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Studies on Monosodium Glutamate Detection Method Using Copper(II) Sulfate Pentahydrate

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Abstract

The purpose of this research is to investigate a relatively simple method to detect the presence of monosodium glutamate in a solution. Monosodium glutamate (MSG) is a flavor enhancer widely used in various foods. MSG is considered safe, but its long-term effects on the human body are still debated. While existing MSG detection methods often require specialized expertise and are high-cost, this research paper proposes a possible alternative MSG detection method with a solution of copper (II) sulfate pentahydrate and sea salt dissolved in deionized water. This reagent solution of MSG changed its color from green to blue when the MSG solution was added, while it did not show a significant color change when salt solutions without MSG were added. The MSG reagent solution also showed distinct color changes corresponding to different MSG concentrations. The color intensities of MSG concentrations of 20, 40, 60, 80, and 100 ppm were analyzed using a vertical path-length photometer at 655 nm wavelength, and a positive correlation between absorbance and MSG concentration was observed, yielding a coefficient of determination (R^2) of approximately 0.99438. However, this study is limited to a laboratory setting rather than real-life applications due to pretreatment challenges with colored and opaque food samples and the need for further pH optimization to alkaline conditions. This research can assist those who seek an accessible and affordable visual detection method of MSG.

Keywords: Copper (II) sulfate pentahydrate, Himalayan salt, Monosodium glutamate (MSG), Monosodium glutamate (MSG) salt, Sea salt

1. Introduction

Monosodium glutamate (MSG) is a monosodium salt of glutamic acid and a food additive widely used to enhance the flavor of various foods (Stańska & Krzeski, 2016; T. Wifall et al., 2006). The U.S. Food and Drug Administration (FDA) has approved MSG to be generally recognized as safe (GRAS), but its long-term effects on the human body are still debated in several studies. Those studies suggest that the GRAS designation of MSG should be revised based on its acceptable daily intake (ADI) and advanced toxicity tests (Barraj et al., 2016; Burdock et al., 2006; Hartung, 2018). Reports on MSG intolerance, known as Chinese restaurant syndrome, and its association with atopic dermatitis have weak supporting evidence, so MSG is not classified as an allergen (Freeman, 2006; Williams & Woessner, 2009; Yang et al., 1997). However, individuals may have minor reactions to MSG, including headaches, numbness, and heart palpitations. This research paper will not determine the harmfulness of MSG, but it suggests a relatively simple method to detect the presence of MSG for those who might experience such symptoms and are cautious about MSG intake.

Examples of analytical methods developed to detect glutamate are high-performance liquid chromatography (HPLC) and amino acid analyzer. Although these instruments offer high sensitivity, they have notable limitations, including intensive labor requirements, time-consuming procedures, and high costs (Liu et al., 2021). To address these drawbacks and provide a potential alternative, this study developed a more intuitive and low-cost reagent solution for

Vol. 2026 (3) 236 – 242
ISSN 2688-3651 [online]

MSG detection based on Cu (II) complexation interaction with glutamate and chloride ions. Compared to conventional detection methods, this proposed colorimetric approach enables rapid and cost-effective on-site screening for MSG.

When copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) is dissolved in water, it dissociates into hexaaquacopper (II) ion ($[Cu(H_2O)_6]^{2+}$) and sulfate ion (SO_4^{2-}).

$$CuSO_4 \cdot 5H_2O(s) + H_2O(l) \rightarrow [Cu(H_2O)_6]^{2+}(aq) + SO_4^{2-}(aq) \quad (1)$$

Subsequent addition of sodium chloride (NaCl) results in ligand substitution, in which the copper (II) ion (Cu^{2+}) reacts with chloride ions (Cl^-) to form the tetrachlorocuprate (II) complex ($[CuCl_4]^{2-}$).

$$[Cu(H_2O)_6]^{2+}(aq) + 4Cl^-(aq) \rightleftharpoons [CuCl_4]^{2-}(aq) + 6H_2O(l) \quad (2)$$

Hexaaquacopper (II) ion ($[Cu(H_2O)_6]^{2+}$) absorbs light in the red region of the visible spectrum and therefore appears blue. The solution appears green when sodium chloride (NaCl) is added due to the formation of tetrachlorocuprate (II) complex ($[CuCl_4]^{2-}$), which absorbs light in the violet region (Katzin & Sullivan, 1951; McConnell & Robertson, 1963).

The addition of MSG to a mixture of copper (II) sulfate pentahydrate and sodium chloride results in the dissociation of the tetrachlorocuprate (II) complex ($[CuCl_4]^{2-}$) due to the strong chelate effect between the copper (II) ion (Cu^{2+}) and the glutamate ion ($C_5H_8NO_4^-$) (Biswas, C. et al., 2009; Antolini, L. et al., 1985). As a result, the concentration of the tetrachlorocuprate (II) complex ($[CuCl_4]^{2-}$) in the solution decreases, while the concentration of the complex molecule ($Cu(C_5H_8NO_4)^+$, Copper (II) ion with one glutamate molecule) increases. The formation of this complex molecule was assumed to be responsible for the observed color change of the MSG reagent solution from green to blue when the MSG solution was added.

$$[CuCl_4]^{2-}(aq) + C_5H_8NO_4^-(aq) \rightleftharpoons [Cu(C_5H_8NO_4)]^+(aq) + 4Cl^-(aq) \quad (3)$$

Considering that most food products contain both sodium chloride (NaCl) and MSG, directly reacting the copper (II) ion solution with these samples can result in a color change influenced by both components. In this research paper, it was hypothesized that preparing the MSG reagent solution by dissolving sea salt and copper (II) sulfate pentahydrate in deionized water would mitigate this interference. By pre-adjusting the solution's color to green, the reagent solution exhibited a distinct color change only when additional MSG was added.

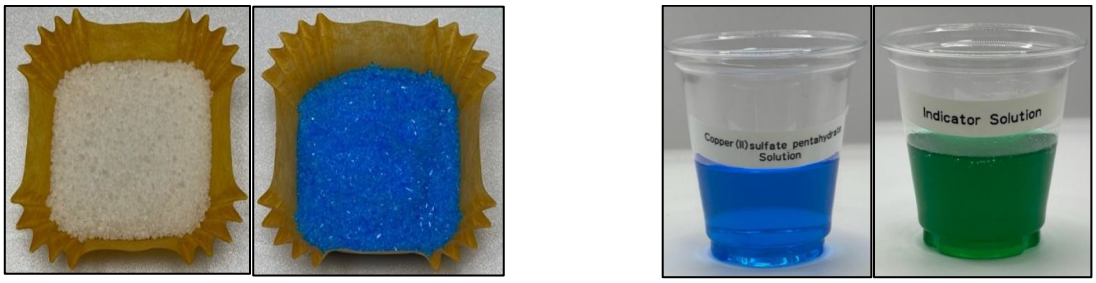
2. Materials and Methods

In this study, copper (II) sulfate pentahydrate (Doungsung, $CuSO_4 \cdot 5H_2O$), different types of salt, monosodium glutamate (Daesang, monosodium glutamate 97.3%, disodium 5′-ribonucleotides 2.7%), and deionized water were used. For the experimental equipment, a precision scale of 0.001g (Ohaus, SPX223KR), magnetic stirrer (IKA, Mini MR stand), pH meter (Hanna Instruments, HI 98494), vertical pathlength photometer (BIO-RAD, Model 550 Microplate Reader) at 655nm wavelength, vortex mixer (INTLLAB, VM-370), beaker (500 mL), and digital pipette (Eppendorf, Xplorer) were used. In addition, a one-way check valve, needleless connector, and microtubes (1.5 mL) were used. Three types of salt used were sea salt (Daesang, sea salt 100%), Himalayan salt (La Collina Toscana S.p.A, rock salt 100%), and MSG salt (Daesang, refined salt 90.3%, monosodium glutamate 9.6%, disodium 5′-ribonucleotides 0.1%). The measuring range of the thermometer is -50°C to 300°C, and the magnetic stirrer can agitate a maximum of 1 L of solution at 0 to 2500 rpm. The experiment environment temperature was 25.1°C.

2.1 Preparation of MSG Reagent Solution

15 grams of sea salt were dissolved in 50 mL of deionized water. The solution's temperature was 23°C. The amount of sea salt, consisting mainly of sodium chloride (NaCl), was set close to its solubility point, which is approximately 35.9 grams in 100 grams of water at 20°C. This salt solution was agitated for five minutes using a magnetic stirrer. Next, 10 grams of copper (II) sulfate pentahydrate were added to the salt solution and agitated for an additional five minutes using a magnetic stirrer.

Vol. 2026 (3) 236 – 242
ISSN 2688-3651 [online]



A B C D

Figure 1. (A) Sea salt; (B) Copper (II) sulfate pentahydrate powder; (C) Pure copper (II) sulfate pentahydrate solution; (D) MSG reagent solution.

Figure 1 shows the photos of the pure copper (II) sulfate pentahydrate solution and the MSG reagent solution. The color of pure copper (II) sulfate pentahydrate solution is blue due to the copper (II) sulfate pentahydrate powder's color, referring to Figure 1B. As for the MSG reagent solution, which is a salt solution mixed with saturated copper (II) sulfate pentahydrate, the color is green (Figure 1D).

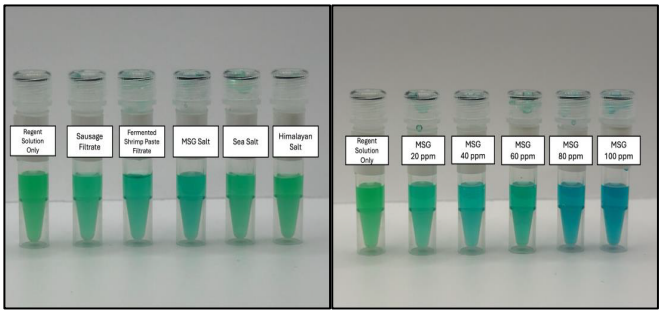
2.2 Preparation of Salt and MSG Solutions and Food Sample Filtrates

As for the salt solutions, sea salt, Himalayan salt, and MSG salt were used. Sea salt and Himalayan salt consist mostly of sodium chloride, while MSG salt contains an amount of monosodium glutamate and disodium 5'-ribonucleotides. 5 grams of each type of salt were dissolved in 50 mL of deionized water and agitated for five minutes using a magnetic stirrer. As for MSG solutions of different concentrations, 1, 2, 3, 4, and 5 grams of MSG powder were each added to 50 mL of deionized water and agitated for five minutes using a magnetic stirrer. The resulting concentrations were 20, 40, 60, 80, and 100 ppm, respectively. Before reacting with the reagent solution, all prepared salt and MSG solutions were colorless.

Sausage and Korean fermented shrimp paste were selected as food samples for the actual application, as MSG is commonly present in processed meats and fermented shrimp paste (Abdel Moneim et al., 2018; P, 2013). Since the color and opacity of such food samples can interfere with the colorimetric measurements, a filtration step was employed to mitigate this limitation. For sample preparation, 5 grams of each sausage and Korean fermented shrimp paste were finely chopped and transferred into separate containers containing 50 mL of deionized water. The resulting mixture was agitated for five minutes, then passed through filter paper to obtain filtrates for analysis.

2.3 Preparation of Measurement Samples

First, 0.4 mL of the reagent solution was added to six 1.5 mL microtubes using a digital pipette. Five of these microtubes separately received 0.048 mL of each food filtrate and MSG salt, sea salt, and Himalayan salt solution. The liquids inside the microtubes were thoroughly mixed using a vortex mixer (Figure 2A). Next, 0.4 mL of the reagent solution was added to a second set of six microtubes. Five of these microtubes separately received 0.048 mL of MSG solutions with concentrations of 20, 40, 60, 80, and 100 ppm, respectively, and were mixed with a vortex mixer (Figure 2B). One microtube in each set was kept containing only the reagent solution for comparison purposes. To ensure consistency, each preparation step was repeated three times, resulting in a total of 36 samples for analysis.



A B

Figure 2. (A) MSG reagent solutions after salt solutions and food sample filtrates were added; (B) MSG reagent solutions after MSG solutions were added.

Vol. 2026 (3) 236 – 242
ISSN 2688-3651 [online]

2.4 Absorbance Measurements

All color intensity measurements were performed using a vertical pathlength photometer at 655 nm wavelength. Before the measurement, the 36 samples were transferred from the microtubes into a 96-well plate according to the layout illustrated in Table 1. The peripheral wells were left blank to prevent potential measurement errors. The measurement was repeated three times to ensure experimental consistency.

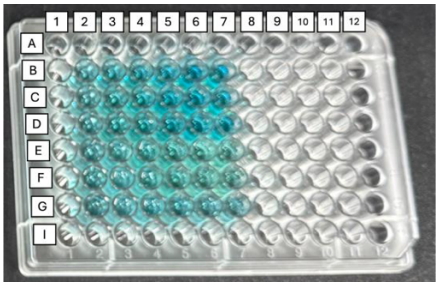


Figure 3. Physical setup of the 96-well plate.

Table 1. 96-well plate layout. In Table 1, "MSG 0 ppm" and "Reagent Only" represent the control group, consisting of six wells containing only the reagent solution.

	1	2	3	4	5	6	7	8
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank	MSG 0 ppm	MSG 20 ppm	MSG 40 ppm	MSG 60 ppm	MSG 80 ppm	MSG 100 ppm	Blank
C	Blank	MSG 0 ppm	MSG 20 ppm	MSG 40 ppm	MSG 60 ppm	MSG 80 ppm	MSG 100 ppm	Blank
D	Blank	MSG 0 ppm	MSG 20 ppm	MSG 40 ppm	MSG 60 ppm	MSG 80 ppm	MSG 100 ppm	Blank
E	Blank	Reagent Only	Sausage	Fermented Shrimp Paste	MSG Salt	Sea salt	Himalyayan Salt	Blank
F	Blank	Reagent Only	Sausage	Fermented Shrimp Paste	MSG Salt	Sea salt	Himalyayan Salt	Blank
G	Blank	Reagent Only	Sausage	Fermented Shrimp Paste	MSG Salt	Sea salt	Himalyayan Salt	Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

3. Results

The "Grand mean absorbance" in Figures 4, 5, and 6 represents the average of nine absorbance measurements obtained from three identical samples for each sample type, when the test was repeated three times (n = 3).

Across the six MSG concentrations, the grand mean absorbance showed a clear positive correlation with increasing MSG concentrations, with an R^2 value of approximately 0.99438. However, for concentrations above 20 ppm, absorbance values exceeded 2.0, which is beyond the reliable detection range of the photometer.

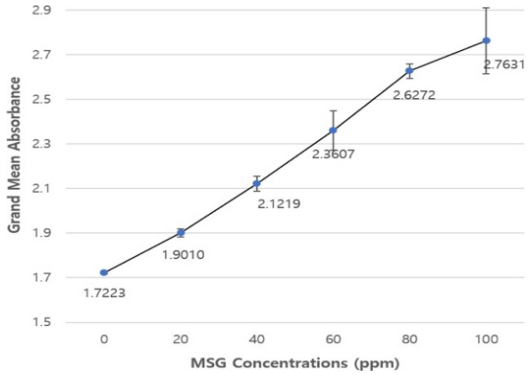


Figure 4. Comparison of mean absorbance values across six MSG concentrations.

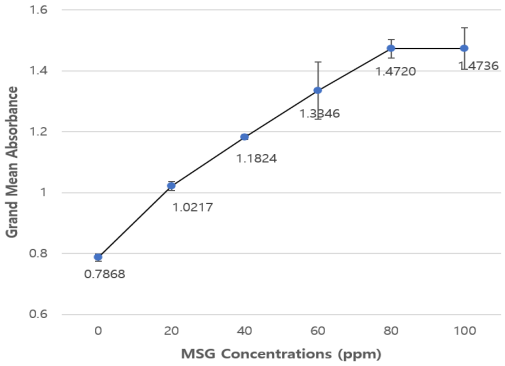


Figure 5. Comparison of mean absorbance values across six MSG concentrations following dilution with deionized water.

To ensure accuracy, the measurements were repeated after diluting half of each sample solution with deionized water. After dilution, the maximum absorbance values remained below 1.5, which falls within the reliable analytical range. As shown in Figure 5, the grand mean absorbance again exhibited an increasing trend with rising MSG concentration, even after dilution.

Figure 6 illustrates the grand mean absorbance values for the food sample filtrates and the different types of salt solutions. Among these, the grand mean absorbance of the sample, in which the MSG salt solution was added to the reagent solution, was relatively higher than that of the other samples, in which salt solutions without MSG (sea salt and Himalayan salt solutions) were added. This suggests that the reagent solution can detect trace amounts of MSG in the MSG salt. However, for the sausage and Korean fermented shrimp filtrates, which contain MSG, the grand mean absorbance value was unexpectedly lower than that of the control group.

Vol. 2026 (3) 236 – 242
ISSN 2688-3651 [online]

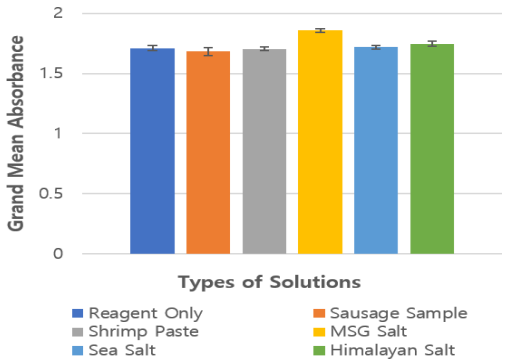


Figure 6. Comparison of mean absorbance values across six samples.

4. Discussion

The MSG reagent solution developed in this research paper provides its user with a simple method to detect MSG by utilizing the reaction between copper (II) sulfate pentahydrate, sodium chloride, and MSG and its corresponding color change. However, an additional safety device is required for its practical use since copper (II) sulfate pentahydrate is generally considered a harmful chemical compound upon contact or inhalation (International Programme on Chemical Safety, n.d.). While safety devices can be applied in various ways, this study aimed to propose one of the ways, as shown in Figure 7.

A B C

Figure 7. (A) Microtubes with a safety device attached; (B) Safety device preventing MSG reagent solution leakage; (C) Injecting MSG solution into microtubes with a syringe.

4. Conclusion

In this research paper, the authors developed an MSG reagent solution with a mixture of copper (II) sulfate pentahydrate and sea salt dissolved in deionized water. It was observed that this reagent solution could detect the presence of monosodium glutamate (MSG) in a solution through a color change. Furthermore, it showed different color changes corresponding to various MSG concentrations.

However, the proposed solution showed potential limitations when testing colored or opaque food samples, such as sausage and Korean fermented shrimp. For both samples, the measured absorbance values of the filtrates were lower than expected. This discrepancy could be attributed to the relatively simple filtration process used in this study, which was constrained by limited laboratory equipment and specialized expertise. In practice, more complex pretreatment steps, such as uniform homogenization and protein removal via centrifugation, are essential for effective extraction of MSG. Therefore, the real-life application of the reagent solution requires further research focused on refining analytical conditions to enhance accuracy and reliability.

The pH of the reagent solution was 2.48, indicating a highly acidic condition under which the chelate-binding effect of MSG is reduced. MSG exhibits optimal chelate binding in neutral to slightly alkaline conditions, typically within a pH range of 6.0 to 8.5 (P. Deschamps et al., 2003). Therefore, future studies will aim to determine the pH at which the reagent solution achieves its optimal performance. To adjust the pH into the alkaline range, buffers such as HEPES, which exhibit minimal binding to metal ions, may be used.

This study on monosodium glutamate (MSG) detection method introduces a simple method to detect MSG in a

Vol. 2026 (3) 236 – 242
ISSN 2688-3651 [online]

solution using copper (II) sulfate pentahydrate and its color change. Although research needs to be improved for practical or commercial applications, it would offer guidance on MSG consumption for those who may experience minor reactions to MSG and are cautious about MSG intake.

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