Kinetic - Potentiometric Determination of Monosodium Glutamate in Soups and Soup Bases and of Glutamic Dehydrogenase*

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A simple and selective procedure has been developed for the determination of glutamic acid and glutamic dehydrogenase by using an ammonia gas-sensing electrode. Glutamic acid is deaminated by bacterial glutamic dehydrogenase in the presence of β-NAD^+. A linear relationship exists between the initial rate of ammonia release and the substrate concentration or the enzyme activity. Optimum conditions for the determinations were established. Glutamic acid in the range 1.0×10^{-4}–1.0×10^{-3} M and enzyme in the range 0.0500–0.750 U can be determined with relative errors of about 2%. A method is given for determining monosodium glutamate in soups and soup bases. The method was compared with the official AOAC method; satisfactory agreement was achieved.

Keywords: *Ammonia gas-sensing electrode; kinetic - potentiometric determination; L-glutamic acid determination; glutamic dehydrogenase determination; soup and soup bases analysis*

Monosodium glutamate (MSG), a widely used food additive, has been claimed to be responsible for the Chinese restaurant syndrome[1,2] and a controversy exists around a report that it produces brain lesions in the neonate mouse.[3,4] Therefore, there is a need for a rapid, simple and sensitive method for the determination of this compound. Non-enzymatic methods for the determination of MSG have been based mainly on chromatographic techniques,[5–7] but most of the proposed procedures are time consuming, cumbersome and, in some instances, irreproducible. On the other hand, potentiometric sensors for the steady-state measurement of MSG have been developed,[8,9] but these methods have not been applied to the determination of this compound in foods.

In this paper, an initial-rate kinetic method for the determination of L-glutamic acid is described. The method is based on the use of glutamate dehydrogenase to deaminate the acid. Deamination is followed by potentiometric measurement of the initial rate of ammonia production. Optimum operating conditions have been established to ensure short measurement times and maximum sensitivity for glutamic acid in the range 1.0×10^{-4}–1.0×10^{-3} M. The method is suitable for the determination of MSG in soups and soup bases. The method has also been used to determine glutamate dehydrogenase in aqueous solutions by adding a constant concentration of the substrate to the sample and measuring the initial rate of the potential change.

Experimental

Apparatus

The apparatus and the electrode assembly used were essentially identical with those described previously.[10] An Orion Model 95-10 ammonia gas-sensing electrode was used and potentials were recorded with a Heath - Schlumberger Model SR-255 potentiometric recorder. A Corning Model 12 research pH meter, acting as a voltage monitor, was inserted between the electrode and the recorder to match the high output impedance of the electrode with the relatively low impedance of the recorder. All measurements were made with a thermostated cell at 37 ± 0.2 °C.

When not in use, the electrode was kept in 0.10 M citric acid buffer (pH 4.5).[11]

Reagents

All solutions were prepared with de-ionised, distilled water from analytical-reagent grade materials and were stored in a refrigerator when not in use.

Tris - HCl buffer, 0.10 M, *pH* 8.5. Dissolve 12.1 g of Tris in water, adjust the pH to 8.5 with 6 M hydrochloric acid solution and dilute to 1 l with water.

β-*Nicotinamide adenine dinucleotide* (β-*NAD^+*) *solution*, 0.0060 M. Dissolve 0.400 g of β-NAD^+ in water and dilute to 100 ml.

Adenosine 5′-diphosphate (*ADP*) *solution*, 0.50 mM. Dissolve 21.4 mg of ADP in water and dilute to 100 ml.

L-Glutamic dehydrogenase stock solution, 25.0 U ml^{-1}. Dilute 0.050 ml of enzyme suspension [L-glutamic dehydrogenase, E.C. 1.4.1.3, from bovine liver (Sigma Chemical), solution in 50% glycerol, 37 U mg^{-1} of protein, defined and measured as recommended by the supplier] with 0.50 mM ADP solution to 10.0 ml. Prepare working standard solutions containing 0.500, 2.50 and 7.50 U ml^{-1} of enzyme from the 25 U ml^{-1} solution by dilution with 0.50 mM ADP solution (prepare just before use).

L-Glutamic acid stock solution, 0.0100 M. Dissolve 0.0735 g of glutamic acid in 1.5 ml of 1 M hydrochloric acid and dilute to 50.0 ml with water. Prepare working standard solutions of concentrations 1.00×10^{-4}, 3.00×10^{-4}, 6.00×10^{-4} and 1.00×10^{-3} M from the stock solution by dilution with water (prepare just before use).

Procedure

Preparation of samples

For products in the dry form, pulverise them uniformly in a mortar and weigh 0.80 g of sample. For undiluted, concentrated soups, homogenise the entire undiluted contents of a can in a blender and weigh 4.0 g of sample. For consommé (clear, condensed) soup, weigh 0.45 g of sample. Dilute the samples to about 35 ml with water at room temperature and mix until all water-soluble substances are in solution. Quantitatively transfer the sample into a 50-ml calibrated flask, dilute to volume with water and mix (solution A). Transfer 1.00 ml of solution A into a 10-ml calibrated flask, dilute to volume with water and mix.

Determination of MSG in soups

Pipette 4.00 ml of 0.1 M Tris - HCl buffer (pH 8.5) and 1.00 ml of 6.00×10^{-3} M β-NAD^+ into a thermostated cell and inject

* Presented at the 2nd International Symposium on Kinetics in Analytical Chemistry, Preveza, Greece, 9–12 September, 1986.

into this solution 0.200 ml of glutamic dehydrogenase stock solution (about 5 U). Immerse the ammonia electrode in the solution and start the stirrer; when the electrode potential has stabilised (1–2 min), start the recorder and pipette 1.00 ml of soup sample into the reaction cell. Record the potential, which decreases during the reaction, for 4–6 min.

Determination of the blank

Treat standard aqueous solutions containing 1.00×10^{-4}, 5.00×10^{-4} and 10.0×10^{-4} M glutamic acid by the above procedure, and plot the initial reaction rates against the concentration of the standards. The intercept of the linear plot on the abscissa gives the blank value.

Determination of glutamic dehydrogenase

Pipette 4.00 ml of 0.1 M Tris - HCl buffer (pH 8.5), 1.00 ml of 6.0×10^{-3} M β-NAD^+ and 1.00 ml of 0.0100 M glutamic acid into the thermostated cell. Immerse the ammonia electrode in the solution and proceed as in the determination of MSG. Start the reaction by injecting 0.100 ml of glutamic dehydrogenase standard solution or sample solution into the cell with a 0.1-ml Hamilton microsyringe.

Calculations

Calibration is carried out by plotting the initial slope (ΔE min^{-1}) against the molar concentration of glutamic acid or glutamic dehydrogenase activity (U).

Results and Discussion

L-Glutamic acid is specifically deaminated in the presence of glutamic dehydrogenase according to the equation

$$\text{L-glutamate} + NAD^+ + H_2O \longrightarrow \text{2-oxoglutarate} + NADH + NH_4^+$$

When the glutamic acid sample solution is pipetted into the buffered system containing the enzyme and β-NAD^+, the potential of the ammonia gas-sensing electrode decreases with time and the initial rate of potential change is directly related to the concentration of substrate added. The same reaction can be used to determine the enzyme activity.

The effects of buffer composition and pH were studied in order to establish conditions that provide good sensitivity and short measurement times. The initial rates of ammonia production in different buffers and at various pH values were monitored at 37 °C, as shown in Fig. 1. Borate and glycine buffers (pH 8.0) were found to inactivate the enzyme completely, whereas phosphate buffers (pH 8.0) inhibited the enzyme. The enzyme acitivity in imidazole buffers (pH 8.0) was relatively low, and Tris - HCl and triethanolamine buffers seemed the most satisfactory. With Tris - HCl buffer, there was an increase in the initial rate with increase in pH. Reaction rates were measured at pH 8.5, however, to ensure a better precision and larger buffer capacity.

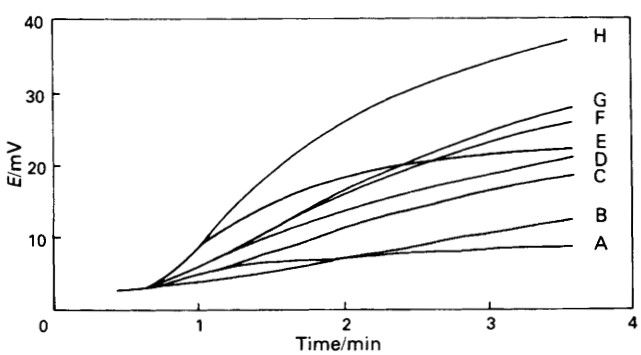

Fig. 1. Recorded graphs of potential *vs.* time for the glutamic dehydrogenase catalysed hydrolysis of glutamic acid, in the presence of NAD^+ at (A) pH 9.5 (carbonate buffer); (B) pH 8.0 (phosphate buffer); (C) pH 8.0 (imidazole buffer); (D) pH 7.5 (Tris - HCl buffer); (E) pH 9.0 (Tris - HCl buffer); (F) pH 8.0 (triethanolamine buffer); (G) pH 8.0 (Tris - HCl buffer); (H) pH 8.5 (Tris - HCl buffer). Enzyme activity 0.625 U; other conditions as in procedure for glutamic dehydrogenase determination

The effect of variations in the NAD^+ concentration on the sensitivity of the enzyme determination was studied. The sensitivity of the method increased (whereas the blank remained almost constant) as the concentration of NAD^+ was increased up to 6.0×10^{-3} M, which was chosen for the recommended procedure. Similar results were obtained for substrate determinations. The sensitivity of the method increases with increase in the amount of enzyme used up to 5 U of glutamic dehydrogenase, which was used in the recommended procedure.

Variations in the glutamic acid concentration used in the enzyme determination showed that the slope of the calibration graph increased with increasing concentration of substrate used, up to 0.010 M glutamic acid, whereas the blank remained almost constant; a further increase in substrate concentration did not improve the sensitivity or the blank. A study of the effect of temperature in the range 26–37 °C showed that the initial rate of potential change and the sensitivity of the method increased sharply with increasing temperature. A temperature of 37 °C is recommended.

The selectivity of the method is good. Although glutamic dehydrogenase has been reported to catalyse oxidations with hydroxy acids (in the presence of phenylhydrazine), only L-glutamic acid reacts in the glutamic dehydrogenase system if a Tris buffer is used and there are no interferences from D-isocitric, L-malic, gluconic, oxalic, L-lactic and DL-citric acids.[12] The interference from other amino acids (*e.g.*, DL-norvaline, L-valine, L-methionine, L-α-aminobutyrate, DL-norleucine, L-alanine, L-leucine and L-isoleucine) is negligible, as the oxidation of L-glutamate is stimulated by ADP. The oxidation of the other amino acids, in contrast, is inhibited by ADP.[13] It was also found that when starch, lactose and glucose are present with glutamic acid in a 10:1 excess, no effect on the glutamic acid determination is observed.

The stability of the reagents used has been discussed previously.[14] It is well known that glutamic dehydrogenase in solution and the absence of protective agents is rapidly inactivated.[15,16] Therefore, the stability of the enzyme in the presence of various stabilisers was examined. It was found that a 200-fold dilution of the enzyme suspension with a solution containing ADP at concentrations higher than 0.50 mM stabilised the enzyme for at least 1 d. It was further found that the initial reaction rate was a maximum for this ADP concentration, as the deamination of glutamate is stimulated by ADP.[13,15] The addition of bovine serum albumin and human serum is also said to stabilise the enzyme.[17] A 200-fold dilution of the enzyme suspension with human serum stabil-

Table 1. Determination of glutamic acid in aqueous solutions

Amount taken*/ 10^{-4} M	Amount found†/ 10^{-4} M	Relative error, %
1.00	1.02	+2.0
3.00	3.07	+2.3
5.00	4.91	−1.8
6.00	5.91	−1.5
8.00	8.12	+1.5
9.00	9.21	+2.3
10.0	9.80	−2.0

* Initial concentrations.

† Single runs; the regression equation is ΔE $min^{-1} = 2.57 \times 10^4$ [glutamic acid] + 3.32; correlation coefficient = 0.9997.

Table 2. Determination of glutamic dehydrogenase (GDH) in aqueous solutions

Amount taken*/ U	Amount found†/ U	Relative error, %
0.0500	0.0495	−1.0
0.0750	0.0759	+1.2
0.125	0.122	−2.4
0.250	0.254	+1.6
0.375	0.368	−1.9
0.500	0.508	+1.6
0.675	0.611	−2.2
0.750	0.766	+2.1

* Activities in 0.1 ml of sample.

† Single runs; the regression equation is ΔE min^{-1} = 30.3(GDH activity) + 2.10; correlation coefficient = 0.9996.

Table 3. Determination of monosodium glutamate in soups and soup bases

Product	MSG found, % *m/m*	
	Proposed method*	AOAC method[20]
Beef bouillon cubes (A) . .	9.83	9.70
Beef bouillon cubes (B) . .	9.86	9.80
Chicken bouillon cubes (A) . .	9.76	9.70
Chicken bouillon cubes (B) . .	9.72	9.95
Minestrone soup mix	2.38	2.30
Beef noodle soup mix	4.22	4.40
Vegetable soup mix	10.62	10.57
Tomato cream	3.92	4.00
Condensed chicken broth . .	1.24	1.28
Condensed beef broth	0.76	0.74

* Double runs; standard additions method.

ised the enzyme, for at least 90 min. The use of 5% bovine serum albumin for dilution resulted in stability of the enzyme preparation for about 60 min. The activity of these preparations. however, decreased by 22 and 34% in 90 and 120 min, respectively. When bovine serum albumin was used for dilution, the activity of the resulting preparation was about 66% of the initial value.

Immobilisation of the enzyme on the electrode was further attempted in an effort to reduce the cost per analysis and simplify the procedure. When an enzyme suspension containing 0.50 mM ADP was placed directly on the electrode surface, the enzyme activity was lost in 1 h. Other procedures were investigated for the immobilisation of the enzyme,[18,19] such as cross-linking with glutaraldehyde on albumin or on activated glass beads (in both instances an amount of 0.50 mM ADP was added). These procedures stabilised the enzyme, but the activities of these preparations were about 10% of the initial enzyme activity in solution.

Applications

Under the optimum conditions described, there was a linear relationship between the initial rate of evolution of ammonia and glutamic acid concentration up to 1×10^{-3} M. Results for the determination of glutamic acid in aqueous solutions are given in Table 1. Glutamic acid in the range 1.00×10^{-4}–1.00×10^{-3} M was determined with an average error of 1.9%. The relative standard deviation was 2.0% for a 5.00×10^{-4} M glutamic acid sample (six results).

Similarly, there was a linear relationship between the initial rate of evolution of ammonia and enzyme activity. With aqueous solutions, glutamic dehydrogenase was determined in the range 0.0500–0.750 U in 0.1 ml of sample with an average error of 1.8% (Table 2). The relative standard deviation for 0.375 U of glutamic dehydrogenase was 2.2% (six results).

Application of the procedure to commercially available soups and soup bases gave the results in Table 3.

Monosodium glutamate was determined by both the proposed method and the official AOAC method.[20] Because of the cost of preparing calibration graphs and because a reference soup without MSG is not available for calibration, a standard additions procedure was used to determine MSG in soups by the proposed method. In general, there was satisfactory agreement between the results obtained by the two methods. The proposed method is faster than chromatographic procedures (4–6 min compared with more than 2 h using chromatography) and there is no need for separation steps.

The accuracy of the proposed method was checked indirectly by means of recovery experiments carried out with three representative soup samples in which MSG was added to the sample. Subtraction of the MSG content of the soup showed that the recovery of added MSG (1.00×10^{-4}–7.00×10^{-4} M) was satisfactory (97.3–102.9%, mean 100.5%).

The author is indebted to the State Chemical Laboratory (Athens, Greece) for the analysis of the soups and soup bases. This work was supported by research grants from the University of Athens.

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Paper A6/329
Received September 10th, 1986
Accepted December 8th, 1986