



Direct Assay of Monosodium Glutamate in Multi-Sourced Bouillon Cubes by First Derivative Potentiometric Titration

*¹ALONGE, PO; ¹IDEMUDIA, OS; ²ODOKUMA-ALONGE, O

¹Department of Science Laboratory Technology, Faculty of Life Sciences, ²Department of Geology, Faculty of Physical Sciences, University of Benin, Benin City, Nigeria

*Corresponding Author Email: alongepeter59@gmail.com

ABSTRACT: In this study, a simple, cheap and accurate analytical method was developed for the direct assay of monosodium glutamate (MSG). First derivative potentiometric assay was adopted in the determination of MSG content in ten brands of Bouillon cubes (coded P₁, P₂, P₃, P₄, P₅, P₆, P₇, P₈, P₉ and P₁₀) sourced from different local markets. Formic acid extract of MSG was used for the determination. The result obtained for MSG standard using this method gave 99.40% which agrees with 99.94% also obtained for the non-extracted method. Results for all the coded samples show that sample P₈ (75.14%) gave the highest value of MSG content while sample P₆ (55.96%) gave the lowest value. Though, the World Health Organization (WHO) requirement for MSG consumption per day is 0.12g, the values obtained in this study cannot be related to daily consumption because the amounts of bouillon cubes and consequently the amount of MSG used by consumers differ from one individual to another. However, the result obtained for the estimation of MSG in bouillon cubes can be adopted by regulatory agencies such as National Agency for Food, Drugs and Administration Control (NAFDAC) and International Standard Organization (ISO).

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Monosodium glutamate (MSG) which was discovered in 1908 by Kikunae Ikeda in Tokyo Imperial University elicits a taste that is distinct from the four known primary tastes. Ikeda reported the discovery and his hypothesis about a fifth taste in 1909 in the *Journal of the Chemical Society of Tokyo* (Maureen, 2003). Acceptance of this “fifth delicious taste” which he termed “Umami” as a basic taste came only decades after Ikeda laid out his hypothesis. This was after other “umami” substances were identified as inosine 5'-monophosphate (IMP) and Guanosine 5'-monophosphate (GMP) as taste responses in humans and animals. Identification of L-glutamate taste receptors in the year 2000 by two researchers Nirupa Chaudhari and Stephen Roper at the University of Miami, School of Medicine dispelled the lingering doubts (Maureen, 2003). On its own, MSG does not have much taste, but its effect is noticeable when added to soups, stews, and snacks, among other foods. Synergisms between MSG and IMP or GMP are known. Adding a pinch of MSG to food containing these nucleotides enhances the umami taste up to eight times the original. Food products claiming “No MSG” may contain disodium salts of IMP and GMP as alternative flavour enhancers (Maureen, 2003). With the help of

Ajinomoto Group of company in Japan, Ikeda patented monosodium glutamate in 1909 and it was commercially available from that time (Lindeman *et al.*, 2002), thus MSG has been around and in commercial use for almost 100 years.

MSG is a white crystalline substance of sodium salt of the non-essential amino acid- glutamic acid. It is one of the most abundant amino acids found in nature (Wijayasekara and Wansapala, 2017). Glutamate is one of the most common amino acids in nature and is the main component of many proteins and peptides of most tissues (Inuwa *et al.*, 2011). Glutamate produced in the body plays an important and essential role in human metabolism. It is a major component of many protein rich food products such as meat, fish, milk and some vegetables (IFIC, 1994). MSG is a substance widely used as flavouring agent. It contains 75% glutamic acid, 22% sodium and 3% water (Andrienne, 1999). In the early part of the twentieth century, MSG was extracted from sea weed and other plant sources, produced through fermentation processes using molasses from sugar cane or sugar beet as well as starch hydrolysis from corn, tapioca, etc. Prior to the development of the fermentation process, MSG was produced by hydrolysis of natural

*Corresponding Author Email: alongepeter59@gmail.com

proteins such as wheat gluten and defatted soybean flakes (IFT, 1994; Fuke and Shimizu, 1993). Most food additives act either as preservatives, or enhancer of food palatability. One of such food additive is MSG. In Nigeria, MSG is sold in the open market as "Ajinomoto" marketed by West African Seasoning Company Limited as "Vedan" or "White Maggi" and indirectly as constituent of food seasoning (Inuwa *et al.*, 2011). It has been calculated that a 70-kg man has a daily glutamic acid intake of 28g that is derived from the diet and from the breakdown of gut proteins. The daily glutamic acid turnover in the body is 48g.

Despite this large turnover, the total pool of glutamic acid in blood is quite small; 20mg, because of its rapid extraction and utilization by various tissues, particularly the muscles and liver (Munro 1979). The methods used for MSG studies include spectrophotometric technique (Beutler, 1990), amperometric techniques (Manas and Miltiades, 2001), chromatographic techniques (Pascal *et al.*, 2000) and potentiometric techniques (Kissinger and William, 1996). In this study, a simple, cheap, accurate and reproducible method for direct assay of MSG is presented.

MATERIALS AND METHODS

Sample Collection: Ten (10) different brands of Bouillon cubes were sourced from various local markets in Benin City, Edo State of Nigeria.

Sample Preparation: 10 cubes from each of the different brands of the sample were randomly selected from their packs and ground into powdered form using mortar and pestle. The powdered samples were transferred into a dry clean crucible and placed in an oven set at temperature of 110°C for 2 hours to remove the moisture. This was repeated until constant weight of the sample was obtained.

Stoichiometric Bases of the Analysis

$C_5H_8NNaO_4 + NaOH \rightarrow C_5H_7N(Na)_2O_4 + H_2O$	1
187.13g $C_5H_8NNaO_4 = 1M NaOH = 1000ml$	2
187.13g $C_5H_8NNaO_4 = 1N NaOH = 1000ml$	3
18.713g $C_5H_8NNaO_4 = 0.1N NaOH = 1000ml$	4
0.18713g $C_5H_8NNaO_4 = 0.1N NaOH = 1ml$	5
0.46725g $C_5H_8NNaO_4 = 0.1N NaOH = 25ml$	6

Extraction of Samples: 0.4673g of the dried and powdered cubes (corresponding stoichiometrically to 25ml 0.1N NaOH solution) was dissolved in 25ml 0.1N formic acid solution. The solution was filtered into a beaker and the filtrate was placed on a boiling water bath set at 100°C. The beaker was stirred briskly at 3 minutes intervals. The solution was evaporated to dryness and allowed to cool at room

temperature. This procedure was repeated for all the coded ten samples.

Direct Assay of Monosodium Glutamate: 25ml of 0.1N formic acid was added to the extracted MSG in the beaker and stirred thoroughly until complete dissolution was attained. The solution was titrated potentiometrically with 0.1N NaOH, using a pH meter which was standardized with a phosphate buffer solutions of pH 4, 7 and 9. Aliquots of 2ml of 0.1N NaOH solution were added progressively followed by 0.2ml aliquots when inflexion point was observed. The end point of the titration was indicated with observed constant pH reading even when 2.0ml aliquot of the 0.1N NaOH solution were added. This procedure was repeated for the analyzed samples (P₁-P₁₀).

Calculation of the amount of MSG in Standard and Test Samples: The titre value (V) obtained for the standard and each test sample was used to calculate the amount of MSG using equation 6. Thus, the weight of MSG is calculated from the formula:

$$\text{Weight of MSG} = (V - 13) \times F \times G \quad .7$$

Where, V = Equivalent titre value and 13 is the back titrated volume for excess formic acid; F = Factor of standardization of NaOH and G = Weight of MSG equivalent to 25 ml 0.1N NaOH (From equation 6)

The percentage purity of MSG in both the standard and bouillon cubes were calculated with the formula:

$$\%p = \frac{\text{wt of MSG in sample}}{\text{Theoretical wt of MSG}} \times 100 \quad .8$$

RESULTS AND DISCUSSION

The first derivative potentiometric titration curves of both standard MSG and formic acid extracted MSG from each of the coded (P₁-P₁₀) brands of Bouillon cubes available in the various markets in Edo state are shown in Figures 1 to Figure 12. Figures 1 and 2 represent the first derivative potentiometric titration of the non-extracted and the formic acid extracted standard samples of MSG respectively, while Figures 3 to 12 represent the formic acid extracted MSG from bouillon cubes titrated with 0.1N NaOH solution.

The peaks of the curves correspond to the equivalent titre values. For each of the derivative plots, the differences in the volume of 0.1N NaOH solution was taken as due to the effect of the formic acid used for the extraction of the various coded brands of MSG used in this study.

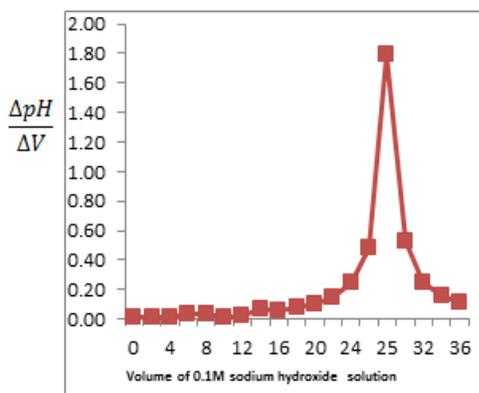


Fig 1: First derivative potentiometric titration plot for standard 0.1M MSG with 0.1M NaOH solution with equivalent volume at 25 .00ml.

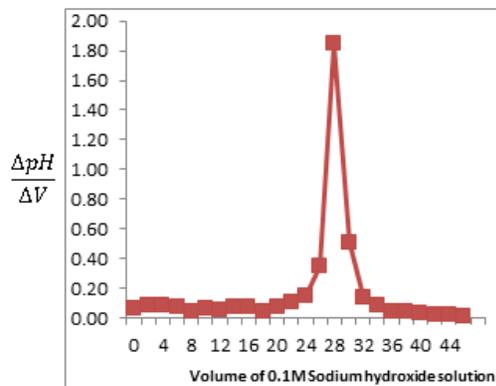


Fig 4: First derivative potentiometric titration plot for P₂ extracted with formic acid and titrated 0.1M NaOH solution with equivalent point at 28.00ml.

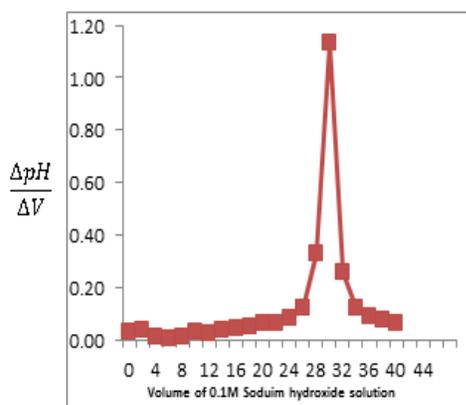


Fig 2: First derivative potentiometric titration plot for formic acid extracted standard MSG and titrated with 0.1M NaOH solution with equivalent volume at 38ml.

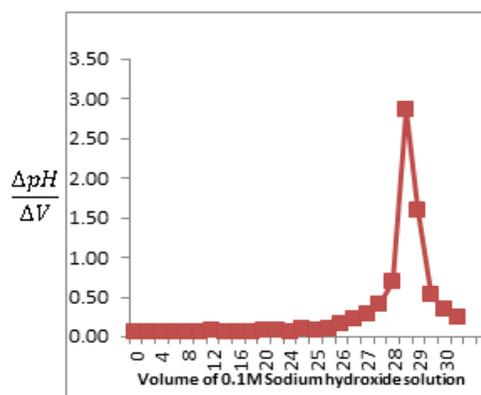


Fig 5: First derivative potentiometric titration plot for P₃ extracted with formic acid and titrated 0.1M NaOH solution with equivalent volume at 29.00ml

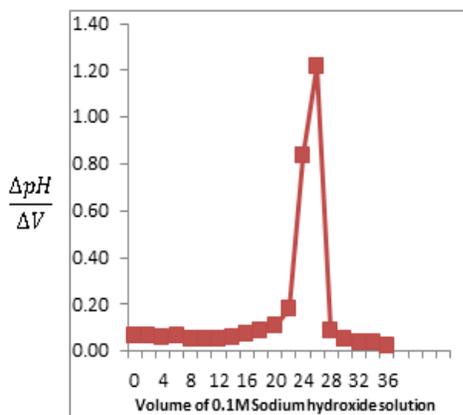


Fig 3: First derivative potentiometric titration plot for P₁ extracted with formic acid and titrated with 0.1M NaOH solution with equivalent volume at 26.00ml.

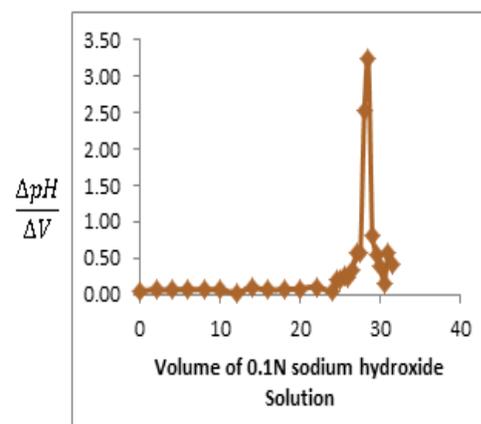


Fig 6: First derivative potentiometric titration plot for P₄ extracted with formic acid and titrated with 0.1M NaOH solution with equivalent volume at 28.50ml.

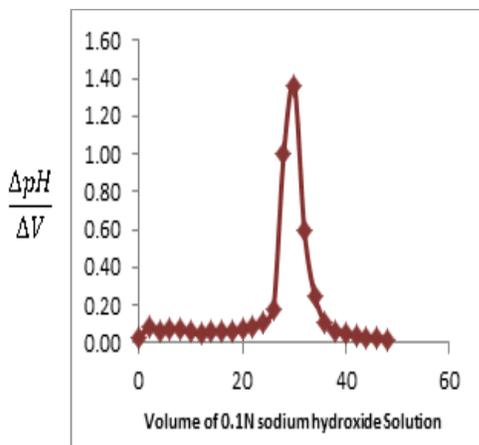


Fig 7: First derivative potentiometric titration plot for **P₅** extracted with formic acid and titrated with 0.1M NaOH solution with equivalent volume at 30.00ml

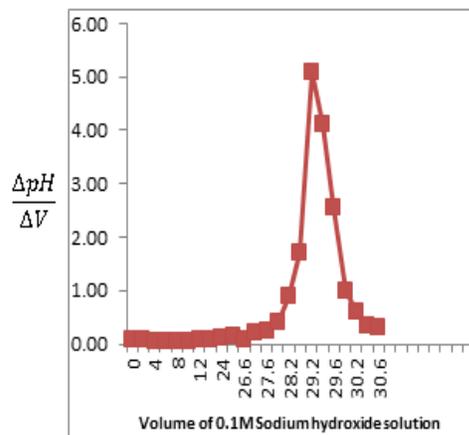


Fig 10: First derivative potentiometric titration plot for **P₈** extracted with formic acid and titrated with 0.1M NaOH solution with equivalent volume at 31.80ml.

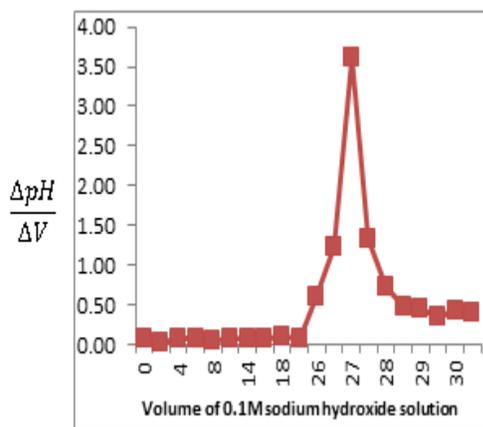


Fig 8: First derivative Potentiometric titration plot for **P₆** extracted with formic acid titrated with NaOH solution with equivalent point at 27.00ml.

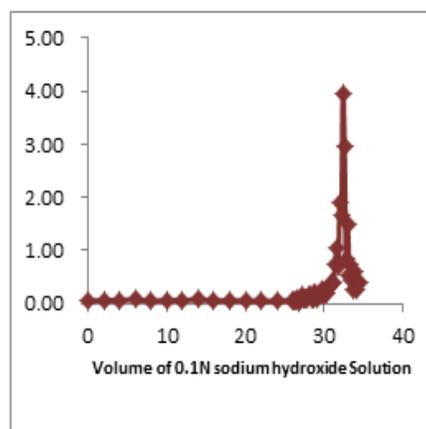


Fig 11: First derivative potentiometric titration plot of **P₉** extracted with formic acid and titrated with 0.1M NaOH solution with equivalent volume at 29.40ml

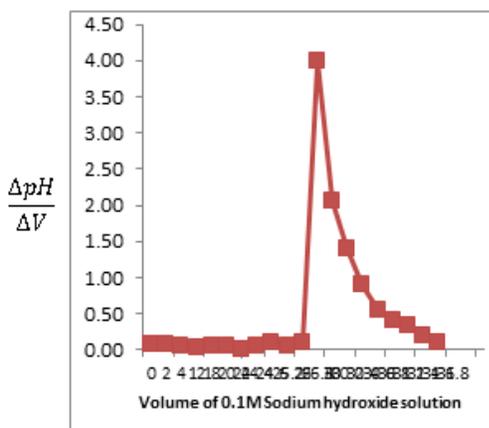


Fig 9: First derivative potentiometric titration plot for **P₇** extracted with formic acid titrated with 0.1M NaOH solution with equivalent volume at 30.00ml

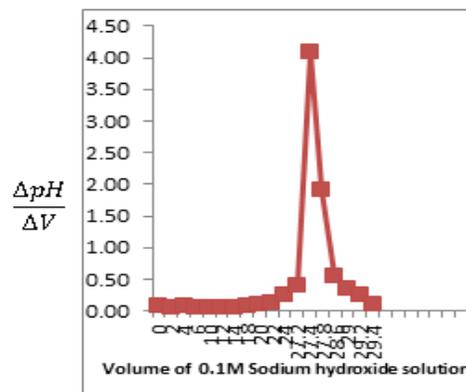


Fig 12: First derivative potentiometric titration plot for **P₁₀** extracted with formic acid and titrated with 0.1M NaOH solution with equivalent volume at 27.40ml

Table 1: Percentage of MSG in both Standard and Different Samples of Bouillon Cubes

Sample	Standard		P ₁	P ₂	P ₃	P ₄	P ₅	P ₆	P ₇	P ₈	P ₉	P ₁₀
	NE	EX										
% MSG	99.94	99.40	67.96	59.96	63.69	61.95	67.95	55.98	67.95	75.14	65.08	57.56

NE = Non Extracted; EX= Extracted

The extraction method employed in this study differs greatly from that used by the Association of Official Analytical Chemist (AOAC,1970). The several steps in the extraction procedure were replaced by formic acid selective dissolution of MSG facilitated by the common ion effect in both formic acid and MSG molecules (Figures 1 and 2). The common carboxylic group (COO⁻) aided the solubility of MSG in formic acid. This fact is also expressed in the observed affinity of formic acid for MSG. This affinity is also supported and revealed by the presence of formic acid in MSG even after evaporation of the extracted sample to dryness. The presences of this excess acid, pushes the pH of the solution downward and hence the volume difference as shown by the equivalent points of the standard MSG titrated with 0.1N NaOH solution and the formic acid extracted standard sample also titrated with 0.1N NaOH solution. The difference in this equivalent points volumes was taken as the value due to the affinity of formic acid for MSG standard and samples used in this study. Hence, 13 units represents the back titrated volume for formic acid. The percentage purity of 99.94% obtained in this study for the standard MSG used in this research work compared favourably with the findings of Andarwulan *et al.*, (2011). The MSG content of P₈(75.14%) was observed to be the highest, while the MSG content of P₆ (55.96%) was the lowest (Table 1). In all the samples studied, the highest MSG values were found to be 75.14% which is lower than that obtained by Andarwulan *et al.*, (2011) in their study titled "Free glutamate content of condiment and seasoning and their in-take in Bogor and Jakarta communities in Indonesia".

Conclusion: This study has successfully developed a cheap, simple, accurate and reproducible method for the direct determination of MSG in bouillon cubes available in various markets in Benin City. Furthermore, it presents the use of simple reagents, a pH meter with basic knowledge in standardization and application of potentiometric titration.

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