学位論文（博士）

Prediction of color changes using the time-temperature superposition principle in liquid formulations

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List of publication

1. Prediction of color changes using the time-temperature superposition principle in liquid formulations: Koji MOCHIZUKI and Kozo TAKAYAMA, Chem. Pharm. Bull. 62(12), 1225-1230 (2014), < presented in Chapters 1 and 2 of this dissertation >

2. Prediction of color changes in acetaminophen solution using the time-temperature superposition principle: Koji MOCHIZUKI and Kozo TAKAYAMA, Drug Dev. Ind. Pharm., Published online 11. Nov. 2015, DOI:10.3109/03639045.2015.1107091, < presented in Chapters 1 and 3 of this dissertation >

Abbreviations

A;	Frequency factor
AIC;	Akaike's information criterion
a_T;	Time-temperature shift factor
CIE;	Commission Internationale de l'Eclairage
DO;	Dissolved oxygen
E_a ;	Apparent activation energy
HS;	Head space
ICH;	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
JIS;	Japanese Industrial Standards
k;	Rate constant of the chemical reaction
$k_{ref;}$;	Rate constant of the chemical reaction at the reference temperature
k_T;	Rate constant of the chemical reaction required to produce the same response at the test temperature
R;	Universal gas constant
RSD;	Relative standard deviation
RMSE;	Root mean square error
T ;	Absolute temperature
T_{ref};	Reference temperature
t_T;	Time required to produce the same response at the test temperature
t_{ref};	Test time at the reference temperature
TTSP;	Time-temperature superposition principle

General introduction

Color changes in pharmaceutical and medical products give indication of deterioration of their commercial value and quality[1),2)]. Color changes during storage may occur as a result of contamination or the presence of impurities or degradation products[3)]. Therefore, color is an important aspect of the quality of pharmaceutical products. Color assessment is one type of quality assurance that should be carried out during the drug development process[4)]. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline recommends that if the color of drug products changes during storage, observation of the color change using a quantitative approach may be appropriate[3)]. At present, the standard quantitative color evaluation by the instrumental approach is performed utilizing the Commission Internationale de l'Eclairage (CIE) LAB color space (a^*, b^*, L^* and ΔE^*ab)[5)]. The CIELAB color parameters can objectively represent all the colors visible by the human eye. A quality by design approach for the color appearance control in pharmaceutical products using objective color parameters has been reported by the pharmaceutical industry[6), 7)].

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.[8)] In some cases, however, color changes occur very slowly over time. If a color change that does not satisfy the quality standard has been revealed in a commercial product before the expiration date, the product must 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,[9] apple puree,[10] and green asparagus.[11]

In the pharmaceutical development of solid dosage forms, kinetic analyses of the color changes have been reported for various purposes, including the discoloration prediction[12], evaluation of the color changes of photosensitive drugs under various light sources[13], investigation of the discoloration kinetics of uncoated white tablets[14, 15], examination of color changes during long-term stability tests[16], evaluation of colorants stability as components in tablets[17], and elucidation of color stabilization through the use of film coating[18, 19]. In the quality control of coated tablets, the parameters of surface color were applied[20, 21]. On the other hand, few methods for kinetic analyses of the color changes in liquid formulations have been reported yet. 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. Kitamura et al. calculated the reaction order of color changes using Matsuda's equation[13] and data on the total color difference (ΔE).[22] 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.[23] These reports did not describe the property of color changes and required complicated calculations based on reaction models. 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-hit models can be difficult to identify. In this study, the time-temperature superposition principle (TTSP) was applied to the data, instead of the conventional use of complicated kinetic models.

Chapter 1

Theory

1. Color evaluation using CIELAB color parameters

The CIELAB color parameters (a^*, b^*, L^*, and ΔE^*ab) authorized in JIS Z8729[24] and JIS Z8730[25] 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[24),25)]:

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

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

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

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

where a_0^*, b_0^*, and L_0^* are the initial values without heat treatment.

These color parameters were measured by a CM-3500d spectrophotometer (KONICA MINOLTA, INC.) under the standard illuminant D65 light source at an observed angle of 10 degrees. The color parameter measurements were represented as the mean of three determinations. The relative standard deviation of any measurement was less that 0.2%, suggesting a high reproducibility.

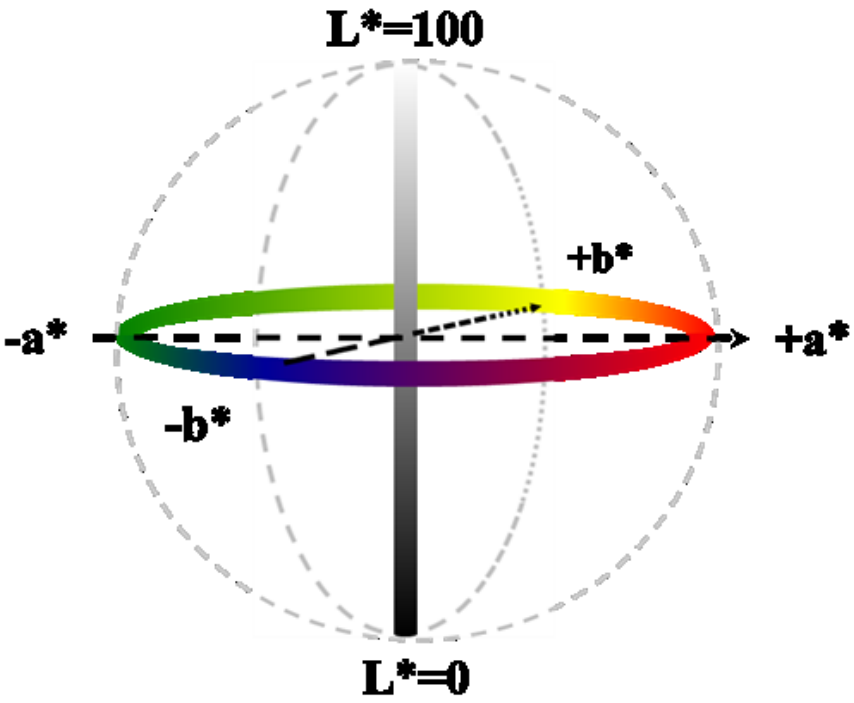

Figure 1. CIELAB Color Space Diagram

2. Kinetic analysis using the time-temperature superposition principle

In order to predict the color changes over time, kinetic analysis is a useful tool. The Arrhenius equation is used as a basic method for performing kinetic analysis of chemical reactions[26]:

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

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

where E_a is the apparent activation energy, k is the rate constant of the reaction, A is the pre-exponential factor (frequency factor), R is the universal gas constant, and T is the temperature (in Kelvin). The slope of the Arrhenius plot gives the apparent activation energy, defined by Eq. 6. The rate constant at any given temperature was obtained by the regression line of the Arrhenius plot. In general, most of chemical reactions are complex and involve several elementary steps. Consequently, the most appropriate reaction model can be difficult to determine. In the present study, I attempted to use the time-temperature superposition principle (TTSP), instead of the conventional kinetic models. Most reports involving kinetic analysis based on the TTSP are related to the mechanical response of various high molecular compounds [27), 28)]. In recent years, the TTSP has been applied in other areas, such as for study of the natural aging of paper, cellulose and wood specimens[29)-33)]. The TTSP deals with both time and temperature as equal; i.e. the values of a color parameter obtained

for a short time at a given temperature are identical to those measured for a longer time at a lower temperature. The curves of changes in the measured color parameters versus 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 determined as follows:

$$a_T = \frac{t_T}{t_{ref}} \tag{7}$$

where t_{ref} is the test time at the reference temperature (T_{ref}), and t_T is the time required to produce the same response at the test temperature (T). In the present study, I selected the intermediate temperature (65°C) of the experimental data for the prediction as the T_{ref}. A polynomial regression equation was used to calculate the values of each color parameter at 65°C. In addition, the best suitable of the polynomial order was determined by using Akaike's Information Criterion (AIC) as a judging standard[34]. The value of a_T was obtained in order to minimize the value of the root mean square error (RMSE) of the difference between the experimental and calculated data at 65°C. The experimental data was superposed by a_T on the logarithmic time axis, and the regression equation was recalculated for the best fitting using all the data plots. This regression model was defined as the master curve. In general, the Arrhenius equation has established a high reputation for the determination of the value of a_T with a reasonably good accuracy [27), 28)]. By combining Eqs. 5 and 7, a_T can be defined as follows:

$$a_{\mathrm{T}} = \frac{t_{\mathrm{T}}}{t_{\mathrm{ref}}} = \frac{k_{\mathrm{ref}}}{k_{\mathrm{T}}} = \exp\left[\frac{E_{\mathrm{a}}}{R}\left(\frac{1}{T} - \frac{1}{T_{\mathrm{ref}}}\right)\right] \tag{8}$$

where both T and T_{ref} are temperatures (in Kelvin). From Eq. 8, plotting $\ln(a_{\mathrm{T}})$ versus $1/T$ allows us to calculate a shift factor at any temperature, and the E_{a} value can then be calculated. Once the shift factors of the sample solution have been estimated, these values can be used to adjust the shifting and extrapolation of the accelerated aging data at any desired temperature.

Chapter 2

Prediction of color changes of Maillard reaction in liquid formulations using the time-temperature superposition principle

1. Introduction

In this chapter, 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.[35] 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 CIELAB color parameters (a^*, b^*, L^*, and ΔE^*ab) to measure color differences in a quantitative manner.[24] The CIELAB color parameters can describe all the colors visible to the human eye.

In this chapter, 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.

2. Materials and methods

2.1 Materials

The liquid formulation used for the Maillard reaction model was composed of glucose as the reducing sugar (San-ei Sucrochemical Co., Ltd.) and L-tryptophan as the amino acid (KENEI Pharmaceutical Co., Ltd.). The following compounds that were used to prepare a citrate buffer solution were obtained commercially: citric acid (Iwata

Chemical Co., Ltd.), sodium citrate (SATUMA KAKO Co., Ltd.), and sodium benzoate (Aioi ChemiScience Co., Ltd.). 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.) was used to store the 15 mL liquid formulation at various temperatures.

2.2 Methods

The sample solutions were stored in a stability chamber (Nagano Science Co., Ltd.) 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. The color measurement, calculation of apparent activation energy and prediction color changes were performed based on the theory described in Chapter 1.

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). Microsoft Excel 2010 was used for all data analysis.

Table 1. Heat treatment conditions: sample storage times at various temperatures

Temperature	Storage time (hour)								
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 *	336	672	1344	1680	2016	2688	3360	4032	-

*The results obtained at 40°C were used for comparison with the predicted data.

3. Result and discussion

3.1 Color change of sample solution

The experimental results showing the appearance of the sample solution at 80°C are presented in Figure 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 Figure 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°C~80°C). It might be caused by relatively short reaction time and slow reaction rate of color 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.

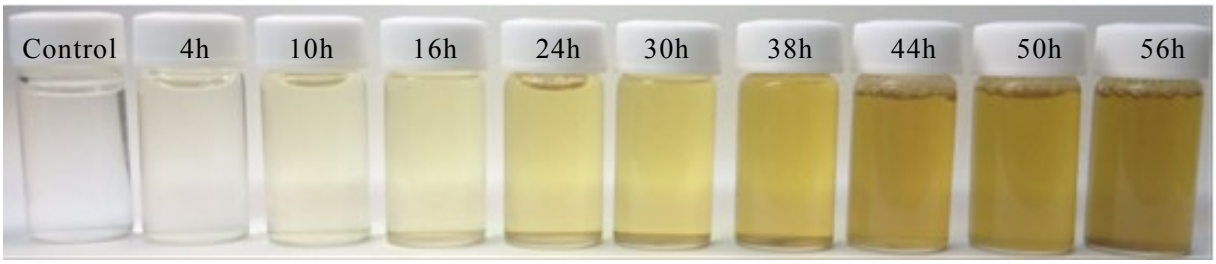

Figure 2. Sample color changes in response to thermal treatment at 80°C according to the storage time (h)

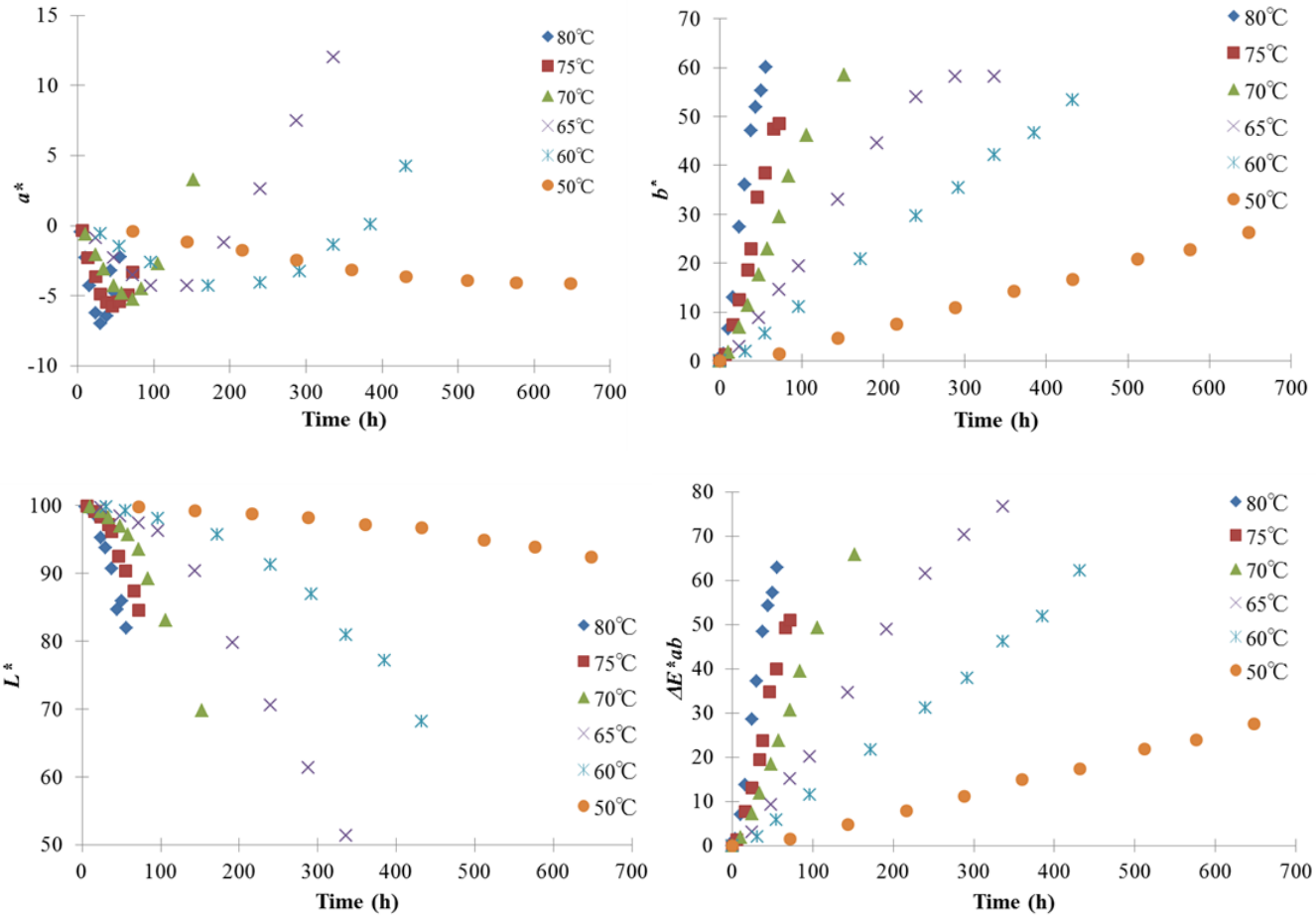

Figure 3. Time courses for color parameters (a^*, b^*, L^* and ΔE^*ab) at various temperatures

Each datum represents the mean of three determinations.

3.2 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 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 Figure 5 as an example. Table 3 shows the Ea 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 Ea values were accurately calculated. As the results calculated from Eq. 8 in Chapter 1, the Ea value of ΔE^*ab was 103.7 (kJ/mol). In the same way, the Ea 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 Ea values had been estimated from color parameters such as a^*, b^* and L^*.

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

The bold value represents the minimum AIC value of each color parameter.

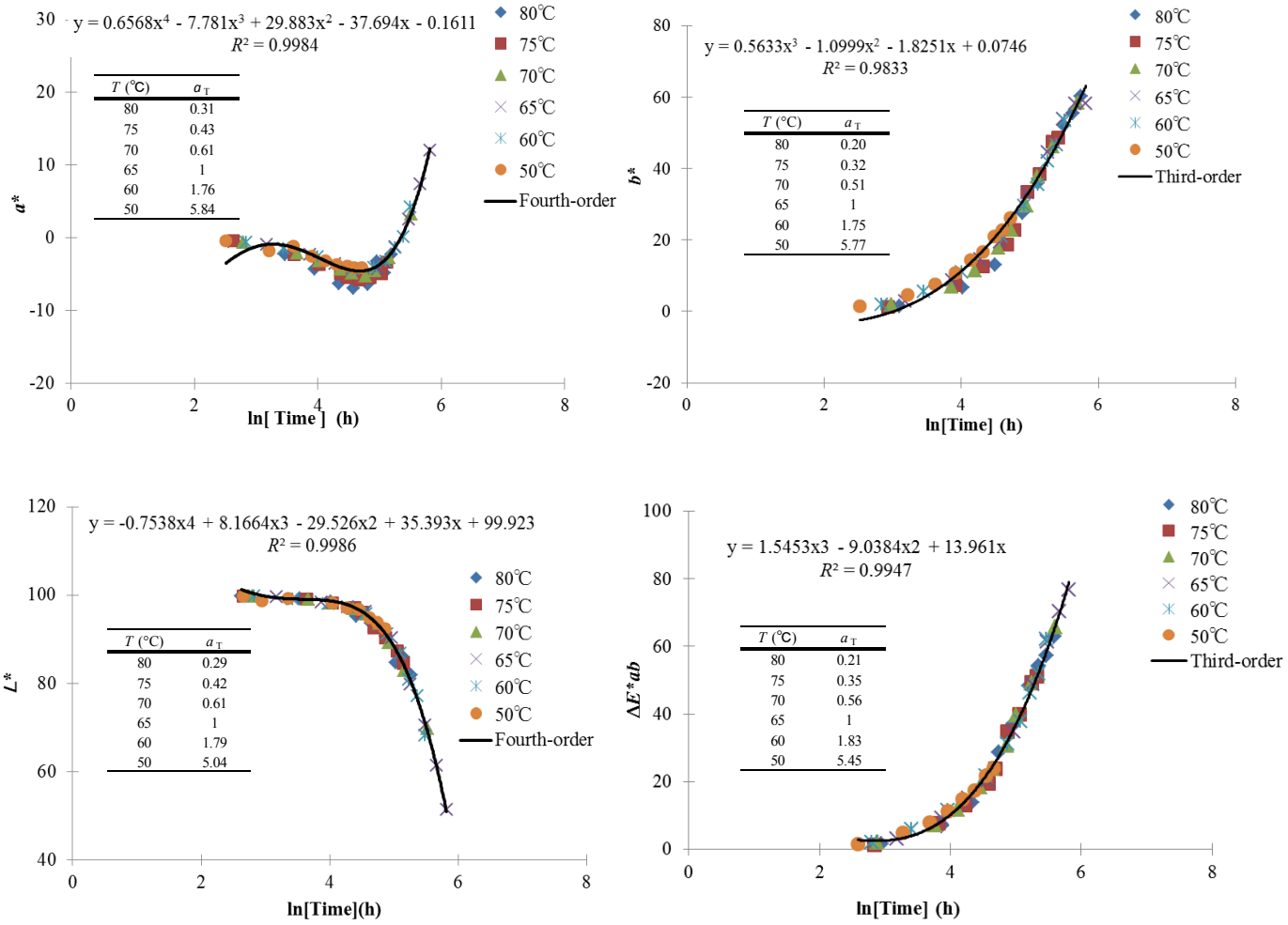

Figure 4. Time course model of color parameters (a^*, b^*, L^* and ΔE^*ab) superposed with the best-fit regression curves at 65°C using the a_T value shown on the figure

Each datum represents the mean of three determinations.

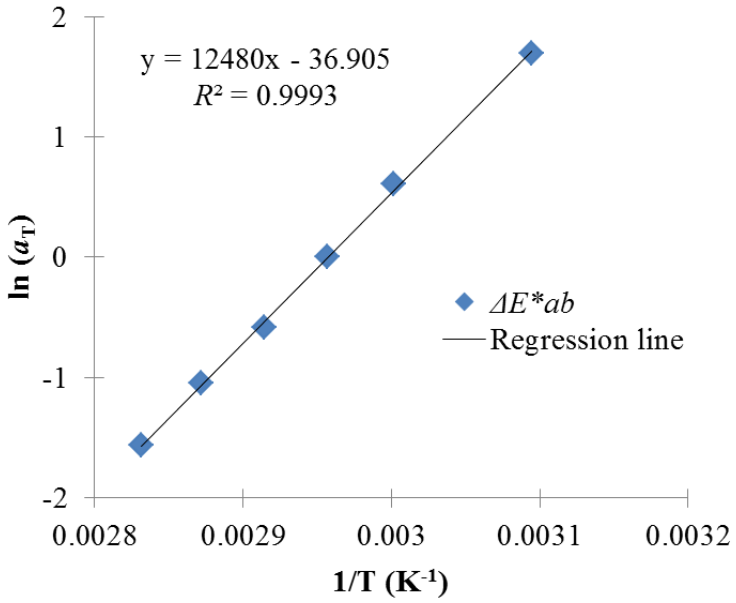

Figure 5. Arrhenius plot for Δ*E*ab* using the a_T of the TTSP

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
Δ*E*ab*	103.7	0.999

3.3 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 hours of storage at 40°C were evaluated, and the relationships between the experimental and predicted values of the color parameters were investigated. These results are shown in Figure 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 primacy 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. I 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 extrapolating 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 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.

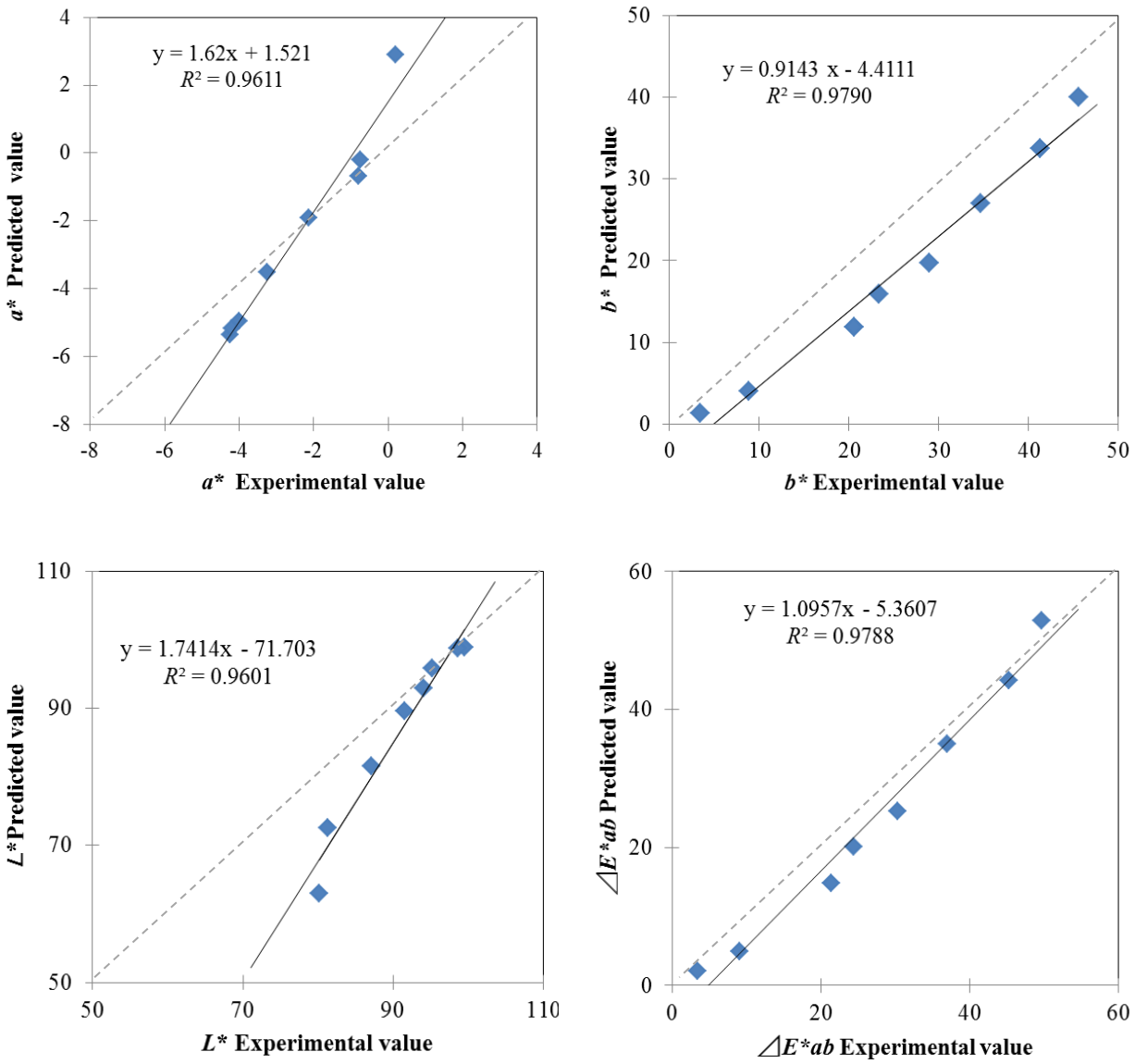

Figure 6. Relationships between experimental and predicted values of color parameters

4. 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. The TTSP is helpful as a novel method of predicting color changes, compared with conventional techniques using complicated reaction models.

Chapter 3

Prediction of color changes in acetaminophen solution using the time-temperature superposition principle

1. Introduction

In Chapter 2, I proposed the analysis of color changes of the Maillard reaction in liquid formulations using a kinetic approach based on the time-temperature superposition principle (TTSP), which allowed easy and precise prediction of the color changes as compared to conventional techniques involving the use of complicated reaction models. However, the validity of TTSP to the coloring reaction of active ingredients is yet to be investigated. In Chapter 3, I focused on the color changes caused by the degradation of an active ingredient which was high purity, low molecular weight and had multiple degradation pathways. There are no reports of a method for the prediction of these color changes using the TTSP. As an example, the color changes of acetaminophen solution were comprehensively investigated and a method for prediction of the color changes was developed using the TTSP. This may be useful for the development of acetaminophen liquid formulations in short period.

Acetaminophen (paracetamol) has remained one of the most widely used drugs in many pharmaceutical products for decades. It is commonly used as an analgesic and antipyretic[36]. Acetaminophen is usually administered in the oral dosage form, e.g., in the tablet, capsule or syrup forms. However acetaminophen can be decomposed through a plurality of degradation pathways (Figure 7)[37),38)]. It is known that acetaminophen in aqueous solution hydrolyzes to p-aminophenol, and then easily oxidizes further, resulting in the formation of the pink-colored quinine imines[39)]. The rate of degradation of acetaminophen increases with increasing temperature and light intensity[40)]. The degradation process is indicated by a gradual color change from light pink to dark brown. The degradation

of acetaminophen in solution by hydrolysis and oxidation is complex, the degradation products occurring in very small amounts and being unstable, so that it is not easy to identify and determine the quantities of the coloration materials. Therefore, only the quantitative determination of the degradation products is not enough to evaluate the color changes of acetaminophen solution. Many attempts have been made to improve the stability of acetaminophen solution, for example, by the addition of sodium metabisulfite in an aqueous composition[41], and addition of polyols like mannitol, sorbitol or inositol[42]. However, it has not been possible to stabilize and suppress the coloration of acetaminophen solution sufficiently. Therefore, the color changes of acetaminophen solution during long-term storage sometimes represent a technical problem during the development products. The method of prediction of color changes is needed for the quality control of acetaminophen products.

In this chapter, first, the influence of oxygen on the color changes was investigated. It is easily expected from the degradation pathway that the color changes of acetaminophen solution are influenced by oxidation. Little is known about the contribution of different levels of oxygen on the color changes of acetaminophen solution in the bottle. Each brown glass bottle of acetaminophen solution contains dissolved oxygen (DO) in the solution and gaseous oxygen in the head space (HS). Variations in the amount of DO as well as in the amount of HS oxygen could be responsible for differences in the chemical changes occurring during storage[43]. The influence of different filling volumes, and therefore different levels of HS oxygen, on the changes in the color of the solution was examined. Subsequently, while the oxygen amount was kept controlled, the prediction method for color changes of the acetaminophen solution was investigated. For this purpose, the

kinetic analysis was performed using the TTSP rather than with the conventional kinetic model.

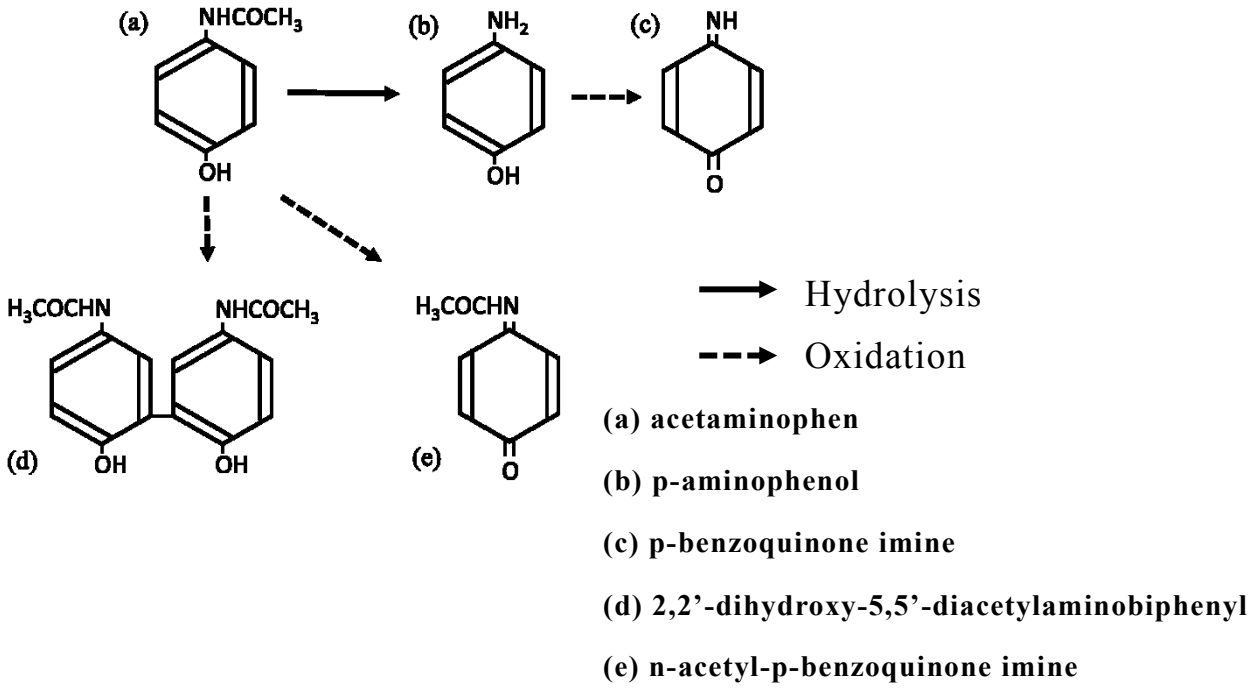

Figure 7. Degradation products of acetaminophen in solution

2. Materials and methods

2.1 Materials

Acetaminophen (pharmaceutical grade) was obtained from ZHEJIANG KANGLE PHARMACEUTICAL Co., Ltd. The following chemicals that were used as components of a citrate buffer were purchased commercially: citric acid (Iwata Chemical Co., Ltd.), sodium citrate (SATUMA KAKO Co., Ltd.) and sodium benzoate (Aioi ChemiScience Co., Ltd.). The sample solution was prepared by dissolving acetaminophen in a citrate buffer solution (25 mM citrate buffer, 4.2 mM sodium benzoate) to obtain final concentrations of 1 % (66.2 mM) of acetaminophen, with a pH of the solution of 3.5.

The bottling set up was as follows. The filling level was adjusted manually using a measuring cylinder. For studying the influence of oxidation, two HS volume samples were prepared. 57 mL and 20 mL of the sample solution were filled into 60-mL brown glass bottles (Daiichi glass Co., Ltd.), and the bottles were closed with aluminum caps (CSI Japan Co. Ltd.). As a result, the HS consisted of 3 mL and 40 mL of ambient air respectively. For prediction of the color changes, 15 mL of the sample solution was filled into a 15.8-mL clear glass container No. 4 (Maruemu Co. Ltd.). The HS volume was 0.8 mL. These samples with different HS volumes were stored at various temperatures.

2.2 Methods

At first, in order to grasp the influence of oxidation on the color changes, the two different HS samples (3 mL and 40 mL) were kept in a stability chamber (Nagano Science Co., Ltd.) at 60, 70, and 80°C. The length of storage time at each treatment temperature shows in

Table 4. Next, for prediction of the color changes, the sample solutions in the 15.8-mL clear glass container were stored in the stability chamber at 40, 50, 60, 65, 70, 75, and 80°C. The length of storage time at each treatment temperature shows in Table 5. Both the sample treatments were conducted under a light-shielded condition.

The color measurement, calculation of apparent activation energy and prediction color changes were performed based on the theory described in Chapter 1.

The predicted color parameters at 40°C were compared with experimental results for the lowest temperature (40°C) to confirm the accuracy of the prediction method. Microsoft Excel 2010 was used for all data analysis.

Table 4. Heat treatment conditions for determining the influence of HS on the color changes: sample storage times at various temperatures

Temperature	HS (mL)	Storage time (hour)						
80°C	3	12	24	48	72	96	120	144
	40	-	-	48	-	96	-	144
70°C	3	29	48	96	144	168	216	240
	40	-	-	96	-	168	-	240
60°C	3	72	144	216	312	360	434	504
	40	-	-	216	-	360	-	504

Table 5. Heat treatment conditions for the kinetic analysis: sample storage times at various temperatures

Temperature	Storage time (hour)								
80°C	4	10	18	24	30	40	50	62	82
75°C	6	18	24	34	40	50	62	72	82
70°C	10	24	34	48	57	72	82	106	144
65°C	24	48	72	96	144	216	240	288	346
60°C	33	58	100	192	240	292	336	456	508
50°C	76	192	240	288	474	526	720	-	-
40°C *	332	714	2184	2688	3408	4176	-	-	-

*The results obtained at 40°C were used for comparison with the predicted data.

3. Result and discussion

3.1 Influence of oxygen on the color changes

In order to verify the influence of oxygen on color changes of acetaminophen solution, the aging test at three temperatures (60, 70, and 80°C) was conducted using brown glass bottles with two different amounts of oxygen (3 mL and 40 mL) in the HS (small and large HS samples, respectively). The appearances of the sample solutions after exposure to 80°C for 144 h are shown in Figure 8. The sample solutions turned from colorless to dark brown. The different HS volumes, and therefore variations of the oxygen amount, resulted in significant color differences. The large HS sample was darker in color than the small HS sample. These findings suggest that the oxidation reaction product increased the color changes in the acetaminophen solution.

The time-courses of changes of the total color difference (ΔE^*ab) at different storage temperatures are shown in Figure 9. The ΔE^*ab values of the large HS samples increased linearly with the storage time. On the other hand, the ΔE^*ab values of the small HS samples, while initially changing at the same rate as that in the large HS samples, changed more and more slowly with increasing storage time. This indicated that the small HS samples consumed the oxygen earlier than the large HS samples, with the result that the oxidation reaction causing the color changes in the small HS samples stopped much earlier. It became apparent that oxygen is important for the development of pigment.

The above results reveal the impact of the amount of oxygen in a container on the color variations of acetaminophen solution. Further studies are needed to fully elucidate the implications of the amount of

oxygen in a quantitative manner on the color changes of acetaminophen solutions. These results suggest that in order to maintain the quality of a product uniformly, it is necessary to manage the amount of oxygen in the container (e.g., by controlling the filling volume) and ensure appropriate packaging selection, because the quantity of oxygen transferred varies according to the packaging material. A method of deaeration under reduced pressure or replacement of the oxygen with an inactive gas, such as gaseous nitride, is required. For the evaluation of the color changes, it is desirable to use the same container and the same oxygen condition as the commercially available product.

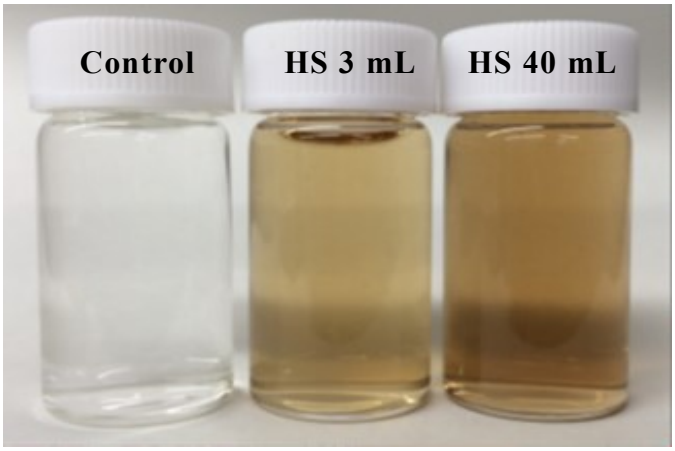

Figure 8. Influence of the headspace volume on the color changes of the sample in response to thermal treatment at 80°C for 144 h
The containers were changed from brown glass bottles to 15.8-mL clear glass bottles before taking the pictures.

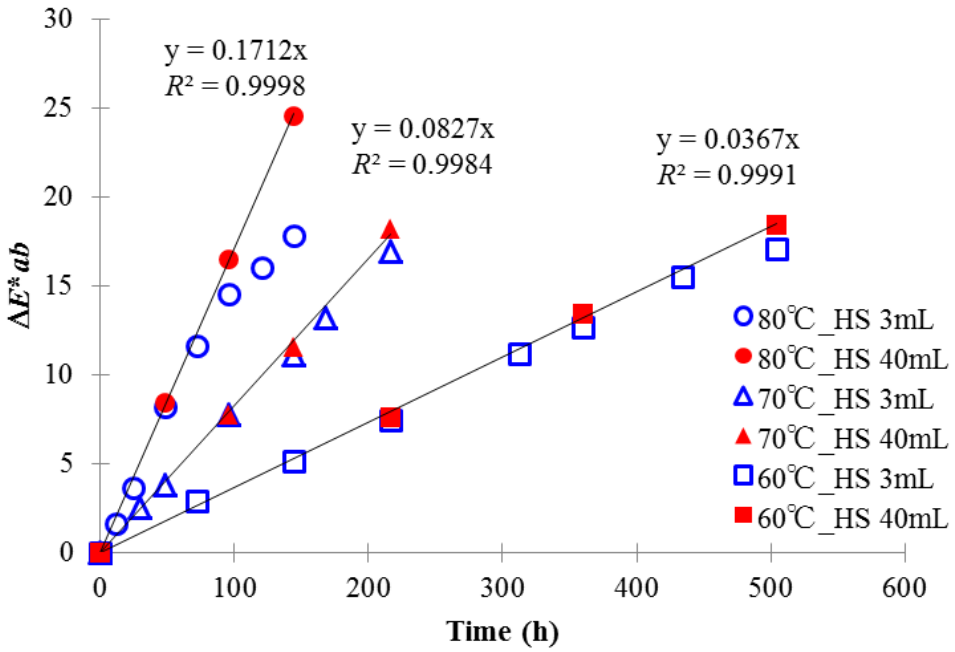

Figure 9. Time-courses of changes in the color differences (ΔE^*ab) in different headspace samples at various temperatures

Each datum represents the mean of three determinations and the relative standard deviation (RSD) was less than 0.2%.

Regression line was derived from the HS40-mL data at each temperature.

3.2 Prediction of the color changes based on the TTSP

3.2.1 Influence of storage temperature and time on the color changes

The influence of storage temperature and time on the color changes was investigated using 15.8-mL clear glass containers filled with the same volume of the sample solution (15 mL). The HS volume was uniform at 0.8 mL. Figure 10 shows the time-courses of changes in the color parameters (a^*, b^*, L^* and ΔE^*ab) at different storage temperatures. The shapes of the time-courses of changes of the color parameters were similar, except for L^*. The a^* and b^* values increased monotonically at first, however, with increasing storage time, the rates of change became slower. As described in the previous section, this slowing of the rate of change indicated that the HS oxygen was consumed and production of the coloring materials decreased. In the CIELAB color space, the changes of the a^* and b^* values with the storage time represented a browner color of the sample solution. The same color changes were observed under all the storage conditions. A steady decrease in the L^* values represented darkening of the color's lightness of the sample solution. This finding indicated that the color of the sample solution gradually became darker and darker brown. The total color difference (ΔE^*ab) was defined as the three-dimensional Euclidean distance composed of the Δa^*, Δb^*, and ΔL^*values (Eq.4 in chapter 1). At all the temperatures, the ΔE^*ab values increased with increasing storage time in the stability chamber. The ΔE^*ab values were largely dominated by increasing b^* values.

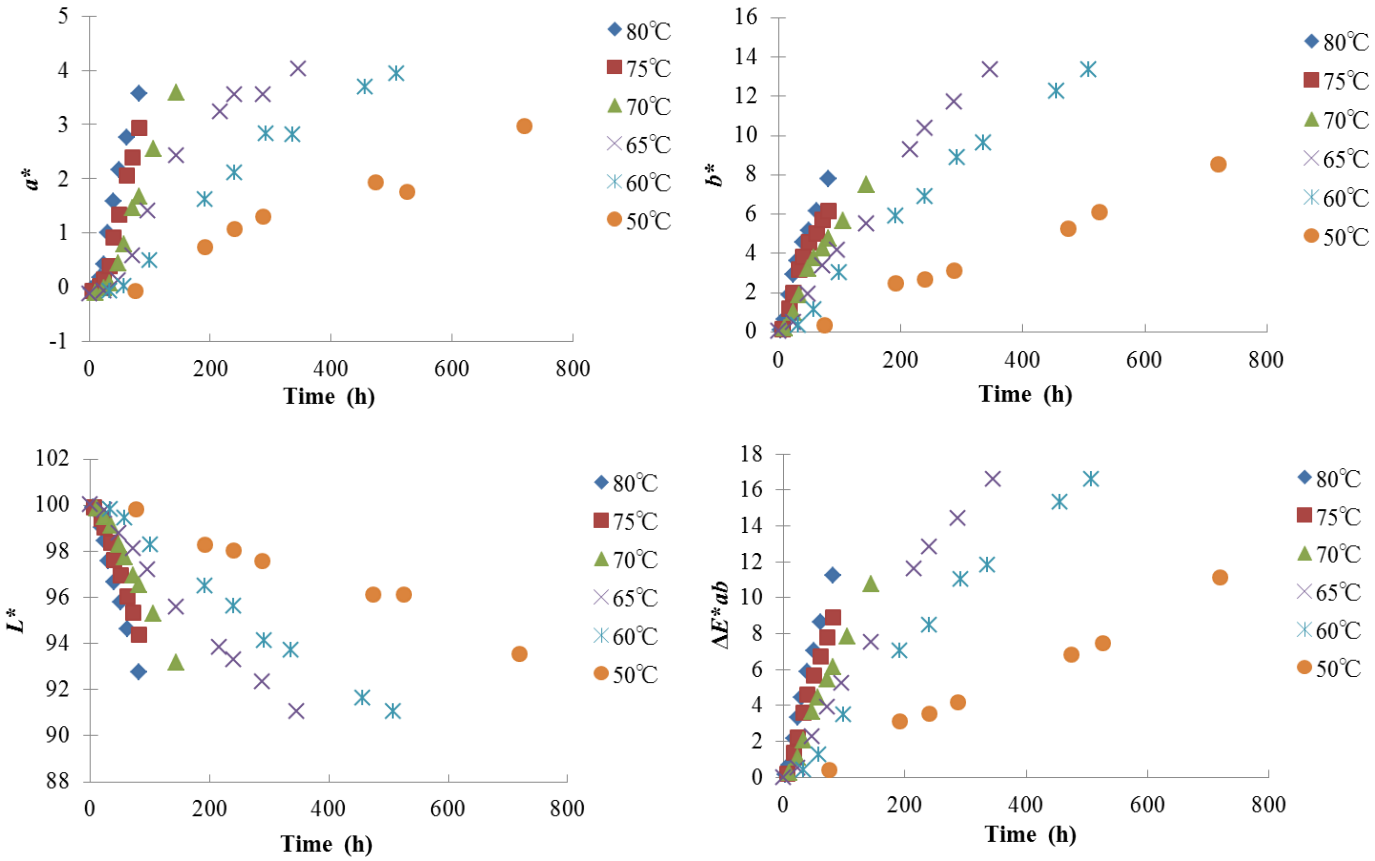

Figure 10. Time-courses of changes in the color parameters (a^*, b^*, L^* and ΔE^*ab) at various temperatures

Each datum represents the mean of three determinations and the relative standard deviation (RSD) was less than 0.2%.

3.2.2 Calculation of the apparent activation energy

In this study, for simplicity, the TTSP was adopted to evaluate the apparent activation energy. In many cases, the coloring materials are generated by very complex reaction mechanisms and the concentrations are quite low. Sometimes the color changes could be detected before the chemical degradation products accumulated to a noticeable extent[44), 45)]. Application of the TTSP does not require the determination and quantity of the coloring materials, as is also the case with the kinetic model.

The temperature of 65°C was selected as the T_{ref}, because the biggest values for each parameter during the storage time were obtained at 65°C. Polynomial regression equations were applied to the values of each color parameter at 65°C expressed as a function of time. The lowest AIC value is used to determine the regression order which provides the best fitting. These regression curves may have no meaning in terms of the reaction mechanism. The AIC values for each color parameter are shown in Table 6. As a result, the third-order polynomial was chosen for the a^* and L^* values, while the fourth-order polynomial was chosen for the b^* and ΔE^*ab values. The value of a_T was estimated in order to minimize the RMSE of the difference between the experimental color parameters and the regressions curves at 65°C. As a result, well fitted data with the regression curve and highly reliable a_T values were obtained. Figure 11 shows the time-course model for each of the color parameters superposed with the best-fitting regression curves at 65°C and the a_T values at various temperatures.

Figure 12 shows the Arrhenius plots ($\ln(a_T)$ versus K^{-1}) for the ΔE^*ab and its linear regression line, as an example. The coefficient of determination (R^2) of the linear regression line was 0.995. In this

sample solution, the same chemical reactions evidently occurred at all temperatures, since the temperature dependence of the shift factor was linear. From the slope, the *E*a value of ΔE^*ab was calculated as 70.9 (kJ/mol) using Eq. 8 in Chapter 1. Table 7 shows the *E*a and R^2 values for each parameter. For each of the color parameters, the R^2 values were sufficiently high ($R^2 > 0.99$); therefore, the *E*a values were accurately calculated. The *E*a values of a^*, b^*, and L^* were 72.4, 69.2 and 72.3 (kJ/mol), respectively. These values of the *E*a were similar to the values for acetaminophen hydrolysis reported in the literature[40),46)]. From these results, the main cause of the color changes was considered to be degradation of acetaminophen by hydrolysis.

Table 6. Comparison of the AIC value using various polynomial regression curves for the color parameters

	Second-order	Third-order	Fourth-order	Fifth-order
a^*	36.4	**31.1**	35.9	67.3
b^*	113.9	49.5	**40.2**	159.6
L^*	77.0	**27.1**	28.2	35.1
ΔE^*ab	119.8	7.9	**7.2**	123.4

The bold value represents the minimum AIC value of each color parameter.

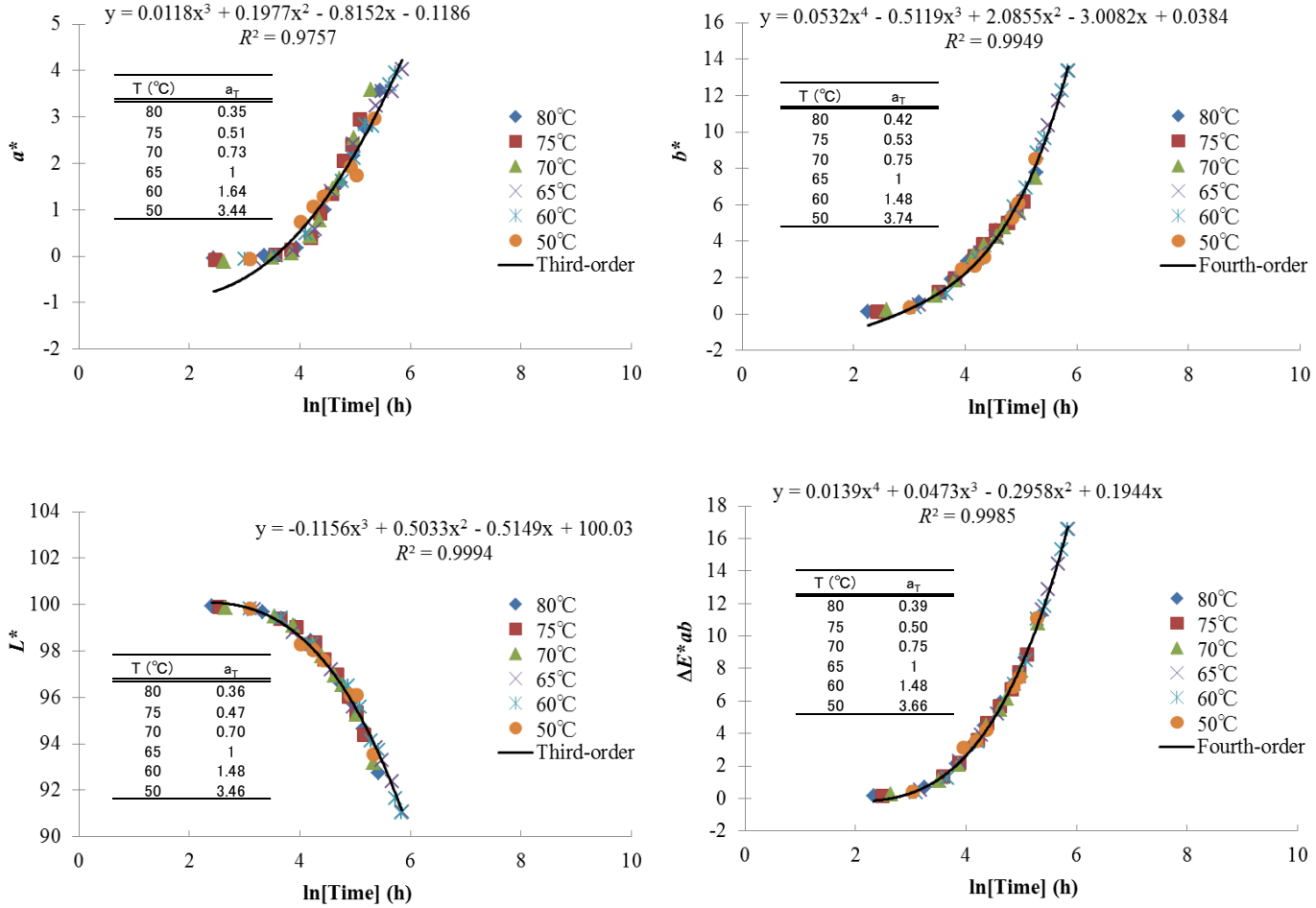

Figure 11. Time-course model of the color parameters (a^*, b^*, L^* and ΔE^*ab) superposed with the best-fit regression curves at 65°C using the a_T value shown on the figure

Each datum represents the mean of three determinations and the relative standard deviation (RSD) was less than 0.2%.

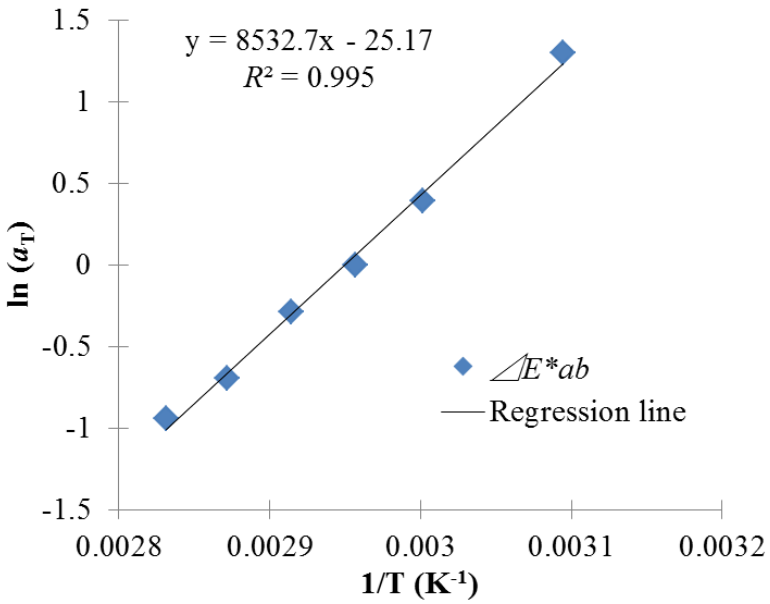

Figure 12. Arrhenius plot for ΔE^*ab using a_T based on the TTSP

Table 7. Apparent activation energy and coefficients of determination according to an Arrhenius plot for the color parameters

	*E*a (kJ/mol)	R^2
a^*	72.4	0.998
b^*	69.2	0.991
L^*	72.3	0.999
ΔE^*ab	70.9	0.995

3.2.3 Prediction of the color changes

Using a_T values calculated from the Arrhenius plots allows prediction of the color changes after exposure to any temperature and for any length of time. In this Chapter, each of the color parameters until 4176 h at 40°C in the stability chamber were evaluated. The comparisons between the experimental and predicted values of the color parameters are shown in Figure 13. The coefficients of determination (R^2) for a^*, b^*, L^* and ΔE^*ab values were 0.996, 0.985, 0.982 and 0.997, respectively. All of the R^2 values were sufficiently high ($R^2 > 0.98$), suggesting the high reliability of the prediction. The degree of accuracy of ΔE^*ab was excellent, because the slope in Figure 13 was almost equal to 1, although the slopes of a^*, b^* and L^* deviated somewhat from 1. It is likely that the small differences between the predicted and experimental values of a^*, b^* and L^* arose from the long-term storage. That is, some errors of measurement may arise as a result of accelerated changes of the color parameters with increasing storage time.

From the above results, color changes associated with acetaminophen degradation can be successfully evaluated by kinetic analysis based on the TTSP. However, some points must be borne in mind before applying the TTSP, as follows. First, it must be verified that the color changes are caused by the same reaction in the temperature range examined between the experiment and prediction. This is an important point, in general, for prediction by extrapolation using the Arrhenius plots. When the reaction mechanism depends on the temperature, the time-courses of changes of the color parameters will exhibit different shapes for each temperature. When using the TTSP, determination of whether there are any differences between the master curve and the data for each temperature is carried out to verify

if the color changes are caused by the same reactions at each temperature. Secondly, in principle, the TTSP is only applicable to thermal aging. These experimental results showed that the time-course of the color changes was also influenced by the amount of oxygen in the bottle. The CIELAB color parameters are influenced by many chemical reactions. It is necessary to strive to control the amount of oxygen in containers, like managing the filling volume or selection of the package. Recently, the modified time-temperature superposition principles were proposed[47)-49)], in which the TTSP was expanded and applied to many other accelerated factors e.g. moisture and external stress. In the case of revealing quantitatively the color changes by the oxygen amount in the bottle at different temperatures, the modified time-temperature superposition might be applicable. Furthermore, in the analysis using the TTSP, the accuracy of prediction is influenced by the quality of the master curve and calculation of the shift factor. In this study, enough experimental points were provided to determine the order of the polynomial regression equation for the master curve using the AIC value.

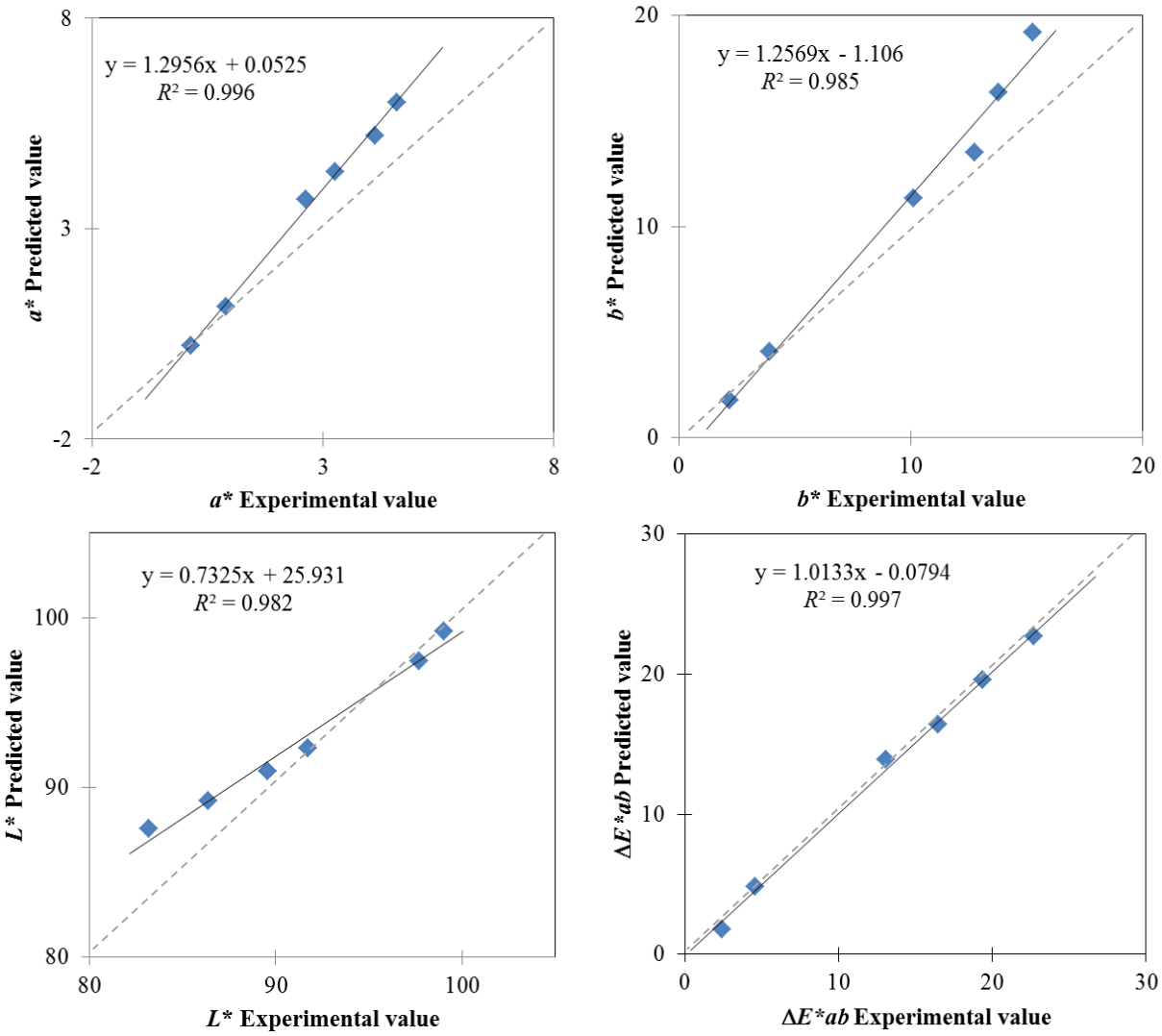

Figure 13. Comparisons between the experimental and predicted values of the color parameters

Each experimental value represents the mean of three determinations and the relative standard deviation (RSD) was less than 0.2%.

4. Conclusion

In this Chapter, the impact of oxygen on the color changes of acetaminophen solution was determined. The results suggested that oxidation reaction products accelerated the color changes of acetaminophen solution. Next, the color changes of acetaminophen solution samples with the same HS volume after accelerated aging at various temperatures were evaluated utilizing the CIELAB color parameters. A kinetic analysis based on the TTSP was successfully adopted to calculate the *E*a value and to predict the color changes after exposure to any temperature and for any length of time. This technique is particularly useful for color changes associated with complex reactions, such as those in acetaminophen solution, because the time-courses of changes of the color parameters in such solutions vary easily depending on the storage condition (e.g., varying temperature, moisture and light exposure period), the formulation, and the packaging of the samples. Hence, this prediction method based on the TTSP is simple and can be easily applied to extrapolating accelerated aging data to any desired temperature and variable mentioned above of the samples.

Summary

A kinetic analysis using the TTSP was successfully applied to calculating the apparent activation energy and to predicting the 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. In addition, this result indicates that the prediction method using the TTSP can also be applied to predicting the color changes of other formulations (e.g., tablets, capsules, and semi-solid dosage forms).

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