

Prediction of Color Changes Using the Time–Temperature Superposition Principle in Liquid Formulations

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This study reports the results of applying the time–temperature superposition principle (TTSP) to the prediction of color changes in liquid formulations. A sample solution consisting of L-tryptophan and glucose was used as the model liquid formulation for the Maillard reaction. After accelerated aging treatment at elevated temperatures, the Commission Internationale de l'Éclairage (CIE) LAB color parameters (a^* , b^* , L^* , and E^*ab) of the sample solution were measured using a spectrophotometer. The TTSP was then applied to a kinetic analysis of the color changes. The calculated values of the apparent activation energy of a^* , b^* , L^* , and ΔE^*ab were 105.2, 109.8, 91.6, and 103.7 kJ/mol, respectively. The predicted values of the color parameters at 40°C were calculated using Arrhenius plots for each of the color parameters. A comparison of the relationships between the experimental and predicted values of each color parameter revealed the coefficients of determination for a^* , b^* , L^* , and ΔE^*ab to be 0.961, 0.979, 0.960, and 0.979, respectively. All the R^2 values were sufficiently high, and these results suggested that the prediction was highly reliable. Kinetic analysis using the TTSP was successfully applied to calculating the apparent activation energy and to predicting the color changes at any temperature or duration.

Key words color change; prediction; time–temperature superposition principle; Maillard reaction

The color of pharmaceutical products is an important quality attribute. Color changes in pharmaceutical products are often indicative of either poor-quality production or product instability.^{1,2} Color measurement is one type of quality assurance test that should be performed during the development of pharmaceutical products.³ Techniques for preventing changes in the color of the product are often used. For example, the use of antioxidant agents and color agents is a common solution for stabilizing the color appearance of products.⁴ In some cases, however, color changes occur very slowly over time. If a commercialized product exhibits a color change before it can be sold, the product should be recalled. Therefore, a method for predicting color changes is important for the development of products within a short time period and for determining the shelf-life of a product. In the food industry, methods for predicting color changes over time have been thoroughly studied as an index of quality. Several reports have discussed the kinetics of color changes in food products, such as broccoli juice,⁵ apple puree,⁶ and green asparagus.⁷ In the pharmaceutical industry, kinetic analyses of changes in the color of solid dosage forms have been reported with a variety of purpose, including the prediction of discoloration,⁸ evaluations of the coloration of photosensitive solid drugs,⁹ elucidation of the discoloration kinetics of uncoated white tablets,¹⁰ examinations of coloration during long-term stability tests,¹¹ and the examination of color stabilization through the use of film coating.¹² On the other hand, few methods for predicting color changes in liquid formulations have been developed. Previous studies of liquid formulations have concentrated on the color changes of a single component, such as ascorbic acid, thiamin hydrochloride, reserpine, cefazolin sodium, or ceftizoxime sodium.^{13,14} In general, the color of a liquid formulation can undergo changes more easily than a solid formulation because

of hydrolysis reactions, oxidation reactions, and other chemical reactions of active ingredients and other additives. Seki *et al.* reported a method of predicting color changes using a Weibull probability plotting paper and data on the reduction in percent transmittance at 430 nm.¹⁴ Kitamura *et al.* calculated the reaction order of color changes using Matsuda's equation⁹ and data on the total color difference (ΔE).¹³ However, these reports did not describe the property of color changes and required complicated calculations based on reaction models.

In this study, a simple model system based on the Maillard reaction of a liquid formulation composed of an amino acid and a reducing sugar was used to develop a method for predicting color changes in liquid formulations. The Maillard reaction is one of the most common non-enzymatic browning reactions in liquid formulation. During this reaction, hundreds of different compounds are created.¹⁵ Since the causal compounds of the browning reaction are difficult to specify, the change in appearance was monitored as one of the physical properties. Such color studies are traditionally performed using Commission Internationale de l'Éclairage (CIE) LAB color parameters (a^* , b^* , L^* , and ΔE^*ab) to measure color differences in a quantitative manner.¹⁶ The CIELAB color parameters can describe all the colors visible to the human eye. In principle, the application of numerous kinetic models should be attempted, and the best-fit model for each color parameter should be defined. However, the search process for identifying the most appropriate kinetic model is time-consuming, and best-fit models can be difficult to identify. In the present study, the time–temperature superposition principle (TTSP) was applied to the data, instead of the conventional use of complicated kinetic models. The superiority and limitations of the TTSP method are discussed from the perspective of solving real-life problems.

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Experimental

Materials The liquid formulation used for the Maillard reaction model was composed of glucose as the reducing sugar (San-ei Surochemical Co., Ltd., Japan) and L-tryptophan as the amino acid (KENEI Pharmaceutical Co., Ltd., Japan). The following compounds that were used to prepare a citrate buffer solution were obtained commercially: citric acid (Iwata Chemical Co., Ltd., Japan), sodium citrate (SATUMA KAKO Co., Ltd., Japan), and sodium benzoate (Aioi ChemiScience Co., Ltd., Japan). The sample solution was prepared by dissolving glucose and L-tryptophan in a solution (25 mM citrate buffer, 4.2 mM sodium benzoate) to yield final concentrations of 10 mM L-tryptophan and 300 mM glucose, with a pH of 6.0. A 15-mL glass container (Maruemu Co., Ltd., Japan) was used to store the 15 mL liquid formulation at various temperatures.

Sample Treatment The sample solutions were stored in a stability chamber (Nagano Science Co., Ltd., Japan) at 40°C, 50°C, 60°C, 65°C, 70°C, 75°C, and 80°C. Table 1 shows the duration of storage at each treatment temperature.

Color Measurements The color parameters were measured using a spectrophotometer (CM-3500d; KONICA MINOLTA, INC., Japan) under a D65 light source and an observed angle of 10°. The CIELAB color parameters (a^* , b^* ,

L^* , and ΔE^*ab) authorized in JIS Z8729¹⁶⁾ and JIS Z8730¹⁷⁾ were used to express the color changes. Figure 1 shows the CIELAB color space diagram. L^* expresses the lightness from white (100) to black (0). Both a^* and b^* express the hue and the chromaticness, *i.e.*, a^* is green ($-a^*$)–red ($+a^*$) axis, and b^* is blue ($-b^*$)–yellow ($+b^*$) axis. The differences in the parameters (Δa^* , Δb^* , and ΔL^*) and the total color differences (ΔE^*ab) were calculated using the following equations^{16,17)}:

$$\Delta a^* = a^* - a_0^* \quad (1)$$

$$\Delta b^* = b^* - b_0^* \quad (2)$$

$$\Delta L^* = L^* - L_0^* \quad (3)$$

$$\Delta E^*ab = (\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2})^{1/2} \quad (4)$$

where a_0^* , b_0^* , and L_0^* are the initial values without heat treatment. The color parameter measurements were represented as the mean of three determinations. The relative standard deviation of any measurement was less than 0.2%, suggesting a high reproducibility.

Prediction of Color Changes A kinetic analysis is a useful method of predicting color changes over time. The data analysis was performed using Microsoft Excel 2010. The Arrhenius equation is a fundamental approach to performing a kinetic analysis of chemical reactions¹⁸⁾:

$$k = A \exp\left(-\frac{E_a}{RT}\right) \quad (5)$$

$$\ln k = \ln A - \left(\frac{E_a}{RT}\right) \quad (6)$$

where k is the rate constant of the chemical reaction, A is the frequency factor, E_a is the apparent activation energy, R is the gas constant, and T is the absolute temperature. The apparent activation energy is then obtained from the slope of the Arrhenius plot, defined in Eq. 6. The reaction rate constant at any temperature was given by the regression line of the Arrhenius plot. For studies involving the basic theory of chemical reactions, the reaction kinetics must be defined. In general, most real chemical reactions are complicated and involve numerous elementary steps. Consequently, a best-fit reaction model can be difficult to identify. In this study, we adopted the time–temperature superposition Principle (TTSP). The TTSP is a well-known alternative method that is useful for extrapolation. Most studies involving a kinetics analysis based on the TTSP are related to the mechanical and electrical relaxation behavior of various polymers.^{19,20)} Recently, the TTSP has been adopted in other fields, such as the natural aging of cellulose. Here, we employed a method previously reported by Ding and Wang^{21,22)} and Matsuo *et al.*^{23,24)} The TTSP regards both time and temperature as equals; that is, the values of a color parameter obtained for a short time at an accelerated aging temperature are identical to those measured for a longer time

Table 1. Heat Treatment Conditions: Sample Storage Times at Various Temperatures

Temperature	Storage time (h)								
80°C	4	10	16	24	30	38	44	50	56
75°C	6	16	24	34	38	46	55	62	70
70°C	10	24	34	48	58	72	84	106	152
65°C	24	48	72	96	144	192	240	288	336
60°C	31	55	96	168	240	288	336	384	432
50°C	72	144	216	288	360	432	504	576	648
40°C ^{a)}	336	672	1344	1680	2016	2688	3360	4032	—

^{a)} The results obtained at 40°C were used for comparison with the predicted data.

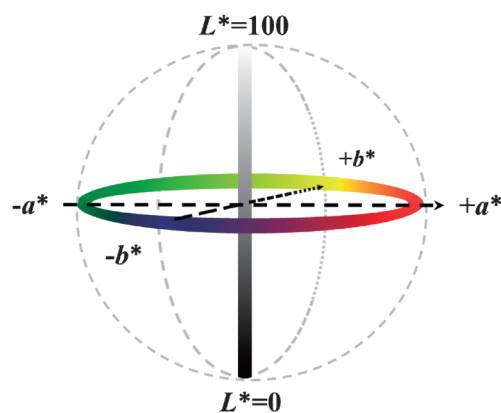


Fig. 1. CIELAB Color Space Diagram

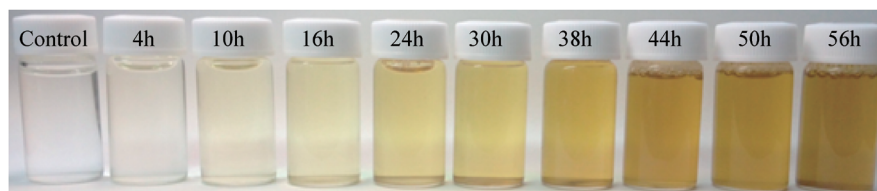


Fig. 2. Sample Color Changes in Response to Thermal Treatment at 80°C According to the Storage Time (h)

at a moderate temperature. The time courses of the measured color parameter against the logarithmic time at different temperatures can be superimposed by proper scale changes on the logarithmic time axis. The shift distance along the logarithmic time axis is called the time-temperature shift factor (a_T) and is given as follows:

$$a_T = \frac{t_T}{t_{ref}} \quad (7)$$

where t_{ref} is the test time at a reference temperature (T_{ref}), and t_T is the time required to produce the same response at the test temperature (T). A value of 65°C was selected as the T_{ref} value because this value corresponded to the middle temperature of the experimental data for the prediction. A polynomial regression equation was used to predict a^* , b^* , L^* , and ΔE^*ab at 65°C. Additionally, the best-fit of the polynomial order was defined using Akaike's Information Criterion (AIC) as a judging standard.²⁵⁾ The value of a_T was calculated so as to minimize the value of the root mean square error (RMSE) of the difference between the experimental and predicted data at 65°C. The measured color parameters were superposed by a_T on the time axis, and the constants of the regression curves were recalculated for the best fit using all the data plots. This regression model was defined as the master curve. Generally, the Arrhenius equation is acknowledged as having a reasonably good accuracy for determining a_T .^{19,20)} By combining Eqs. 6 and 7, a_T can be defined as follows:

$$a_T = \exp\left[\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \quad (8)$$

where both T and T_{ref} are absolute temperatures. From Eq. 8 a shift factor at any temperature can be given by the slope of the plot between the logarithm a_T and $1/T$, and the apparent activation energy (E_a) can then be estimated. Once the shift factors of the sample solution have been determined, these values can be used to adjust the shifting and extrapolation of the accelerated aging data at any desired temperature. To confirm the accuracy of the prediction, the color parameters at 40°C were evaluated and compared with the experimental result for the lowest temperature (40°C).

Results and Discussion

Color Change of Sample Solution The experimental results showing the appearance of the sample solution at 80°C are presented in Fig. 2. The color of the sample solution turned from colorless to dark brown with an increasing storage time in the stability chamber. The same color changes were observed in the other samples under all the storage conditions. The time courses of the color parameters (a^* , b^* , L^* , and ΔE^*ab) at various storage temperatures are shown in Fig. 3. The shapes of the time courses in the graph differed for each of the color parameters. The a^* values (upper left) immediately decreased and then increased thereafter. The shape of time course for a^* at 50°C seems to be not the same as those at the other temperatures (60–80°C). It might be caused by relatively short reaction time and slow reaction rate of color

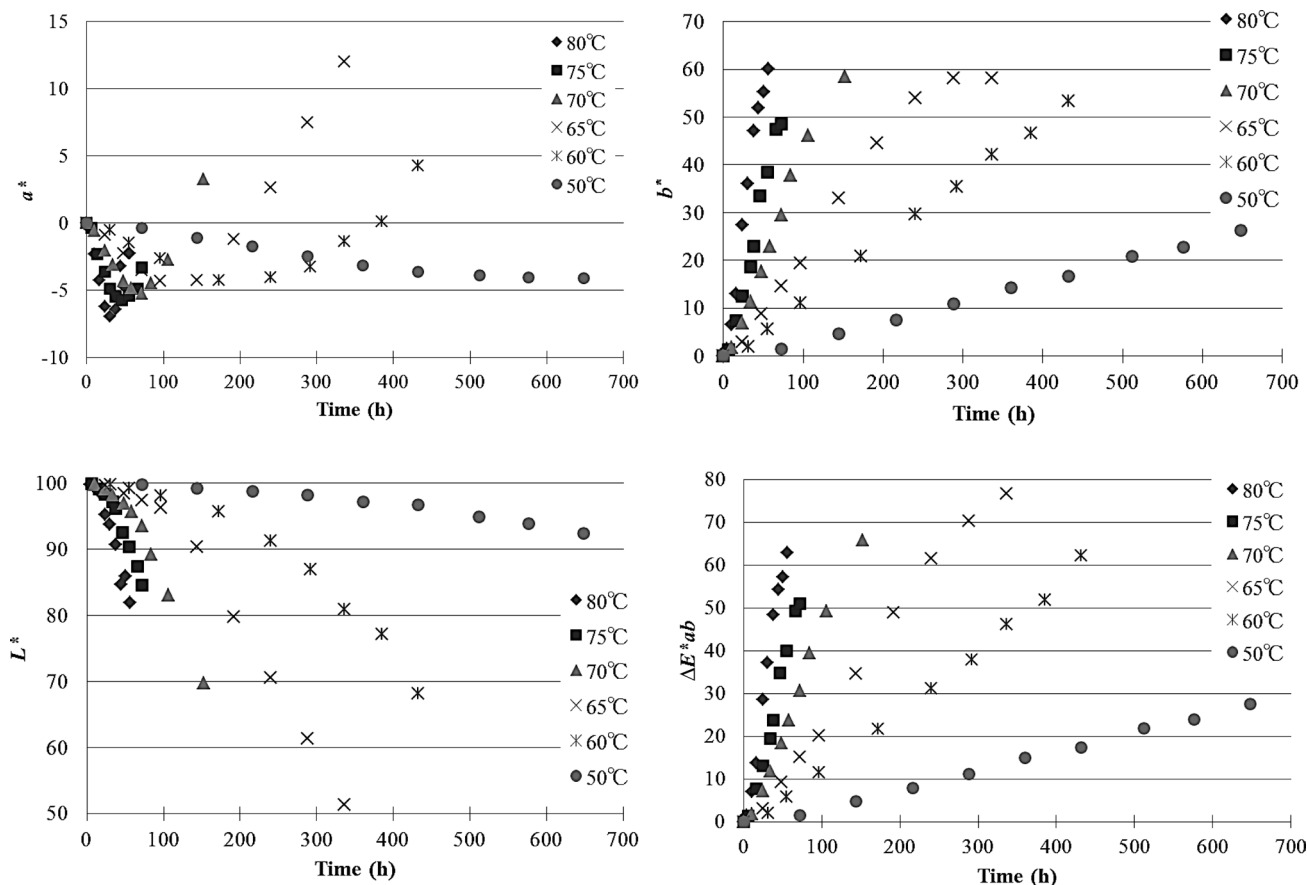


Fig. 3. Time Courses for Color Parameters (a^* , b^* , L^* and ΔE^*ab) at Various Temperatures

Each datum represents the mean of three determinations.

change at 50°C. This complicated behavior indicated that at first the color of the sample solution turned green, then the direction of the reaction changed and the color became red. The b^* values (upper right) monotonically increased at all the temperatures. This finding indicated that the color of the sample solution steadily became more yellow. A steady decrease in the L^* values (lower left) indicated that the color of the sample solution darkened. The total color change (ΔE^*_{ab}) was defined as the Euclidean distance in three dimensions composed of the Δa^* , Δb^* , and ΔL^* values. At all the temperatures, the ΔE^*_{ab} values (lower right) increased with an increase in the storage time, and the appearance turned from colorless to brown.

Calculation of Apparent Activation Energy To evaluate the apparent activation energy, the TTSP was adopted. A temperature of 65°C was selected as T_{ref} . Polynomial regression equations were applied to the values of a^* , b^* , L^* , and ΔE^*_{ab} at 65°C expressed as a function of time. The regression order providing the best fit was selected based on the one

Table 2. Comparison of the AIC Value Using Various Polynomial Regression Curves for the Color Parameters

	Second-order	Third-order	Fourth-order	Fifth-order
a^*	212.1	209.8	154.7	171.0
b^*	262.4	231.6	236.3	256.7
L^*	285.9	234.0	136.0	222.6
ΔE^*_{ab}	280.3	179.8	197.6	273.3

with the lowest AIC value. Table 2 shows the AIC for each parameter. As a result, the third-order was selected for the b^* and ΔE^*_{ab} values, while the fourth order was selected for the a^* and L^* values. The value of a_T was calculated so as to minimize the RMSE of the difference between the measured color parameters and the regressions curves at 65°C. Figure. 4 shows the best-fit regression curve of each of the color parameters at 65°C and the value of a_T at various temperatures. The Arrhenius plots of the ΔE^*_{ab} values using the a_T value and its linear regression line are shown in Fig. 5 as an example.

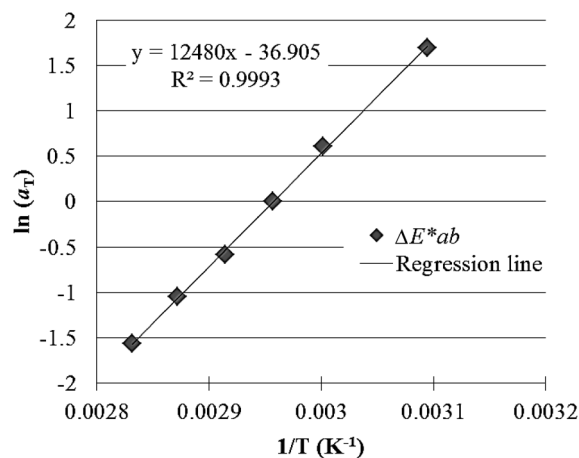
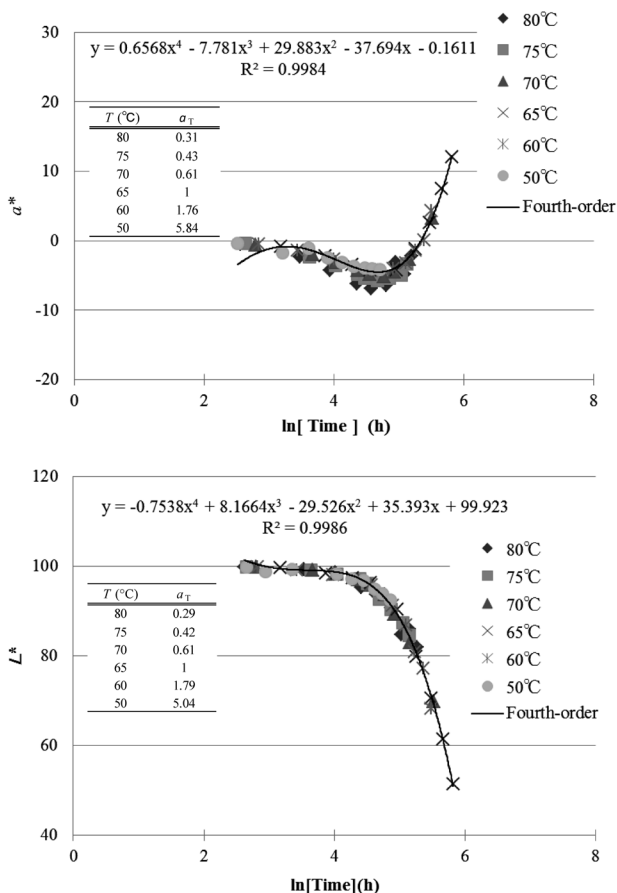


Fig. 5. Arrhenius Plot for ΔE^*_{ab} Using the a_T of the TTSP

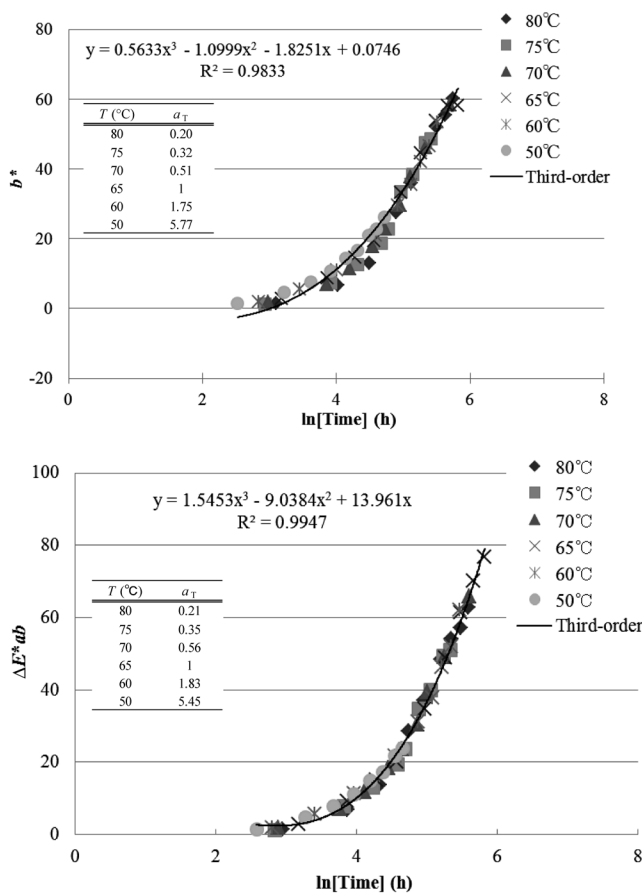


Fig. 4. Time Course Model of Color Parameters (a^* , b^* , L^* and ΔE^*_{ab}) Superposed with the Best-Fit Regression Curves at 65°C Using the a_T Value Shown on the Figure

Each datum represents the mean of three determinations.

Table 3 shows the E_a and R^2 values for each parameter. For each of the color parameters, the R^2 values for the Arrhenius plot were sufficiently high; therefore, the E_a values were accurately calculated. As the results calculated from Eq. 8, the E_a value of ΔE^*_{ab} was 103.7 (kJ/mol). In the same way, the E_a values of a^* , b^* , and L^* were 105.2, 109.8, and 91.6 (kJ/mol), respectively. In this sample solution, the same chemical reactions might occur at all temperatures, since the temperature dependence of the shift factor was linear. The coloring of the sample solution is based on the chemical species and concentration of compounds produced by chemical reactions. For the same reason the change of color parameters is also dependent on the chemical species and concentration of compounds. As consequence, it was thought that the E_a values had been estimated from color parameters such as a^* , b^* and L^* .

Prediction of Color Change To predict the color changes at 40°C, the shift factor a_T at 40°C was calculated from the Arrhenius plots for each of the color parameters. The color parameters for 4032 h of storage at 40°C were evaluated, and the relationships between the experimental and predicted val-

ues of the color parameters were investigated. These results are shown in Fig. 6. The coefficients of determination (R^2) between the experimental and predicted a^* , b^* , L^* , and ΔE^*_{ab} values were 0.961, 0.979, 0.960, and 0.979, respectively. All the R^2 values were sufficiently high ($R^2 > 0.96$), suggesting a highly reliable prediction. However, the prediction ability of a^* and L^* is somewhat poor compared with that of b^* and ΔE^*_{ab} . It is likely that the signal-to-noise ratio is one of the primary causes of lowering the prediction ability of a^* and L^* .

Using this method, the accuracy of prediction is influenced by the selection of the polynomial regression equation for the master curve and the calculation of the shift factor. We optimized the order of the polynomial regression equation for the master curve based on the AIC. Using the AIC, the shift factor was adequately calculated because the RMSE values between the master curves and the experimental data were minimized for all the color parameters. The consideration of experimental errors, such as sample variability, temperature control in the stability chamber, and the spectrophotometer measurements, are also important for maximizing the accuracy.

According to the above results, color changes associated with Maillard browning in a liquid formulation can be successfully evaluated using a kinetic analysis incorporating the TTSP. This prediction method using TTSP is simple and can easily be applied to extrapolate accelerated aging data to any desired temperature. However, the color prediction using TTSP does have some limitations. The different behaviors of different color parameters are likely caused by the chemical reactions of each component. The CIELAB color parameters

Table 3. Apparent Activation Energy and Coefficients of Determination According to an Arrhenius Plot for the Color Parameters

	E_a (kJ/mol)	R^2
a^*	105.2	0.997
b^*	109.8	0.999
L^*	91.6	0.997
ΔE^*_{ab}	103.7	0.999

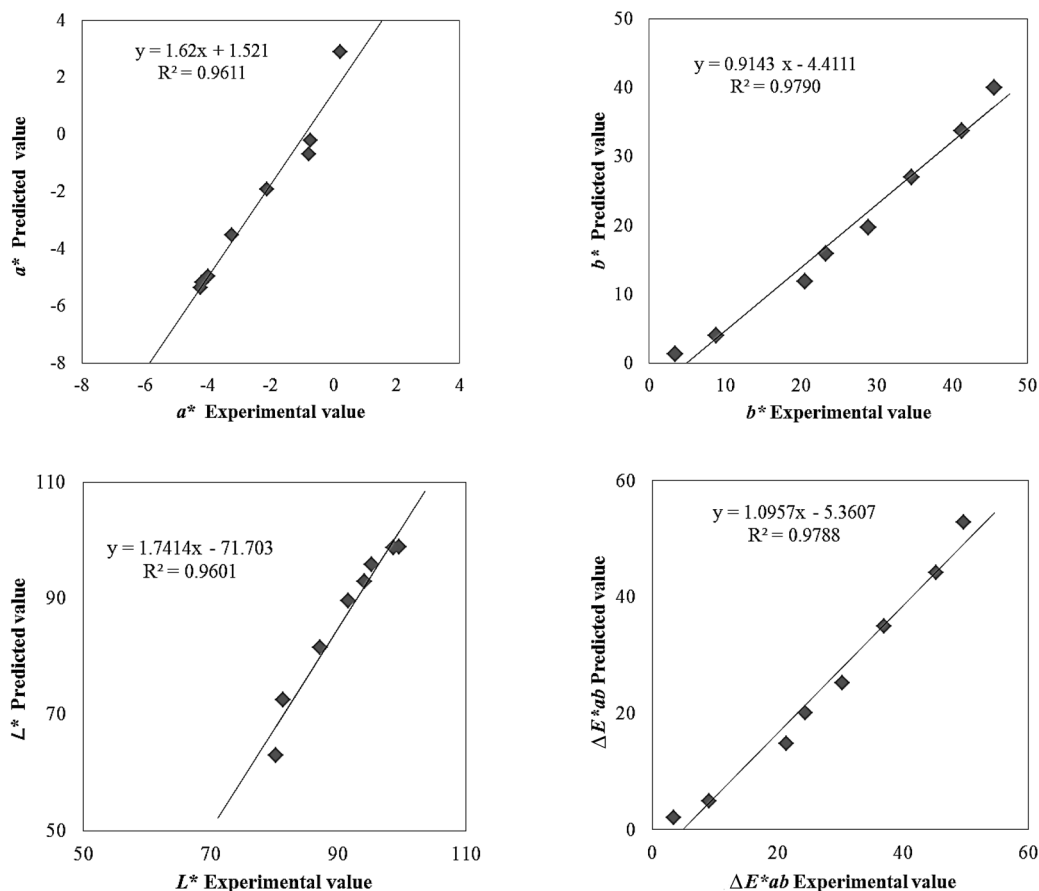


Fig. 6. Relationships between Experimental and Predicted Values of Color Parameters

are reflected by many chemical reactions. To understand the details of the chemical reactions, quantitative analysis at different wavelength spectra and the identification of the chemical components would be needed. When the behavior of color changes varies with temperature, the time courses for the color parameters will exhibit different shapes. Furthermore, the TTSP can only be applied to thermal aging. In the case of oxidation, the color changes are mainly influenced by the concentration of oxygen. Nevertheless, the TTSP has superior merits beyond a conventional kinetic analysis. No matter how complicated, the process underlying the reaction models does not need to be identified for the application of the TTSP.

Conclusion

The Maillard reaction of a model liquid formulation after accelerated aging treatment at elevated temperatures was measured using the CIELAB color parameters (a^* , b^* , L^* , and ΔE^*ab). The a^* values initially decreased and then increased. The L^* values steadily decreased, while the b^* and ΔE^*ab values increased linearly throughout the storage period. A kinetic analysis using the TTSP was successfully applied to calculate the apparent activation energy and to predict color changes at any temperature and duration. To predict color changes, the CIELAB parameters are useful for quantifying color changes after long periods of aging at ambient conditions, since the CIELAB parameters can be used to describe all colors visible to the human eye. Therefore, the simultaneous evaluation of these three color parameters is preferable. Even though the time courses for each of the color parameters exhibited a different behavior, the TTSP was easily applied to all the color parameters. The TTSP is helpful as a novel method of predicting color changes, compared with conventional techniques using complicated reaction models.

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