCl_4$ solution on the surface of the carbon-containing electrode within 30 seconds.

Before each experiment CCEMG were mechanically cleaned by polishing their surfaces with a pH meter. The electrode was calibrated with commercially available buffer reference solutions.

Reagents and solutions

L-Glutamate was purchased from Fluka and used without further purification. All other chemicals were Merck pro analysis grade and all solutions were prepared using distilled water.

Stock solutions of $1.00 \cdot 10^{-1}$ M glutamate were prepared by dissolving an exact amount of the pure compound in water. That solution was stored at +4ºC in the dark to prevent chemical alterations and considered stable for one week. Buffer reference solutions (potassium hydrogen phthalate pH 4.01, disodium phosphate pH 6.86, sodium tetraborate pH 9.18, hydroxide calcium pH 12.43) were selected as background electrolytes.

Procedures

A supporting electrolyte (20.0 mL) was placed in the electroanalytical cell. The solution was deoxygenated before analysis by purging with purified nitrogen for 2 minutes. A known volume of stock glutamate standard solution was transferred to the electrochemical cell and that solution was purged with purified nitrogen for 30 seconds. That procedure also ensured homogenization of the resulting solution.

Electrochemical studies of glutamate on a CCEMG were carried out by means of cyclic voltammetry (CV).

3. Results and discussion

The electrochemical behavior of glutamate on the CCEMG was investigated by CV over the potential range of +0.8 to -1.4 V. Borate buffer with pH 9.18 was used as a supporting electrolyte. Voltammograms of glutamate show a single peak in a borate buffer solution (Figure 1) at -0.80 V, applied over a CCEMG surface versus an AgCl/Ag reference electrode.

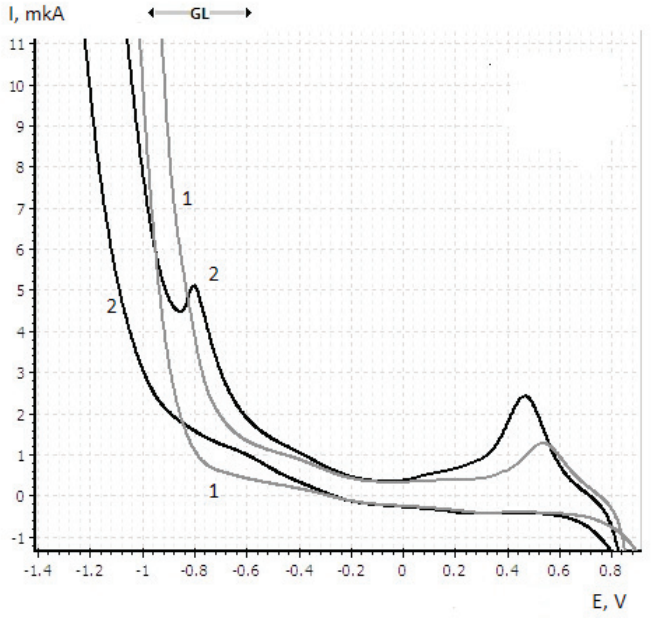

Fig.1. Cyclic voltammogram of the phosphate borate buffer with pH 9.18 in the absence (1) and in the presence of glutamate ($4.5 \cdot 10^{-3}$ M) (2) on the CCEMG measured in the range from +0.8 to –1.4 V. The number of cycles was 3. The scan rate was 50 $mV \cdot s^{-1}$

The absence of any anodic peak on the reverse scans indicates the irreversible nature of the electrode reaction. This irreversible nature is recorded for all scan rates from 10 to 300mV/s. The influence of the scan rate on the peak current (Ik) is studied within the range of 10 and 300 mV s^{-1} (Figure 2).

The cycles carried out within the increased values of scan rate produced a linear relationship with the square root of the scan rate, indicating that the process on the surface of the electrode was mainly controlled by diffusion.

The influence of pH on the glutamate reduction current at a potential of -0.8 V has been investigated. Voltammograms indicate that Ik depends on the pH of the medium (Figure 3). Glutamate presents eletroreduction activity only at pH values from 7.0 to 12.43. Glutamate in the pH range from 2 to 5 is not reduction. Thus, a value of pH 9.18 has been selected for subsequent studies.

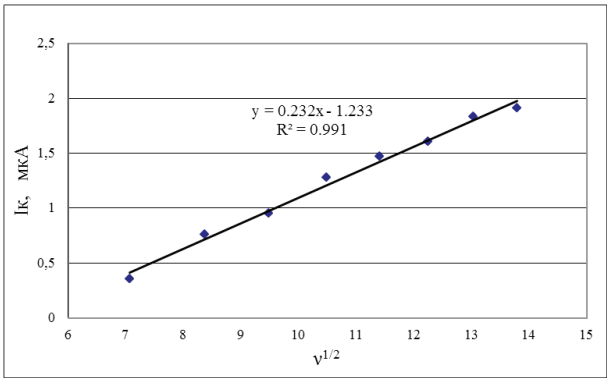

Fig.2. Cathodic peak current of glutamate ($4.5 \cdot 10^{-3}$ M) as functions of $V^{1/2}$

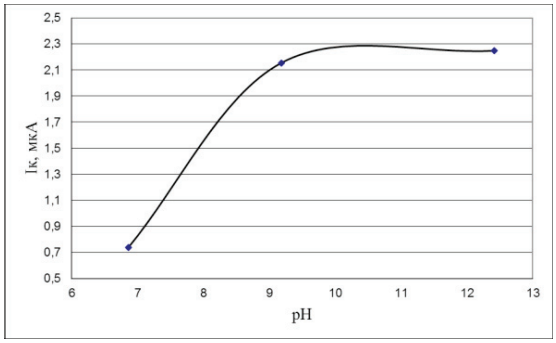

Fig.3. Influence of pH on the reduction peak current (Ik) of glutamate ($4.5 \cdot 10^{-3}$ M) on CCEMG

Consequently, electroanalytical procedures were established for the CV determination of glutamate. Table 3 shows the main analytical features obtained after the calibrations have been carried out with previously selected experimental conditions and with standard solutions of $(0.1 - 3) \times 10^{-3}$ M.

Table 3. Main analytical features of CV glutamate measurements

Analytical feature	CCEMG
pH	9.18
V, $mV \cdot s^{-1}$	150
Ep (V)	-0.8
Linear range ($\times 10^{-4}$ M)	3.2 −25
Correlation coefficient	0.9965
LOD ($\times 10^{-4}$ M)	0.42
LOQ ($\times 10^{-4}$ M)	1.9

The CV response shows a linear behavior for glutamate standard solutions ranging from $3.2 \cdot 10^{-4}$ to $25 \cdot 10^{-3}$ M. The limits of detection (LOD) and quantification (LOQ) are $4.4 \cdot 10^{-5}$ and $2.0 \cdot 10^{-4}$ M, respectively. These are calculated from the calibration plots using the equations: LOD = 3s/m and LOQ = 10s/m, where s is the standard deviation of the intercept and m is the slope of the calibration plot. Relative standard deviation is of 3.0 %, thus suggesting that the method is of good precision.

Glutamate was successfully determined in pure solutions after calibration by CV carried out under previously selected experimental conditions. Different concentration levels of the analyte ranging from about 100.0 to 300.0 mg/L were tried out.

Table 4. Determination of glutamate in spiked solutions

Glutamate (mg/L)		Recovery (%)	RSD[a] (%)	Relative error (%)
Taken	Found			
93.80	91.44	97.49	12.09	-2.70
155.56	155.83	100.18	3.59	0.18
247.06	250.96	101.58	0.45	2.58
307.32	303.64	98.80	0.76	-1.20

[a] RSD: relative standard deviation.

Analytical results of glutamate determination in simulated solutions are presented in Table 4. Mean reduction values are always close to 100 %. Plotting glutamate amount taken versus found gives a linear correlation of slope close to unit (0.9977), a small origin displacement (0.9303), and a squared correlation coefficient of 0.9988. The

overall procedure may take about 15 minutes per calibration with seven standard solutions and 3 minutes per triplicate measurement of a model sample. Regarding reagent consumption, each calibration procedure with eight standard solutions requires about 20 mL of supporting electrolyte and 2.3 mL of standard glutamate solution of 0.10 M. The supporting electrode is composed of nitrate, potassium and/or chloride. Environmental effect of the effluents produced after the analytical procedure is considered to be of a small concern. They contain mostly phosphate and a small amount of glutamate of about 7 mg.

4. Conclusions

The electrochemical behavior of glutamate on a carbon-containing electrode modified with gold has been investigated by CV. Electrochemical reduction of glutamate on a carbon-containing electrode modified with gold is irreversible and diffusion controlled. The potential of the peak is dependent on pH and higher reducing currents are pH 9.19. The CV analytical procedure is fast and simple, of low cost and of small environmental impact, all suitable features when routine measurements are intended. The authors offer a simple, fast and free of enzymes method for the determination of glutamate in simulated solutions. Further studies are required to confirm application of the proposed method to real objects studies.

Acknowledgements

This work was financially supported by the Ministry of Education and Science of the Russian Federation (the project “Science” and a grant of the RFBR, research project No. 15-04-01110.

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