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Stability of Drugs in Aqueous Solutions. II.[1,2] Application of Weibull Probability Paper to Predictions of Coloration of Parenteral Solutions

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For the quality control of drug products, it is usually necessary to predict the coloration of parenteral solutions under marketing conditions. This paper presents a new method for the prediction of coloration of parenteral solutions using as examples thiamine hydrochloride injection, reserpine injection, ascorbic acid injection and ATP injection. The time course of decrease of percent transmittance at 430 nm for each parenteral solution was measured spectrophotometrically. When the decreases at elevated temperatures were plotted against time on Weibull probability paper, straight lines were obtained at all temperatures. The temperature dependence of the decrease in each parenteral solution was obtained as a straight regression line on plotting $(1/m)$ ln k *vs.* $1/T$. The predicted decreases of percent transmittance for each parenteral solution at 25°C were in good agreement with those observed at 25°C except in the case of reserpine injection.

Thus, this method proved to be satisfactory for the quality control of the other three injections and may also be applicable to other injections.

Keywords——prediction; coloration; Weibull probability paper; transmittance; parenteral solution; injection

Many colorless parenteral solutions, *e.g.* ascorbic acid,[3] epinephrine,[4] thiamine hydrochloride, morphine,[5,6] and paraaminosalicylate in aqueous solution, are known to become gradually colored under usual storage conditions. However, in most of these solutions the colored constituents are very difficult to isolate and the mechanisms of coloration have not been elucidated yet. Oxidation is considered to be one of the causes of coloration, and the addition of antioxidants or some chelating agents is effective for the stabilization of coloration in some cases.[4,7] Though the colorations sometimes do not reflect the extent of degradation of active ingredients, and the parenterals are therapeutically almost unaffected, this phenomenon is serious from the viewpoint of quality control in the pharmaceutical industry. Therefore the prediction of coloration is important for determining the shelf lives of drug preparations.

For kinetic treatment of the coloration, the gradual change of the absorption spectrum has often been applied. Awada and Miki[8] measured the coloration of aqueous solutions of ascorbic acid by absorption spectroscopy and showed that the reaction appeared to be an autocatalytic one. Otani[9] adopted the color difference (ΔE) for following the coloration of aqueous solutions of ascorbic acid and showed that ΔE increased in proportion to storage time. Hayashi *et al.*[10] investigated the coloration of aqueous solutions of some sulfonamides by measuring their absorbance at 410 nm, and showed that the color reactions of seven sulfonamides follow zero-order kinetics.

Kinetic treatments of coloring rate are few, and little work has been done on predictions of changes under storage conditions by using the results of short-term experiments at elevated temperatures.

The Weibull distribution is a family of distributions and is capable of describing a wide variety of patterns of variations, including distributions other than normal and exponential.

Weibull probability paper is available for normal, exponential, Weibull and other probability distributions.[11]

Using Weibull probability paper, Okusa[12] proposed a new kinetic treatment which is applicable to any type of degradation of active ingredients of pharmaceutical formulations in both homogeneous and heterogeneous states.

The purpose of the present work was to make an attempt to use Weibull probability paper from the practical point of view and to predict the coloration of parenteral solutions under storage conditions from the results of short-term experiments at elevated temperatures. The decrease of percent transmittance from the initial value was adopted as the index of coloration in this approach.

Experimental

Materials——Thiamine hydrochloride injection (J.P., 10 mg/1 ml), reserpine injection (J.P., 1 mg/1 ml), ascorbic acid injection (J.P., 500 mg/2 ml), ATP injection (20 mg/2 ml).

Accelerated Test——The samples were immersed in constant temperature water baths (accuracy ±0.1°C), and removed from the baths at periodic time intervals. Light was excluded.

Color Measurements——The color intensities of the samples were measured spectrophotometrically in terms of the percent transmittance at the wavelength of 430 nm in a 10 mm layer without dilution.

Results

The decreases of percent transmittance of thiamine hydrochloride injection, reserpine injection, ascorbic acid injection and ATP injection after storage at elevated temperatures were plotted against time on Weibull probability paper (Figs. 1, 2, 3 and 4), and they gave straight lines at each temperature. This relationship can be expressed by eq.(1):

$$\ln\ln\left(\frac{100}{100-\alpha}\right) = \ln k + m \ln t \qquad (1)$$

where α is the decrease of percent transmittance, t is time and m and k are parameters. The parameters m and k obtained from the figures are listed in Table I. The m values were almost the same at different temperatures for a given injection. The prediction of the percent transmittance by means of Weibull probability paper seemed possible, but the m values of the injections were not 1.0. The temperature dependence of each injection is shown in Fig. 5 as a regression line for $(1/m) \ln k$ *vs.* $1/T$. The values of $(1/m) \ln k$ at 25°C were estimated by the

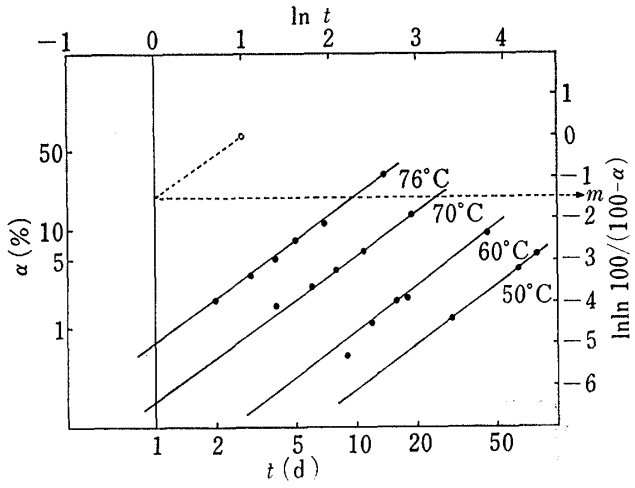

Fig. 1. Regression Lines of the Decrease of Percent Transmittance *vs.* Storage Time for Thiamine Hydrochloride Injection at Various Temperatures

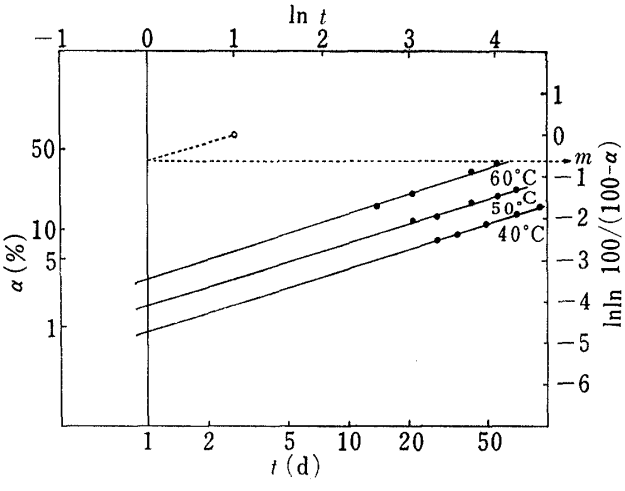

Fig. 2. Regression Lines of the Decrease of Percent Transmittance *vs.* Storage Time for Reserpine Injection at Various Temperatures

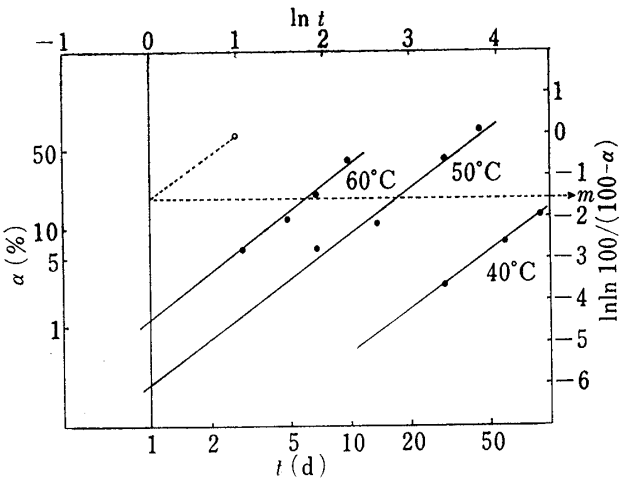

Fig. 3. Regression Lines of the Decrease of Percent Transmittance *vs.* Storage Time for Ascorbic Acid Injection at Various Temperatures

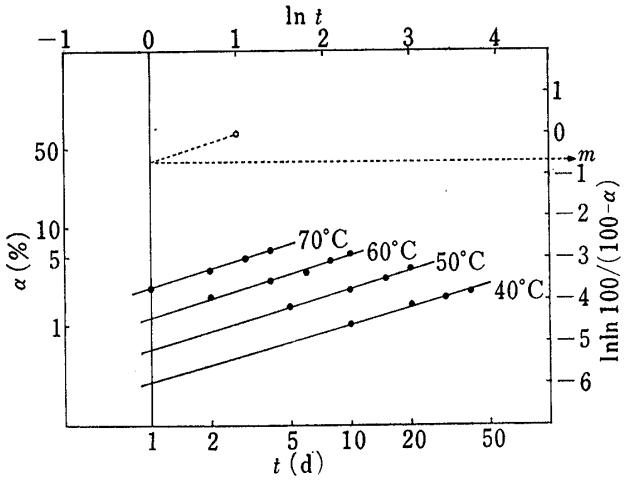

Fig. 4. Regression Lines of the Decrease of Percent Transmittance *vs.* Storage Time for ATP Injection at Various Temperatures

TABLE I. Parameters k and m obtained from Figs. 1, 2, 3 and 4 for the Decrease of Percent Transmittance of Each Injection stored at Various Temperatures

Elevated temp. (°C)	Thiamine hydrochloride injection		Reserpine injection		Ascorbic acid injection		ATP injection	
	ln k	m	ln k	m	ln k	m	ln k	m
40	—	—	−4.76	0.64	−8.18	1.50	−6.02	0.69
50	−9.96	1.50	−4.09	0.64	−5.75	1.50	−5.18	0.69
60	−7.88	1.50	−3.53	0.64	−3.98	1.50	−4.36	0.69
70	−6.25	1.50	—	—	—	—	−3.61	0.69
76	−4.98	1.50	—	—	—	—	—	—

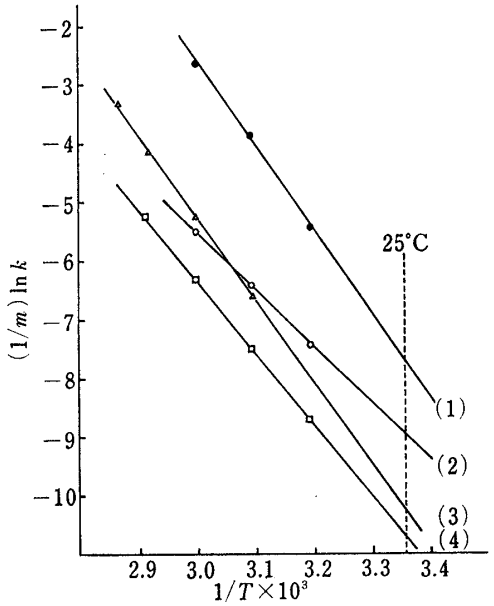

Fig. 5. Temperature Dependence on $(1/m)$ ln k for Each Injection

(1): Ascorbic acid injection, (2): Reserpine injection, (3): Thiamine hydrochloride injection, (4): ATP injection.

extrapolation of these lines, and are shown in Table II. The predicted decreases of percent transmittance for the injections on storage at 25°C were obtained by using the values of $(1/m)$ ln k estimated at 25°C. On the other hand, the observed decreases of percent transmittance for the injection on storage at 25°C and at room temperature (Tokyo, without air conditioning) were measured. Table III shows the predicted decreases of percent transmittance for the parenteral solutions at 25°C and those observed at 25°C and room temperature.

Discussion

The predicted and observed decreases of percent transmittance at 25°C were in good agreement with each other except for reserpine injection (Table III). The temperature dependence of the decreases of percent transmittance of reserpine injection was linear between 40

Table II. Estimated Parameters at 25°C for Each Injection

Injection	Estimated parameters		
	m	$(1/m)\ 1n\ k_{25°C}$	$1n\ k_{25°C}$
Thiamine hydrochloride injection	1.50	−10.27	−15.41
Reserpine injection	0.64	−8.98	−5.74
Ascorbic acid injection	1.50	−7.73	−11.60
ATP injection	0.69	−10.70	−7.38

Table III. Predicted Decreases of Percent Transmittance for Each Parenteral Solution at 25°C and Those observed at 25°C and Room Temperature (Tokyo, without Air Conditioning)

Injection	Period (Years)	Predicted decrease of T(%) at 25°C	Observed decrease of T(%) at 25°C	Observed decrease of T(%) at room temp.
Thiamine hydrochloride injection	3	0.7	1.1	—
	5	1.5	1.8	—
	7	2.6	2.5	—
Reserpine injection	1	13.0	1.3	—
	2	19.5	1.4	—
	3	24.5	2.5	—
Ascorbic acid injection	1	6.2	5.5	2.4
	2	16.6	15.2	8.4
	3	28.4	26.7	14.3
ATP injection	1	3.6	3.4	2.6
	2	5.7	5.6	3.7
	3	7.5	8.3	5.0

and 60°C (Fig. 5), but scarcely any color appeared at 25°C. This suggests that the mechanism of color development above 40°C differs from that at 25°C. However, the prediction of coloration by application of Weibull probability paper is useful for the quality control of some injections. The color was measured as the percent transmittance at 430 nm because some colorless parenteral solutions are known to become yellow or yellowish-brown upon storage. If the colorations are assumed to arise from degradation products, the amounts of which are too small to be detected, and the degradations are of a particular kinetic type, the use of percent transmittance as a factor on Weibull probability paper can be justified theoretically (Appendix). However, the m values of the four parenteral solutions were not equal to 1.0 (Table I) and this means that the mechanisms of coloration of parenteral solutions are not simple. If the coloration of ascorbic acid injection is assumed to be a result of an autocatalytic

Table IV. Predicted Decreases of Percent Transmittance for Ascorbic Acid Injection and ATP Injection stored at 15°C

Injection	Period (Years)	Predicted decrease of T(%) at 15°C
Ascorbic acid injection	1	0.5
	2	1.5
	3	2.8
ATP injection	1	1.3
	2	2.2
	3	2.8

reaction,[8] the n value can be calculated to be 0.33 from eq. (21) (Appendix), somewhat different from the reported value of 0.4—0.8.[8]

Thiamine hydrochloride injection proved to be stable and ascorbic acid injection and ATP injection were not stable as regards coloration. Ascorbic acid injection and ATP injection are required to be preserved in a cool place (under 15°C, J.P.) and when they are preserved at 15°C, they are predicted to show good stability against coloration (Table IV). When the observed ratios at 25°C and at room temperature (Tokyo, without air conditioning) were compared, the effects of storage at 25°C and at room temperature were equally severe. Observed decreases of percent transmittance at room temperature coincided with the predicted values at 21.5°C for ascorbic acid injection and 20.6°C for ATP injection. The activation energies of coloration were calculated[12] to be 29.3 kcal/mol for ascorbic acid injection and 24.8 kcal/mol for ATP injection, and these temperatures are close to the virtual temperatures proposed by Haynes[13] or the kinetic average temperatures of Kinuno[14] or Grimm[15] in Tokyo. Table V shows the predicted values at 21.5°C for ascorbic acid injection and at 20.6°C for ATP injection and observed values at room temperature.

Table V. Predicted Decreases of Percent Transmittance of Ascorbic Acid Injection and ATP Injection at a Fixed Temperature and Observed Data at Room Temperature (Tokyo, without Air Conditioning)

Injection	Period (Years)	Temp. (°C)	Predicted decrease of T(%) at a fixed temp.	Observed decrease of T(%) at room temp.
Ascorbic acid injection	1		3.0	2.4
	2	21.5	8.2	8.4
	3		14.6	14.3
ATP injection	1		2.4	2.0
	2	20.6	3.8	3.7
	3		5.0	5.0

In the quality control of parenterals, color is an extremely important factor. However, it is subjective and dependent on the volume of parenteral solution, light source and other factors. The expression "colorless" or "pale yellow" in the J.P. is thus subjective, and an objective measure such as trasmittance is obviously desirable for quality control in the pharmaceutical industry. However, among manufacturers there are various interpretations of the description in the J.P. since the value of transmittance which corresponds to "colorless" cannot be fixed objectively. Nevertheless, if the transmittance at a fixed wavelength is considered as a measure of coloration of parenteral solutions and the permissible limits of the transmittance are specified, this method of prediction could be useful for predicting the shelf lives of some injections.

Appendix

If the colorations of parenteral solutions are assumed to be due to degradation products, the amounts of which are usually too small to be detected, the concentration of the degradation products is proportional to the absorbance at a fixed wavelength, from Lambert-Beer's law. Because the concentration of the degradation products is proportional to the percent degradation, we have the following equation:

$$a = KA \tag{2}$$

where a is the percent degradation, A is the absorbance at a fixed wavelength and K is the constant. A can be expressed by eq.(3)[16]

$$A = 2 - \log T \tag{3}$$

where T is the percent transmittance at a fixed wavelength. When the initial transmittance of the sample is taken as 100% and α is the difference of percent transmittance from the initial value (*i.e.* the decrease of percent transmittance), eq.(4) is obtained.

$$a = K \log \frac{100}{100-\alpha} \tag{4}$$

Usually the value of a is too small to determine, as mentioned previously.

There are various types of reaction which may be involved in the degradation of drugs, and the reaction order may be taken into consideration as follows:

1) n th order reactions ($n \geqq 2$)

When a drug is decomposed according to an n th order reaction, a differential equation (5) can be written

$$-\mathrm{d}(100-a)/\mathrm{d}t = k(100-a)^n \tag{5}$$

where t is time and k is the n th order rate constant. Integrating eq.(5) between $a=0$ at time $t=0$ and the degradation a at some later time t, eq.(6) is obtained.

$$kt = \frac{1}{(n-1)(100-a)^{n-1}}\left\{1-\frac{(100-a)^{n-1}}{100^{n-1}}\right\} \tag{6}$$

$(100-a)^{n-1}$ can be expanded in the form of eq.(7).

$$(100-a)^{n-1} = 100^{n-1} - \binom{n-1}{1}100^{n-2}a + \binom{n-1}{2}100^{n-3}a^2 - \cdots \tag{7}$$

When $0 < a \ll 100$, eq.(7) can be approximated by eq.(8).

$$(100-a)^{n-1} = 100^{n-1} - \binom{n-1}{1}100^{n-2}a \tag{8}$$

Eq.(6) yields eq.(9) by substituting in eq.(8).

$$kt = \frac{1}{(n-1)(100-a)^{n-1}} \frac{\binom{n-1}{1}100^{n-2}}{100^{n-1}} a \tag{9}$$

As $100-a=100$ approximately, the coefficient of a is almost constant. Therefore

$$kt = k'a \tag{10}$$

where

$$k' = \frac{1}{(n-1)(100-a)^{n-1}} \frac{\binom{n-1}{1}100^{n-2}}{100^{n-1}}$$

Using eq.(4)

$$kt = k'K \log \frac{100}{100-\alpha} \tag{11}$$

Taking logarithms on both sides, eq.(12) is obtained:

$$\mathrm{inln} \frac{100}{100-\alpha} = \ln K' + \ln t \tag{12}$$

where $K' = 2.303\, k/k'K$.

2) 1 st order reactions

The rate equation of a 1st order reaction can be written as follows:[17]

$$kt = \ln 100 - \ln(100-a) = -\ln\left(1-\frac{a}{100}\right) \tag{13}$$

where k is the 1st order rate constant. When $a/100 \ll 1$, eq.(13) can be approximated as eq. (14):

$$kt = a/100 = k'a \tag{14}$$

where $k'=1/100$.
From eq.(14), eq.(12) is obtained in the same way as eq.(10).
3) 0 order reactions

When n is equal to zero in eq.(6), eq.(15) is obtained.

$$kt = a \tag{15}$$

From eq.(15), eq(12) is obtained, in the same way as eq.(10), where $k'=1$.
4) Autocatalytic reactions[8]

A differential equation can be written as follows to express a general autocatalytic reaction:[8]

$$da/dt = ka^n(100-a) \tag{16}$$

where k is the rate constant of the autocatalytic reaction and n is the order of the reaction. When $0<a \ll 100$, eq.(16) can be expressed as eq.(17):

$$da/dt = k'a^n \tag{17}$$

where $k'=(100-a)k$.
i) $n<1$

Integrating eq.(17) between $a=a_0$ at time $t=0$ and the degradation a at some later time t, eq.(18) is obtained.

$$a^{1-n} = (1-n)k't + a_0^{1-n} \tag{18}$$

When $a_0 \fallingdotseq 0$, eq.(18) can be approximated by eq.(19):

$$a^{1-n} = (1-n)k't = k''t \tag{19}$$

where $k''=(1-n)k'$.
Using eq.(4)

$$\left(K \log \frac{100}{100-\alpha}\right)^{1-n} = k''t \tag{20}$$

Taking logarithms on both sides of eq.(20),

$$\ln\ln \frac{100}{100-\alpha} = \ln K' + \frac{1}{1-n} \ln t \tag{21}$$

where $K'=2.303\ k''^{1/1-n}/K$.
ii) $n \geqq 1$

When $n \geqq 1$, the derivation of eq.(17) to eq.(1) is impossible and the application of Weibull probability paper may be difficult.

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