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International Journal of Pharmaceutics 173 (1998) 43–49

international journal of pharmaceutics

Improved stability of 25% vitamin C parenteral formulation

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Received 14 March 1998; received in revised form 22 April 1998; accepted 19 May 1998

Abstract

The rates of chemical degradation and coloration of 25% vitamin C aqueous solutions were studied in the temperature range of 60–90°C and pH range of 0.4–12.7. Curves of pH versus degradation or coloration rate at various temperatures (20–90°C) under anaerobic conditions indicated that the pH of optimal stability of a 25% vitamin C aqueous solution is 9–11, when determined by the degradation rate, compared to 8, when determined by the coloration rate. Adjustment of solutions with Na_2CO_3 resulted in an accelerated rate of degradation and coloration in alkaline pH compared to adjustment by NaOH; pH of optimal stability when adjusted by Na_2CO_3 was 6.5–7, as determined by the coloration rate. Based on the above studies, a parenteral formulation of 25% vitamin C with improved stability was developed. The shelf-life of the improved parenteral formulation is limited by its coloration, and was predicted to be 5 years.

Keywords: Chemical degradation; Coloration; Vitamin C; Parenteral formulation

1. Introduction

Low concentration ($\leq 10\%$) vitamin C aqueous solutions are most stable at pH 6–8 (Finholt et al., 1963; Huttenrauch, 1968; Connors et al., 1979; Motome et al., 1980). To develop a stable high concentration (25%) vitamin C parenteral formulation, it is important to re-determine the pH rate profiles. Since in most parenteral formulations of vitamin C, the pH is adjusted with $NaHCO_3$ or Na_2CO_3, it is also important to know the effect of $NaHCO_3$ or Na_2CO_3 on stability. Because the coloration rate appeared to be a more sensitive assay for degradation of vitamin C, it was used for shelf-life estimation (Yin et al., 1980).

The data indicate that the stability of vitamin C aqueous solutions was compromised by the presence of CO_3^{2-}. Hence a more stable parenteral formulation of a 25% vitamin C can be developed by the following strategies: (a) adjusting the pH with NaOH rather than Na_2CO_3; (b) boiling the water to remove dissolved CO_2; (c) replacing the

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0378-5173/98/$ - see front matter
PII S0378-5173(98)00175-6

Table 1
The degradation of 25% vitamin C aqueous solution at pH 7.28 determined by the single time point method

Temperature (°C)	$1/T$ (1/K)	Time (h)	Concentration (%)	log k
		0	25.04 ± 0.03[a]	
75.0	2.872×10^{-3}	31.00	24.29 ± 0.04	−1.620
76.5	2.860×10^{-3}	26.50	24.30 ± 0.05	−1.558
78.0	2.848×10^{-3}	22.33	24.35 ± 0.05	−1.517
79.5	2.836×10^{-3}	19.00	24.27 ± 0.04	−1.398
81.0	2.824×10^{-3}	17.00	24.27 ± 0.06	−1.347
82.5	2.812×10^{-3}	14.67	24.27 ± 0.05	−1.286
84.0	2.800×10^{-3}	13.50	24.17 ± 0.06	−1.195
85.5	2.788×10^{-3}	10.00	24.30 ± 0.04	−1.135
87.0	2.777×10^{-3}	8.50	24.27 ± 0.06	−1.046
88.5	2.765×10^{-3}	7.75	24.28 ± 0.06	−1.012

[a] Mean ± S.D. of three measurements.

air in the ampoules by nitrogen instead of by CO_2. The shelf-life of the improved parenteral formulation reported here was prolonged to 5 years from the original 2.5 years (Yin et al., 1980).

2. Experimental

2.1. Materials

Vitamin C for parenteral use was received from Beijing Pharmaceutical Factory. Iodometric titration (Anon., 1995) of this product showed that it contained 99.91% vitamin C. All reagents used were of analytical grade.

2.2. Instruments

A UV spectrophotometer (UV-240, Shimadzu, Japan) was used for absorbance and transparency measurements.

2.3. Preparation of 25% vitamin C aqueous solutions

A 250-g quantity of vitamin C was dissolved in water. The pH was adjusted to 0.4–12.7 with NaOH or HCl. Then the solution was diluted with water to a total volume of 1000 ml. The solution was filtered using fused glass funnel and filled into 2-ml ampoules. The air in the ampoules was replaced by nitrogen and the ampoules were sealed.

The preparation of 25% vitamin C aqueous solutions adjusted with Na_2CO_3 is the same as that explained above except the pH was adjusted to 5–8 with Na_2CO_3.

2.4. Preparation of the improved 25% vitamin C parenteral formulation

A 250-g quantity of vitamin C, 0.3 g of Na_2EDTA, 1 g of sodium pyrosulfite, and 1 g of L-cysteine hydrochloride were dissolved in water that was previously boiled to remove CO_2 and cooled to ambient temperature. The solution was adjusted with NaOH to pH 8 and then was diluted with water to a total volume of 1000 ml.

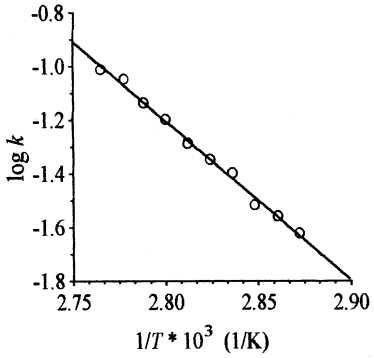

Fig. 1. Arrhenius plot of the degradation rate constant of a 25% vitamin C aqueous solution at pH 7.28 and a temperature range of 75–88.5°C.

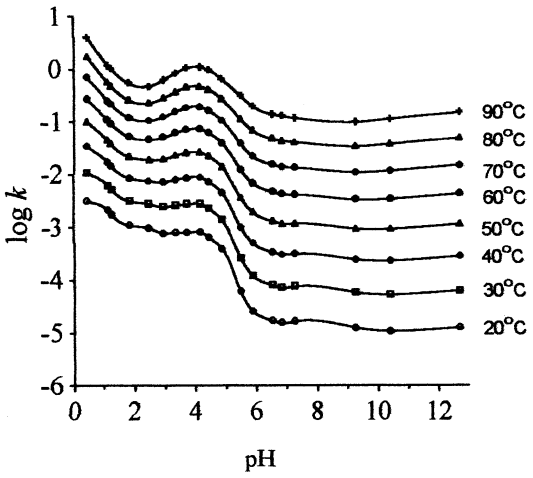

Fig. 2. The pH-degradation rate profiles of 25% vitamin C solutions.

Activated carbon was added and the solution was stirred and filtered. The parenteral formulation was filled into 2-ml ampoules and the air in the ampoules was replaced by nitrogen. The ampoules were sealed and sterilized at 110°C for 15 min.

2.5. Assays

The residual vitamin C concentration was determined iodometrically (Anon., 1995). The transparency of the solution at 430 nm was measured using spectrophotometry (Yin et al., 1980) and a 1-cm quartz cell.

2.6. Stability studies and analyses

The stability of the solutions at different pH values was determined using three groups of incubation temperatures: (a) 60–82.5°C at 2.5°C intervals; (b) 67.5–90°C at 2.5°C intervals; and (c) 75–88.5°C at 1.5°C intervals. Samples were placed in an isothermal water bath that could maintain the temperature to within 0.1°C. Five ampoules were taken out of the bath after each incubation, cooled, and the contents of the ampoules were mixed for three measurements of the residual vitamin C concentration. The predefined time to end each incubation was when the concentration decreased to about 95–97%.

Single time point method (Pang and Lu, 1982a) was used to determine degradation rates. In this method, only one sample under each testing temperature is analyzed at a single time point. The reduced accuracy, resulting from the single time point measurement, was compensated for by increasing the number of test temperatures. The accuracy obtained using this method is similar to the classical multi-time point isothermal method, with the advantage of using only 1/4–1/3 the number of measurements.

The rate constant k_i at temperature i was obtained from zero-order kinetics, as shown by us and previous studies (Yin et al., 1982):

$$k_i = (c_0 - c_i)/t_i$$

where c_0 is the initial concentration of vitamin C, t_i is the incubation time at temperature i, and c_i is the residual concentration after incubation.

The Arrhenius plot of log k against $1/T$ was made with slope equal to E/R, where E is the observed activation energy and T is temperature in Kelvin. The degradation kinetics, and hence shelf-life, at any given temperature could then be estimated from the linear fit of the Arrhenius plot.

2.7. Stability studies for coloration rate determination

Three groups of incubation temperatures were used: (a) 60–82.5°C at 2.5°C intervals; (b) 67.5–90°C at 2.5°C intervals; and (c) 75–88.5°C at 1.5°C intervals. A total of 15 ampoules were taken out of the bath after each incubation, cooled, and the contents each group of five ampoules was mixed for one measurement of absorbance. Since only a 1% decrease in concentration made the coloration obvious enough for assay, the predefined time to end each incubation was set at the time to reach 99% of initial concentration.

Since the coloration, evaluated with either the decrease in transparency or the increase in absorbance, could not be expressed by simple order kinetics, the rate of coloration was determined by using the initial average rate method (Pang and Lu, 1982b). This method for studying the stability of pharmaceuticals is established by using the

Table 2
The coloration of 25% vitamin C aqueous solution with pH 3.70 determined by the initial average rate method

Temperature (°C)	$1/T$ (1/K)	Time (h)	Absorbance	log v
		0	0.00743 ± 0.00005[a]	
60.0	3.002×10^{-3}	3.68	0.05143 ± 0.0005	−1.923
62.5	2.979×10^{-3}	2.87	0.04816 ± 0.0004	−1.847
65.0	2.957×10^{-3}	2.25	0.04655 ± 0.0004	−1.760
67.5	2.936×10^{-3}	1.75	0.04477 ± 0.0004	−1.671
70.0	2.914×10^{-3}	1.40	0.04735 ± 0.0003	−1.545
72.5	2.893×10^{-3}	1.08	0.04300 ± 0.0003	−1.484
75.0	2.872×10^{-3}	0.88	0.04635 ± 0.0004	−1.356
77.5	2.852×10^{-3}	0.67	0.04418 ± 0.0004	−1.259
80.0	2.832×10^{-3}	0.55	0.04497 ± 0.0004	−1.166
82.5	2.812×10^{-3}	0.43	0.04776 ± 0.0004	−1.031

[a] Mean ± S.D. of three measurements.

logarithm of the average rate at the initial stage of reaction (log v). At each testing temperature, only one analysis of the sample in initial stage was made. The initial average rate v_i at temperature i was obtained from:

$$v_i = (A_0 - A_i)/t_i$$

where A is the absorbance of the solutions. An Arrhenius plot of log v against $1/T$ was made for extrapolation of shelf-life.

2.8. Expiration date determination

The rate of coloration was determined by the single time point method. Samples of 25% vitamin C parenteral formulation were incubated isothermally at a temperature range of 65–95°C at 5°C intervals for a predefined period (when the transparency at 430 nm decreased to about 80%). A total of five ampoules per time point were taken out of the bath after each incubation, cooled, and their contents were mixed for subsequent transparency measurements.

The rate of the coloration determined by the decrease in transparency (*Trans*) at 430 nm, can be expressed as $Trans = Trans_0 - kt^2$ when $Trans \geq 70\%$. The $t_{0.8,i}$, the time for the *Trans* to decrease to 80% at temperature i, was obtained from:

$$t_{0.8,i} = t_i[(Trans_0 - 0.8)/(Trans_0 - Trans_i)]^{1/2}$$

where $Trans_0$ is the initial transparency, t_i is the incubation time at temperature i and $Trans_i$ is the transparency after incubation.

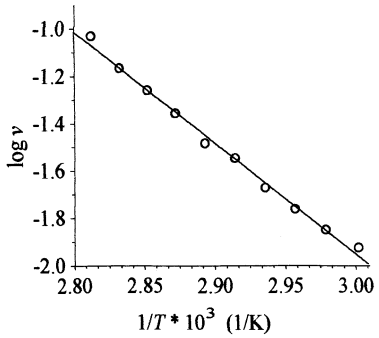

Fig. 3. Arrhenius plot of the coloration rate of a 25% vitamin C aqueous solution at pH 3.70 and a temperature range of 60–82.5°C.

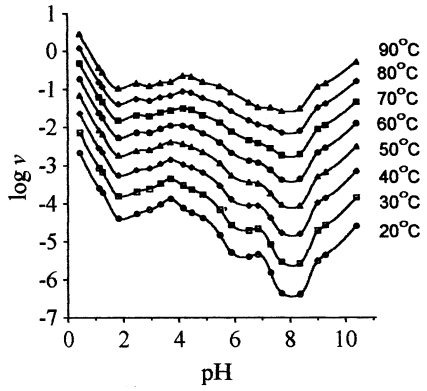

Fig. 4. The pH-coloration rate profiles of 25% vitamin C solutions.

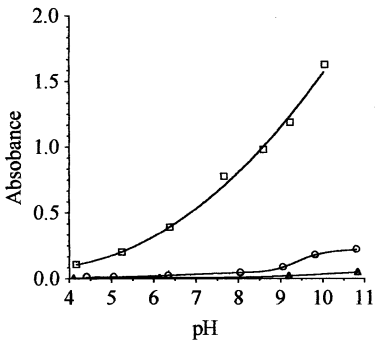

Fig. 5. The pH dependence of the absorbance of 25% vitamin C solution. □–□, vitamin C solution incubated for 0.5 h at 95°C; ○–○, fresh vitamin C solution; △–△, recrystallized vitamin C solution.

3. Results and discussion

3.1. pH-degradation rate profiles of 25% vitamin C aqueous solutions

The rate constants of chemical degradation of 25% vitamin C aqueous solutions were determined in the pH range of 0.4–12.7 using the single time point method. As an example, the data at pH 7.28 are listed in Table 1. By plotting log k against $1/T$, a straight line with correlation coefficient $r = -0.9974$ was obtained, as shown in Fig. 1.

The rate constants of degradation of 19 such solutions in the pH range 0.43–12.7 were determined at a temperature range of 20–90°C. The correlation coefficient of a linear fit of log k versus $1/T$ was in the range of -0.9773 to -0.9997 with a mean value of -0.9976. The resulting pH-degradation rate profiles are shown in Fig. 2.

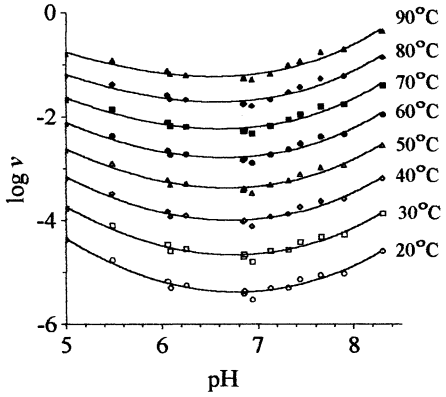

Fig. 6. The pH-coloration rate profile of 25% vitamin C aqueous solutions adjusted with Na_2CO_3.

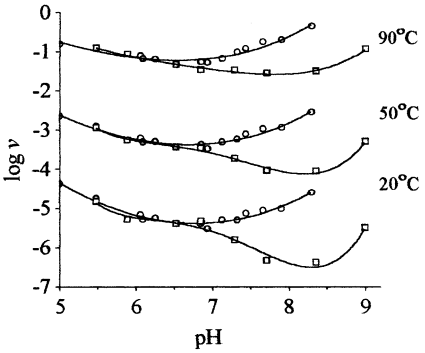

Fig. 7. The effect of Na_2CO_3 on the pH-coloration rates of 25% vitamin C aqueous solutions. ○–○, adjusted with Na_2CO_3; □–□, adjusted with NaOH.

The degradation rate of 25% vitamin C aqueous solutions at alkaline pH observed in Fig. 2 was slower than that reported by others for low concentration ($\leq 10\%$) solutions (Finholt et al., 1963; Huttenrauch, 1968; Connors et al., 1979; Motome et al., 1980). We confirmed this difference by measuring the rate constant $k_{25°C}$ of a 2.5% vitamin C aqueous solution at pH 12.8. The rate constant, 8.831×10^{-2} g/(100 ml·h), is about 3200 times higher than that of the 25% solution under the same conditions.

3.2. pH-coloration rate profiles of 25% vitamin C aqueous solution

The rates of coloration of 25% vitamin C

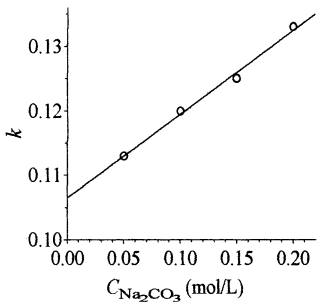

Fig. 8. The effect of Na_2CO_3 on the degradation of 25% vitamin C aqueous solutions at pH 10.24 and 90°C.

Table 3
The coloration of 25% vitamin C parenteral formulation determined by the single time point method

Temperature (°C)	Time (h)	Transparency (%)	$t_{0.8}$ (h)
	0	95.0 ± 0.3[a]	
65	120	83.1 ± 0.5	134.73
70	70	83.0 ± 0.5	78.26
75	40	78.4 ± 0.4	38.02
80	21.32	83.2 ± 0.4	24.03
85	12	81.4 ± 0.5	12.60
90	7	81.1 ± 0.5	7.27
95	4	81.0 ± 0.5	4.14

[a] Mean $\pm$ S.D. of three measurements.

aqueous solutions were determined in the pH range of 0.4–10.4 using the initial average rate method. As an example, data for pH 3.70 solution are listed in Table 2. By plotting log v against $1/T$, a straight line with a correlation coefficient $r = -0.9972$ was obtained, as shown in Fig. 3.

The initial average rates of coloration of 21 such solutions in the pH range of 0.43–10.4 were determined. The correlation coefficient of a linear fit of log v versus $1/T$ was in the range of -0.9790 to -0.9998 with a mean value of -0.9956. The resulting pH-coloration rate profiles are shown in Fig. 4.

A comparison of the data in Figs. 2 and 4 indicates that at alkaline pH the degradation rate is similar to that at neutral pH, while the coloration rate increases. This might suggest that the extinction coefficient of the degradation products

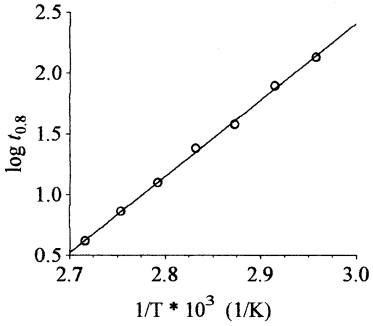

Fig. 9. The relationship between the logarithm of $t_{0.8}$ of 25% vitamin C parenteral formulation and the reciprocal of temperature.

of vitamin C increases dramatically with increasing pH. This was confirmed by comparing the absorbance of aged, fresh and recrystallized 25% vitamin C solution at various pH values adjusted with saturated NaOH solution (Fig. 5). The yellow color formed by aging vitamin C is probably from a dimer of pyruvaldehyde, which is a degradation product of vitamin C (He and Yin, 1986).

3.3. Accelerated coloration rate of vitamin C aqueous solutions by CO_3^{2-}

The initial average rates of coloration of 14 vitamin C aqueous solutions in the pH range 5–8.3 were determined. The correlation coefficient of a linear fit of log v versus $1/T$ was in the range of -0.9988 to -0.9999 with a mean value of -0.9995. The resulting pH-coloration rate profiles are shown in Fig. 6. A comparison of pH rate profiles of samples pH-adjusted by NaOH or Na_2CO_3 (at temperature 20, 50 and 90°C and pH range of 5–9) is shown in Fig. 7. Adjustment of pH with Na_2CO_3 significantly catalyzed the rate of coloration above pH 7.

3.4. Accelerated rate of degradation of vitamin C aqueous solution by Na_2CO_3

The degradation catalysis of vitamin C by Na_2CO_3 was studied at pH 10.24. The rate constants were determined by the classical isothermal method at 90°C. As shown in Fig. 8, the rate of degradation was directly proportional to the concentration of Na_2CO_3.

3.5. Stability improvements of 25% vitamin C parenteral formulation

Based on the above data, the stability of a 25% vitamin C parenteral formulation (Yin et al., 1980) could be improved by minimizing contact with CO_2, for example by: (a) using NaOH to adjust pH, instead of $NaHCO_3$ or Na_2CO_3; (b) boiling the water to remove dissolved CO_2; and (c) replacing the air in the ampoules by nitrogen instead of by CO_2. Under such conditions, the pH of optimal stability is 8 (instead of 6.4).

3.6. Shelf-life determination of the parenteral formulation by coloration rate

During storage of vitamin C parenteral formulations, the color of the solution changed significantly more than the measured concentration. Since the concentration of vitamin C was still $>97\%$ when transparency decreased to 80%, the time to decrease transparency to 80% was set as the expiration limit. Table 3 summarizes the data used for estimation of expiration dating.

By plotting log $t_{0.8}$ against $1/T$, a straight line with correlation coefficient $r=1-0.9993$ was obtained as shown in Fig. 9. A shelf-life ($t_{0.8,25°C}$) of 5.0 years was extrapolated to room temperature from these data. This calculated shelf-life was confirmed to be 5.3 years by a long-term storage testing.

Acknowledgements

This work was financed by the National Natural Science Foundation of China.

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