

Kinetic Analysis of the Glucose/Glycine Maillard Reaction for Entry-Level Student Experiments

Yuma Minami

Department of Nutrition, Faculty of Nursing and Nutrition,
Kagoshima Immaculate Heart University

Keywords: Maillard Reaction, Food Chemistry, UV/Vis Spectroscopy,
Reaction Kinetics, Student Experiments

Abstract

【Introduction】 The Maillard reaction, also called amino-carbonyl reaction, is one of the most important reactions in food chemistry. The Maillard reaction contributes to the browning and flavor in a wide range of foods such as miso, soy sauce, toasts, cookies, roasted coffee, and grilled meats *etc.* It is particularly important for students involved in nutrition and cookery science to learn about the Maillard reaction from a chemical perspective. On the other hand, the use of unfamiliar analytical instruments and rigorous analysis are daunting for beginner students. This bulletin article aims to introduce the easy experiments and the rudimental kinetic analysis of the Maillard reaction based on absorbances for beginner students.

【Methods】 Glucose (Glc) and glycine (Gly) solution were used as simple model substrates. The pH of the solution was adjusted with phosphate buffer. Reaction mixtures were heated in a boiling bath. After every 10 minutes, one test tube was immediately cooled with running water and absorbances (A) at 470 nm were measured. Data were analyzed by Excel.

【Results and Discussion】 Based on Lambert-Beer's law, the rate of melanoidin formation was estimated by absorbances. First, an effect of the concentration of substrates was demonstrated. As the principal of chemical reactions, the greater the concentration of reactants, the faster the reaction proceeded. Next, an effect of pH was also clearly shown. Although the reaction proceeded rapidly at pH 9, almost no reaction was observed in pH 4. This tendency is fundamental in the mechanism of the Maillard reaction. Using the results in pH 7 and 9 cases, a basic kinetic analysis was carried out. Generally, some approximation is useful for the complicated reaction analysis. If the concentration of one reactant (either an amino acid or a reducing sugar) is significantly higher than the other, its concentration can be regarded as almost constant throughout the reaction. Based on these assumptions, the reaction was regarded as a pseudo-first order reaction. A linear approximation of plotting the $\ln(A/A_0)$ to time showed good linearity. The coefficient of the linear equation is the reaction rate constant (k). $k = 9.08 \times 10^{-2} \text{ min}^{-1}$ in pH 9, and $k = 7.14 \times 10^{-2} \text{ min}^{-1}$ in pH 7 were calculated. An estimation of reaction rate constants also confirmed that the Maillard reaction was faster under basic conditions.

【Summary】 In this report, spectroscopic analysis of the glucose/glycine Maillard reaction was investigated as a model reaction for student experiments. These experiments are easy to perform and only require knowledge of the elementary level chemistry and mathematics. By assuming the Maillard reaction to be a pseudo-first order reaction, a simple kinetic calculation was able to be carried out. However, a lot of assumption and calculation were not rigorous for the simplicity. Needless to say, more rigorous and advanced analysis requires a variety of measurements, exact equations, and calculations.

Introduction

The Maillard reaction, named after French chemist Louis-Camille Maillard¹⁾, is a complex series of chemical reactions that occur between reducing sugars and amino acids or proteins to provide melanoidins. It is also called an amino-carbonyl reaction because it is due to the reaction between an amino group and a carbonyl group. The Maillard reaction contributes to the browning and flavor in a wide range of foods such as miso, soy sauce, toasts, cookies, roasted coffee, and grilled meats. Food discoloration or browning is directly tied to not only food properties but also consumer purchasing behavior²⁾. Current various human diet and food culture would not flourish without the effective utilization of the Maillard reaction.

A general mechanism of the Maillard reaction involves several steps. Using glucose as a model, it can be summarized as follows (Figure 1)³⁻⁵⁾. Since there are countless compounds involved in the Maillard reaction even in a simple reaction system, it is not easy to identify the exact reaction mechanism⁶⁾.

- Formation of the Schiff base: The reaction begins with the condensation of a reducing sugar with an amino group of an amino acid or a protein. This

condensation leads to the formation of a Schiff base, which is an imine intermediate.

- Amadori rearrangement: The Schiff base undergoes a rearrangement. This step leads to the formation of highly reactive intermediates known as Amadori compounds which are important intermediates in the Maillard reaction.
- Fragmentation and condensation: Various compounds generated in the Amadori rearrangement undergo further fragmentation and condensation reactions. These reactions involve the formation of a wide range of compounds including furans, pyrroles, pyrazines, and other complex structures. Especially, Strecker degradation produces various volatile compounds concerning the flavor of the final products. These compounds contribute to the characteristic color, flavor, and aroma of the Maillard reaction products⁷⁾.
- Polymerization: As the Maillard reaction proceeds, the intermediate compounds can undergo polymerization reactions, resulting in the formation of high molecular weight melanoidins. Melanoidins are brown pigments which are responsible for the coloration of the Maillard reaction products⁸⁾.

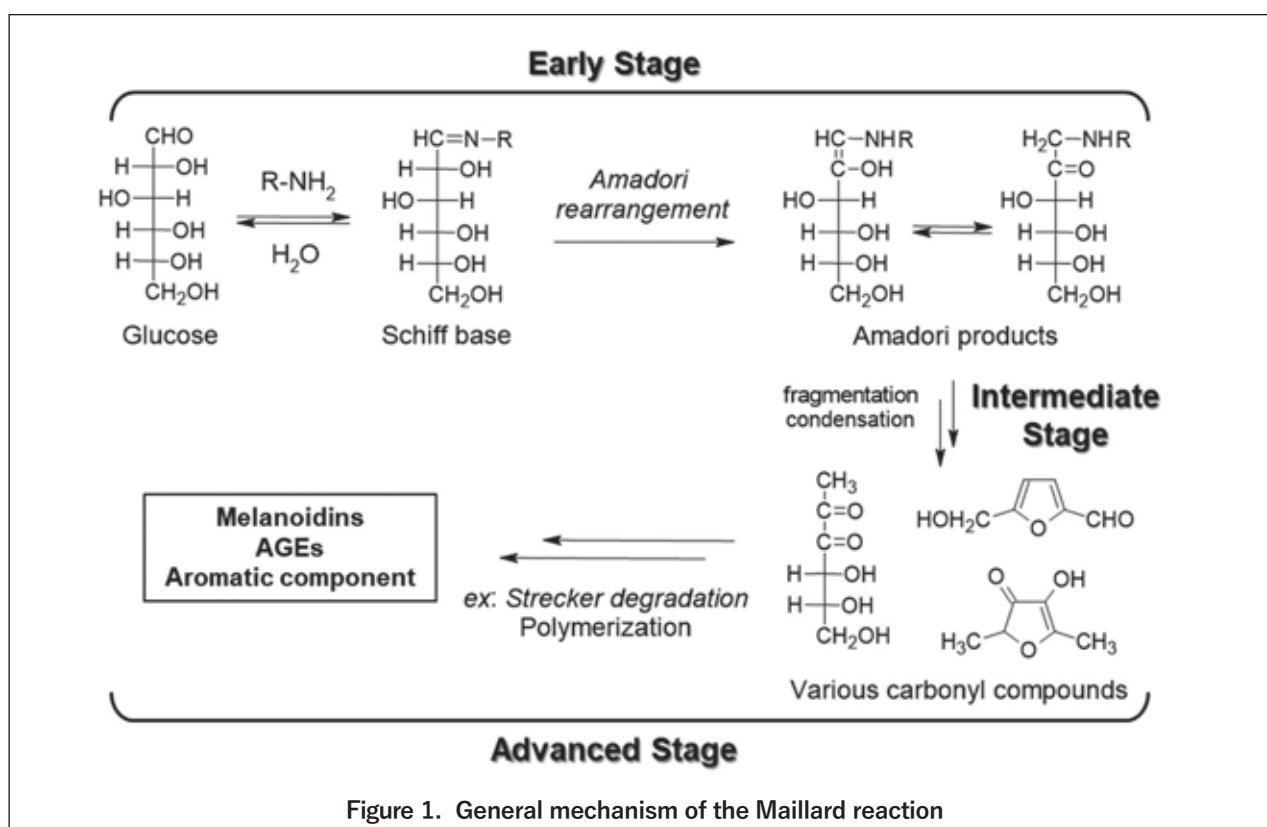


Figure 1. General mechanism of the Maillard reaction

The Maillard reaction occurs not only in foods but also *in vivo*. The end products of the Maillard reaction are known as AGEs (Advanced Glycation End products). Nowadays, research has been conducted on the Maillard reaction and how it relates to the development of aging and aging-related diseases⁹⁾.

It is important to note that the Maillard reaction is influenced by several factors, including temperature, pH, water activity, buffers, presence of catalysts such as metal ions, and the types and concentrations of reactants^{10–11)}. These factors can impact the rate and extent of the reaction and contribute to the diverse range of flavors and colors observed in numerous food products.

The Maillard reaction is also an important part in student experiments to understand the food ingredients from the viewpoints of chemistry¹²⁾. Students confirm that what is the key factor to facilitate or suppress the Maillard reaction. In many cases, the easiest way is checking a degree of browning by color under several reaction conditions.

This bulletin article proposes a spectrometric method and an easy reaction kinetic analysis that can be understood by the beginner students, rather than simply observing color changes. For students who are not familiar with chemistry, it is good opportunity that they get some experience with simple experiments and analyses, even if they are not rigorous. Here, a simple method, for analyzing the Maillard reaction that can be performed using high school-level chemistry and mathematics knowledge, is introduced.

Experimental Section / Methods

【General】

- All chemical reagents were purchased by FUJIFILM Wako Pure Chemical Co. or Nacalai Tesque, Inc. and used without further purification.
- UV/Vis absorbances were measured by AS ONE ASV11D-H against distilled water as a control.
- The graphing and the linear approximation were carried out in Excel (Microsoft 365).

【Reagents】

- 0.2 M, 0.5 M, 1.0 M glucose solution (Glc)
- 2.0 M glycine solution (Gly)
- 0.5 M phosphate buffer (pH 4, 7, 9)

【Procedures】

• Effect of concentration

0.2 or 0.5 or 1.0 M Glc 1 mL and 2.0 M Gly 1 mL and phosphate buffer (pH 7) 1 mL were added to the stoppered test tubes. 6 tubes were prepared in each measurement (every 10 min. up to 60 min.). The reaction mixtures were heated by using boiling bath. To suppress the evaporation of water, test tubes were capped with glass marble (Figure 2). After each reaction time, the test tubes were immediately cooled with running water and absorbances at 470 nm were measured.

• Effect of pH

0.2 M Glc 1 mL and 2.0 M Gly 1 mL and phosphate buffer (pH 4 or 7 or 9) 1 mL were added to the stoppered test tubes. The procedure after mixing these solutions was the same as described above.

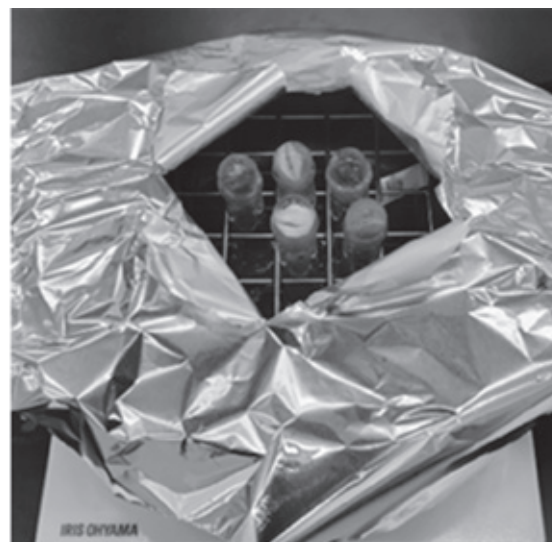


Figure 2. Photo of the reaction setup

Results and Discussion

Glucose and glycine solution were used as simple model substrates. Melanoidins from glucose and glycine have maximum absorption wavelength at 470 nm¹³⁾. Increasing of the absorbance at 470 nm represents the formation of melanoidins. This relationship can be explained by Lambert-Beer's law (Eq. (1)). The Lambert-Beer's law states that the absorbance of a solution is directly proportional to the concentration of the absorbing substance and the path length of the light through the solution.

$$A = \epsilon c L \quad (1)$$

A: Absorbance, ϵ : molar absorption coefficient, c: concentration, L: path length of the light

First, Figure 3 shows the effect of the concentration of glucose in the reaction with 2.0 M Gly. Comparing 1.0 M Glc with 0.2 M Glc, the increase of absorbance in 1.0 M Glc is more rapid than 0.2 M Glc. It means that the greater the concentration of reactants, the faster the reaction proceeds. This is a fundamental law of chemical reactions.

Figure 4 shows the change in absorbance with time using each phosphate buffer (pH 4, 7, 9). 2.0 M Gly and 0.2 M Glc were used as reactants. Obviously, the reaction proceeded rapidly at basic conditions (pH 9) although it is almost suppressed under acidic conditions (pH 4). This tendency is quite important for considering a mechanism of the Maillard reaction. The first step of the reaction is a nucleophilic attack of an amine group to a carbonyl group. A free amino group of amino acids are protonated under acidic conditions and the nucleophilicity is significantly decreased.

Based on these simple experimental results, a rudimentary kinetic analysis was considered. The following equations are detailed in references¹⁵⁻¹⁶⁾.

In a kinetic analysis of chemical reactions, it is important to determine the reaction order. Without the reaction order, the reaction rate kinetics cannot be established. It cannot be overemphasized that the Maillard reaction is very complicated. Its kinet-

ics can be influenced by several factors, including pH, temperature, reactant concentrations, and the presence of other compounds. Although there are many reports on the reaction kinetics of the Maillard reaction^{6,14)}, it is quite difficult for entry-level students to perform strict experiments and calculations. A simpler, if not rigorous, model is needed for the beginner students.

One of the simplest methods is regarding the Maillard reaction as the first order reaction. Especially, pseudo-first order analysis is a powerful tool for complex reactions. The pseudo-first order approximation is a simplification that assumes the concentration of one reactant (either the amino acid or the reducing sugar) is significantly higher than the other, so its concentration remains relatively constant throughout the reaction. In pH experiments, a concentration of glycine is 10 times to that of glucose. It must be noted that over 1000 times difference of the concentration is generally desirable for the pseudo-first order approximation.

When the reaction is first order for each of [Glc] and [Gly], reaction rate (r) can be written as Eq. (2). Furthermore, if [Gly] is assumed to be constant during the reaction, Eq. (3) is introduced ($k' = k[\text{Gly}]$).

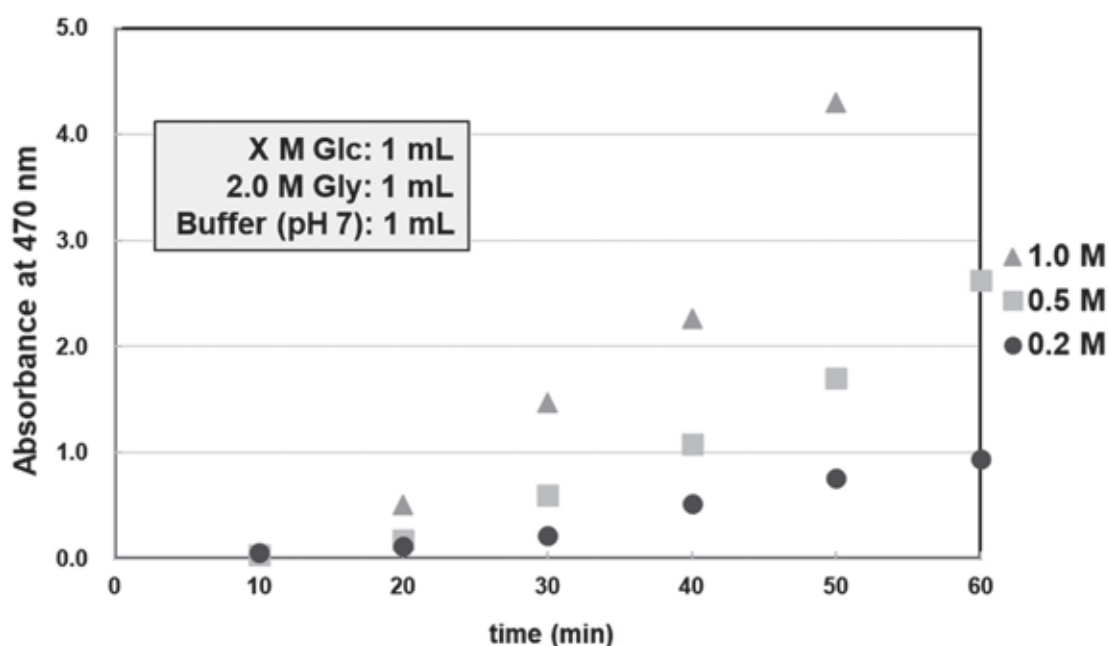


Figure 3. Effect of the concentration of glucose

$$v = k[\text{Glc}][\text{Gly}] \quad (2)$$

$$v = k'[\text{Glc}] \quad (3)$$

k (k') is a rate constant which depends on temperature. Chemical reactions generally proceed faster at higher temperature. These equations imply that the rate of product formation is proportion-

al to the concentration of reactants.

At the same time, the rate equation can be also described by the time variation of reactant concentration $[C]$ (Eq. (4)).

$$v = -\frac{d[C]}{dt} = k[C] \quad (4)$$

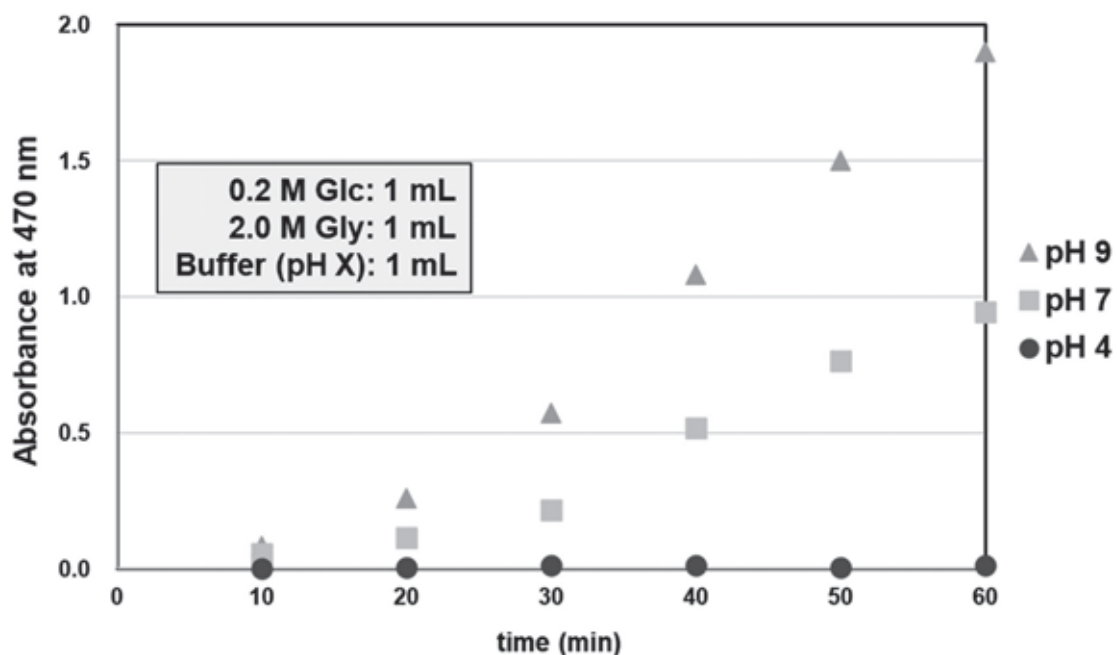


Figure 4. Effect of the pH of reaction mixtures

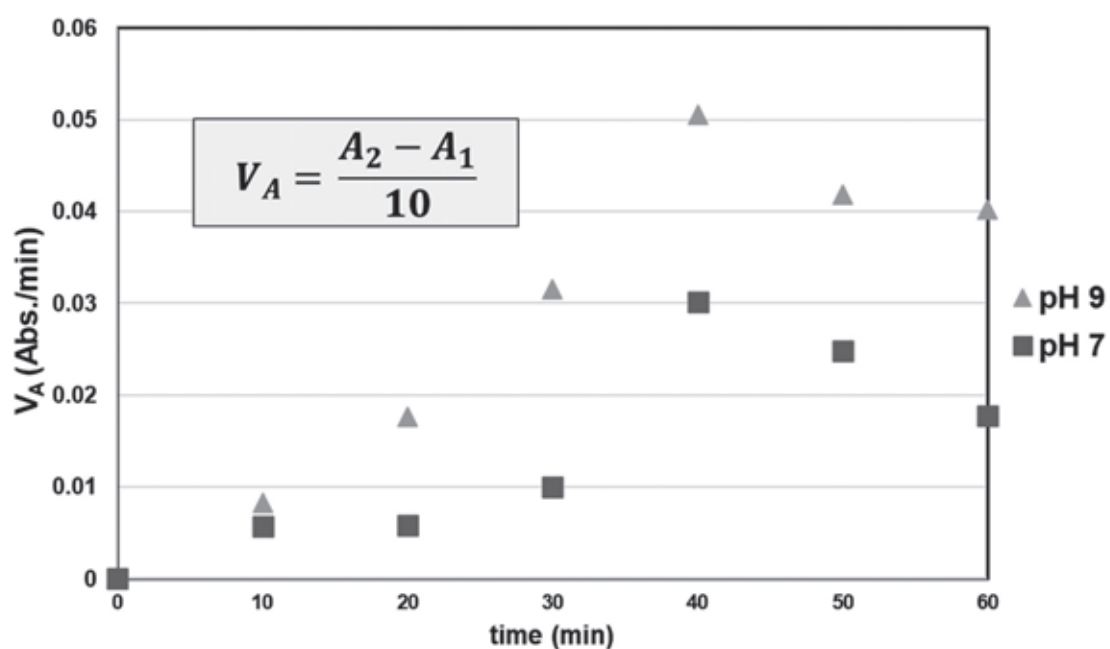
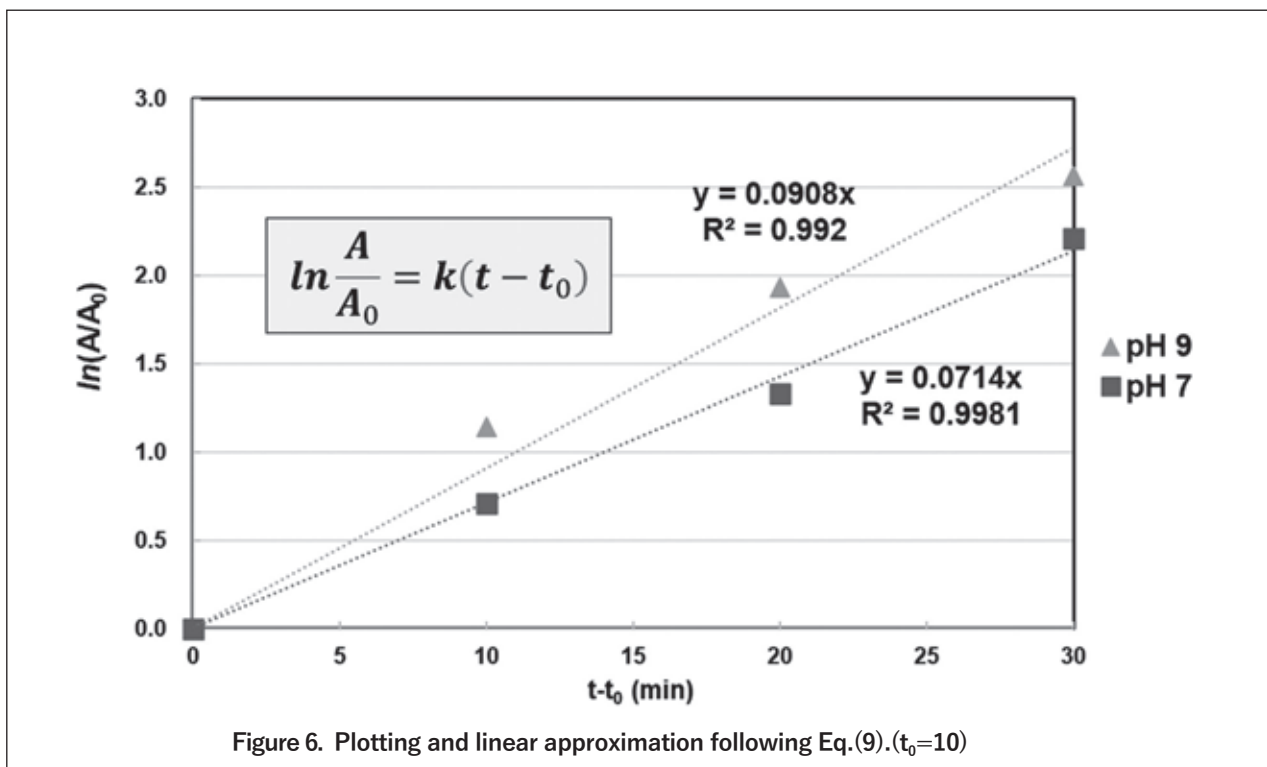


Figure 5. Average increase of absorbance every 10 minutes (V_A , Eq.(10))



After rewriting to the variable separation type (Eq. (5)), integrate from time $t = t_0$ to t , and from the molar concentration $[C] = C_0$ to C . (Eq. (6) ~ (8))

$$\frac{d[C]}{[C]} = -kdt \quad (5)$$

$$\int_{C_0}^C \frac{d[C]}{[C]} = -k \int_{t_0}^t dt \quad (6)$$

$$\ln[C] - \ln[C]_0 = -k(t - t_0) \quad (7)$$

$$\ln \frac{[C]}{[C]_0} = -k(t - t_0) \quad (8)$$

A very important point about rate equations is that they generally cannot be deduced from the chemical reaction equation itself but are experimentally determined. Eq. (8) means if $\ln(C/C_0)$ is proportional to $(t-t_0)$, the reaction is assumed to be the first order reaction.

In this experiment, a reaction rate of the Maillard reaction was considered by using absorbances of reaction mixtures. From Lambert-Beer's law (Eq. (1)), an absorbance is proportional to a con-

centration of the solution. Eq. (8) was applied to absorbances (Eq. (9)). Although it would be accurate if changes in the concentration of the reactant (glucose) could be monitored by absorbances, the absorbance of the products (melanoidins) was used in this experiment. Minus (-) is removed at Eq. (9) because absorbance increases as the reaction proceeds.

$$\ln \frac{A}{A_0} = k(t - t_0) \quad (9)$$

The reaction seems to be pseudo-first order if this relationship holds. In the actual reaction analysis, initial reaction mixtures were transparent, and then $A_0 = 0$ is considered when $t_0 = 0$. However, since the equation is not valid in this case, $t_0 = 10$ was considered. In addition, the changes in absorbance every 10 minutes were focused. It means the average increase of the absorbance (V_A , Eq. (10)).

$$V_A = \frac{A_2 - A_1}{10} \text{ (Abs./min)} \quad (10)$$

In both pH 7 and pH 9, V_A continued to increase up to 40 minutes after the start of the reaction but began to decrease after 40 minutes (Figure 5). Based on these results, the kinetic analysis was performed using the results up to 40 minutes.

Figure 6 plots the $\ln(A/A_0)$ versus $(t-t_0)$ in the pH 7 and pH 9 cases. Linear approximations by Excel gave R-squared values of 0.992 (pH 9) and 0.9981 (pH 7), respectively. Since the linear approximation almost holds, the reactions are considered to follow Eq. (9). The coefficient of the linear function, which is an approximate straight line, is directly the reaction rate constants.

$$\text{pH 9: } k = 9.08 \times 10^{-2} \text{ min}^{-1}$$

$$\text{pH 7: } k = 7.14 \times 10^{-2} \text{ min}^{-1}$$

As mentioned in figure 4, the Maillard reaction proceeds rapidly at higher pH. This tendency is also confirmed by comparing the reaction rate constants. It should be mentioned that an approximate straight line with a lower R-squared value was obtained from the analysis up to 60 minutes.

These experiments can be performed very easily without the need for special laboratory equipment. The analysis of experimental data is also very familiar, as it is done by using Excel. This approach could be applied to a variety of further experiments. For example, experiments with various sugars and amino acids combinations would confirm the tendency of the Maillard reaction in each substrate.

Summary

In this report, rudimentary kinetic analysis of the Maillard reaction for beginner students was introduced. The Maillard reaction is quite important to learn food chemistry, cookery science, nutrition and so on. By setting up a simple reaction system and assuming that the reaction is a pseudo-first order reaction, students can experience the analysis of a chemical reaction with only rudimentary knowledge of chemistry and mathematics. Students not only learn the knowledge of the Maillard reaction but also can get a glimpse of a theoretical chemical analysis.

On the other hand, there is only an introduction to understanding elementary ideas of reaction

kinetics because it ignores exact measurements and calculations. It goes without saying that the various condition settings and assumptions are not rigorous. For more strict and advanced analysis, various measurements, exact equations, and calculations are needed.

References

- 1) Maillard, L. C., "Action of amino acids on sugars. Formation of melanoidins in a methodical way" *C. R. Acad. Sci.*, **1912**, 154, 66-68. (in French)
- 2) 村田容常, "酵素的褐変ならびにメイラード反応に関する食品化学的研究" 日本食品科学工学会誌, 2020, 第 67 巻, 第 1 号, 1-12.
- 3) 村田容常, "メイラード反応と着色・褐変〜糖とアミノ酸を加熱すると茶色くなる化学〜" 化学と生物, 2019, Vol. 57, No. 4, 213-220.
- 4) 須山亨三, "糖尿病とメイラード反応; その基礎と反応機構" 仙台大学紀要, 2006, 第 38 巻, 47-71.
- 5) 白井照幸, "食品におけるメイラード反応" 日本食生活学会誌, 2015, 第 26 巻, 第 1 号, 7-10.
- 6) Harry Nursten, "The Maillard Reaction - Chemistry, Biochemistry and Implications" The Royal Society of Chemistry, **2005**.
- 7) 大畑素子, 横山竜成, 有原圭三, "メイラード反応生成香気成分が有する新たな可能性への挑戦〜食品メイラード反応の最新の香り研究〜" 化学と生物, 2019, Vol. 57, No. 12, 722-727.
- 8) K. Noda and M. Murata, "What are melanoidins, polymers in food?" *J. Biol. Macromol.*, **2022**, 22, 23-36.
- 9) 白河潤一, 永井竜児, "生体におけるメイラード反応の影響" 化学と生物, 2015, Vol. 53, No. 5, 299-304.
- 10) Sara I.F.S. Martins, Wim M.F. Jongen and Martinus A.J.S. van Boekel "A review of Maillard reaction in food and implications to kinetic modelling" *Trends. Food Sci. Technol.*, **2001**, 11, 364-373.
- 11) Y. Mikami and M. Murata, "Effects of sugar and buffer types, and pH on formation of Maillard pigments in the lysine model system" *Food Sci. Technol. Res.*, **2015**, 21, 813-819.
- 12) Kravchuk, O., Elliott, A. J., and Bhandari, B. R., "A Laboratory Experiment: Based on the Maillard Reaction, Conducted as a Project in Introductory Statistics" *J. Food Sci. Educ.*, **2005**, 4, 70-77.
- 13) Sara I.F.S. Martins and Martinus A.J.S. van Boekel, "Melanoidins extinction coefficient in the glucose/glycine Maillard reaction" *Food Chem.*,

- 2003**, 83, 132-142.
- 14) Sara I.F.S. Martins and Martinus A.J.S. van Boekel, "A kinetic model for the glucose/glycine Maillard reaction pathways." *Food Chem.*, **2005**, 90, 257-269.
- 15) 大山秀子, "反応速度論の基礎" 化学と教育, 2008, Vol. 56, No. 12, 630-633.
- 16) 小木修, "微分方程式で理解する反応速度論" ぶんせき, 2014, 94-100.