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Shelf Life Determination of Vitamin C in Aqueous Solutions: Effect of Vitamin C Concentration and Addition of Antioxidants

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Abstract:- Ascorbic acid or Vitamin C (Vit C) is sensitive to oxidation process therefore, its formulation should take its sensibility into consideration for assuring maximum stability.The objective of this work was to stabilize (Vit C) in aqueous solutions by modifying the concentration of Vit C and by the addition of different antioxidant: metabisulfite Na (MBS), hydroquinone (HQ) and vitamin E (Vit E). Kinetic of Vit C degradation was estimated by determining reaction order, rate constants and by calculating shelf lives (t_{90}). The results showed that shelf life of Vit C was enhanced when a higher concentration of Vit C was used (15 days vs. ≈10 days at low concentration). Use of MBS and HQ (both at concentrations: 0.25 and 0.5%) increased Vit C shelf life (≈21, 29 days for MBS and 18, 20 days for HQ). However, lower shelf lives were reported when Vit E (at two concentrations 0.25 and 0.5%) was used.

Keywords:- *Vitamin, Shelf Life, Stability, Antioxidant.*

I. INTRODUCTION

The bioactive form of Vit C, also known as L-ascorbic acid, has positive effects on all parts of the body [1] including the skin[2]. The role of Vit C in collagen formation is well known[3]. Vit C is necessary for the hydroxylation of proline which is necessary for collagen synthesis and it activates fibroblast responsible of new collagen formation[4,5,6,7]. Vit C is a potent depigmenting agent[8,9] and it plays an important role in lowering melanin formation[10]. Vit C is capable to neutralize free radicals[11]. It is an excellent antioxidant because it can donate its electrons and thus prevents other compounds from oxidation[12,13,14,15,16,17].

However, being a water-soluble molecule, Vit C loses its properties by its high poor stability in solution that can result in important loses. It also degrades rapidly in the presence of oxygen, light and free- radical mediated oxidative processes. These processes are strongly catalyzed by transition metal ions, especially iron and copper, leading to rapid destruction of Vit C[18]. Oxidation is also accelerated at neutral pH and above[19].

The ene-diols system of Vit C donates electrons and thus transforms into dehydroascorbic acid in aqueous medium[20]. It is reversibly oxidized into dehydroascorbic acid (Fig. 1) upon exposure to heat, temperature and alkaline medium then dehydroascorbic acid irreversibly hydrolyzes into 2,3-diketoguloronic acid[21].

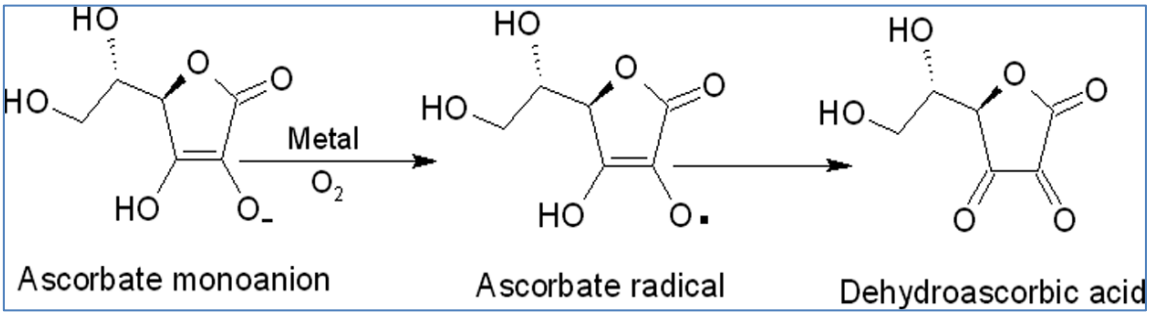

Fig. 1: Oxidation of Vit C in solution

After neutrilizing, Vit C or ascorbic acid converts into dehydroascorbic acid, which can regain antioxidant activity after accepting hydrogen atoms. Further oxidation of dehydroascorbic acid into diketogulonic acid results in complete loss of bioactivity[22]. This process of oxidation is accompanied by a yellowish color of the formulation. Among the several strategies developed to limit Vit C oxidation: controlling oxygen content during formulation and storage, reduction of both pH and water content[23]. The addition of anti-chelating agents and antioxidants like cysteine, glutathione, metabisulfite Na, hydroquinone[24], alpha-tocopherol[24,25,26] prevents the degradation of the Vit C. Antioxidants act by delaying or preventing the oxidation of other chemicals by different mechanisms[27].

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Therefore, antioxidants are sometimes classified as primary by scavenging free radicals and as secondary by chelation of transition metals[28,29].

Acidity and concentration of topical Vit C control its absorption. Concentration of ≤20% is associated with high absorption and tissue saturation[30]. In order to combat the problems related to reactive oxygen species, topical ascorbic acid formulations have been developed in the concentration range of 1 to 20%[31,32,33].

In spite of grand number of researches about enhancing Vit C stability, a limited quantitative data is present in the literature about this stability. Therefore, the aim of this research is to evaluate Vit C stability mathematically by accelerated stability test. The effect of Vit C concentration and the addition of different antioxidants will be studied. The accelerating stability test would permit determination of rate constants of Vit C degradation and calculation of the shelf lives of Vit C in different pure aqueous solutions.

II. MATERIAL AND METHODS

A. Material

Vit C was purchased from Loba chemie, India. Metabisulfite Na, hydroquinone and vitamin E were supplied from Sigma. 2,6-dichorophenol: Indophenol Sodium was obtained from Tmmedia, India. HCl and alcohol were of analytical grade.

B. Methods

➢ *Preparation of Vit C solutions*

Vit C (10 0r 20%) and antioxidants (MBS or HQ) were dissolved in water. When vit E was used, it was first dissolved in alcohol and then added to Vit C aqueous solution. The compositions of formulated solutions under different variables were presented in TABLE 1.

Table 1: Composition of aqueous Vit C solutions

	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
Vit C (g)	5	10	10	10	10	10	10	10
Metabisulfite Na (g)	-	-	0.25	0.5	-	-	-	-
Hydroquinone (g)	-	-	-	-	0.25	0.5	-	-
Vitamin E (g)	-	-	-	-	-	-	0.25	0.5
Alcohol (ml)	10	10	10	10	10	10	10	10
Water up to	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100ml	100ml

➢ *Vit C Solutions Characterization*

- *Visual inspection*

Vit C solutions were inspected for color[34]. A number from 0 to 5 was used to characterize solutions colors from colorless (0) to dark yellow (5).

- *pH Determination*

The pH of solutions were determined by pH meter (Inolab, pH 7110, Germany) in triplicate. The pH of solutions should be suitable for Vit C stability and for better skin penetration.

- *Spreadability Test*

Spreadability is a measure of lubricity[35]. It depends on many factors such as viscosity of the formulation and physical properties of the excipients used[36]. Higher spreadability values increase surface area available for drug [37]. Hence, therapeutic efficacy may be enhanced[38].

This test was carried out according to previous study[39]with little modifications. Briefly, 0.5 g of each solution was placed between two horizontal plates (20 cm × 20 cm) and the weight of the upper plate was standardized at 60g. The mean spreading diameter "d" (in vertical and horizontal axes) was determined after one minute and the areas of circles "S" ($S=d^2\pi/4$) were recorded as spreadability values. Spreadability studies were performed on solutions at room temperature and at 37C in the 14^{th} and 21^{th} days after preparation for monitoring possible changes in viscosities.

- *Vit C Determination*

The active form of Vit C was determined using 2,6-dichlorophenolindophenol (DCPIP) method according to AOAC official method (1984)[40] with little modification in which the metaphosphoric acid was substituted by hydrochloride acid HCl (0.2%). Diluted Vit C solutions from different formulation were titrated by standardized solution DCPIP.

- *Vit C Stability Analysis Using Accelerated Stability test*

Eight closed tubes containing 5 or 10% ascorbic acid were placed in two ovens (A&E Lab, UK)) at two temperatures: 37˚ and 45˚ C. Vit C degradation rate increases with the temperature[41]. These temperatures were chosen based on previous studies of Vit C stability[42,43]. Preparations were stored in the dark and samples were taken periodically at 0 (100 % ascorbic acid), 7, 14, 21 and 28 days. An aqueous solution of Vit C (5 and 10%) without additives was used as a control for determining its stability.

✓ *Kinetic modeling*

In literature, zero and first order models are often used to describe linear and exponential relationships between time and concentration[44].

✓ *Shelf life determination*

The shelf life (t_{90}) is the time after which ascorbic acid concentration decreases by 10 % in the solution[45].

ISSN No:-2456-2165

From the data of the kinetics of degradation and by applying Arrehenius equation[46], various parameters could be calculated: the degradation reaction rate constants of ascorbic acid (k) at two temperature: 37 and 45 °C (K_{37} and K_{45}). Using Arrhenius equation ($K= A\ e^{-Ea/RT}$) and the rate constants: K_{37} and K_{45}, the activation energies (Ea) were then calculated[47]. By substituting Ea and either K_{37} or K_{45} in Arrhenius equation again, rate constant at room temperature (K_{20}) was calculated and the shelf lives of Vit C (t_{90}) were determined.

III. RESULTS AND DISCUSSION

Vit C solutions were yellowish and translucent. As the time passes the color becomes more yellow du to Vit C decomposition. TABLE 2. shows the numbers coding the color as the time passes.

The pH values ranged between 2.61 and 3.35. These values are acidic and probably due to high concentration of Vit C that makes the pH decrease[48]. Under these conditions, Vit C is at its unionized form (pKa=4.2)[20]. Acidic pH < 3.5 (< pKa: 4.2) is required for stability and optimal percutaneous absorption[30,49].

Table 2: Color and pH values of different Vit C solutions

		F1		***F2***		***F3***		***F4***		***F5***		***F6***		***F7***		***F8***	
		Color	**pH**	**Color**	**pH**	**Color**	**pH**	**Color**	**pH**	**Color**	**pH**	**Color**	**pH**	**Color**	**pH**	**Color**	**pH**
0	**25˚C**	0	2.89	0	2.87	0	2.94	0	2.83	0	2.82	0	2.82	0	2.66		2.87
21th day	**37˚C**	2	3.1	2	2.93	1	3.1	1	2.97	2	2.92	2	2.95	3	2.9	3	2.98
	45˚C	2	2.99	2	3.11	2	2.98	2	2.94	3	2.88	3	2.97	3	2.78	3	2.87
28th day	**37˚C**	3	2.98	3	2.87	3	2.56	3	2.76	3	2.78	3	2.85	4	2.95	4	2.95
	45˚C	4	3.35	4	2.81	3	2.79	3	2.71	4	2.61	4	2.83	5	2.83	5	2.89

A. Spreadability Test

TABLE 3. shows spread ability values of Vit C solutions at room temperature and at 37C. No important differences in spreadability values were noticed. Therefore, system viscosity didn`t interfere in explanation of results.

Table 3: Spreadability values (mm^2) of different Vit C solutions

Day	**Temperature**	***F1***	***F2***	***F3***	***F4***	***F5***	***F6***	***F7***	***F8***
7th day	**25˚C**	70.84	70.10	55.39	52.78	41.26	60.10	56.72	56.72
	37˚C	65.72	57.39	44.16	52.14	44.75	65.01	53.43	47.76
14th day	**25˚C**	63.59	73.86	56.72	49.61	53.43	90.72	63.59	69.36
	37˚C	70.85	74.62	62.18	71.59	72.35	65.72	45.94	64.29

B. Determination of Vit C shelf life under different conditions

Table 4. shows R^2 values of degradation rates and shelf lives of Vit C in different formulations at both temperatures: 37˚ and 45˚ C.

Table 4: The determination coefficients: R^2_1 (first order)and R^2o (zero order) of Vit C degradation kinetics at 37˚ and 45˚ C

		F1	***F2***	***F3***	***F4***	***F5***	***F6***	***F7***	***F8***
37˚ C	**R^2_1**	0.98	0.98	0.999	0.994	0.98	0.988	0.97	0.96
	R^2o	0.96	0.96	0.998	0.992	0.96	0.987	0.94	0.93
45˚ C	**R^2_1**	0.93	0.997	0.982	0.98	0.996	0.999	0.97	0.94
	R^2o	0.9	0.992	0.98	0.96	0.994	0.998	0.94	0.9

Fig. 2. illustrates the profiles of Vit C degradation at 37C according to first order. TABLE 4. shows that the degradation of Vit C follows the equation for first-order which agrees with other studies[50,51]. The calculated shelf lives of Vit C varied from 8 to 29 days as shown in TABLE 5.

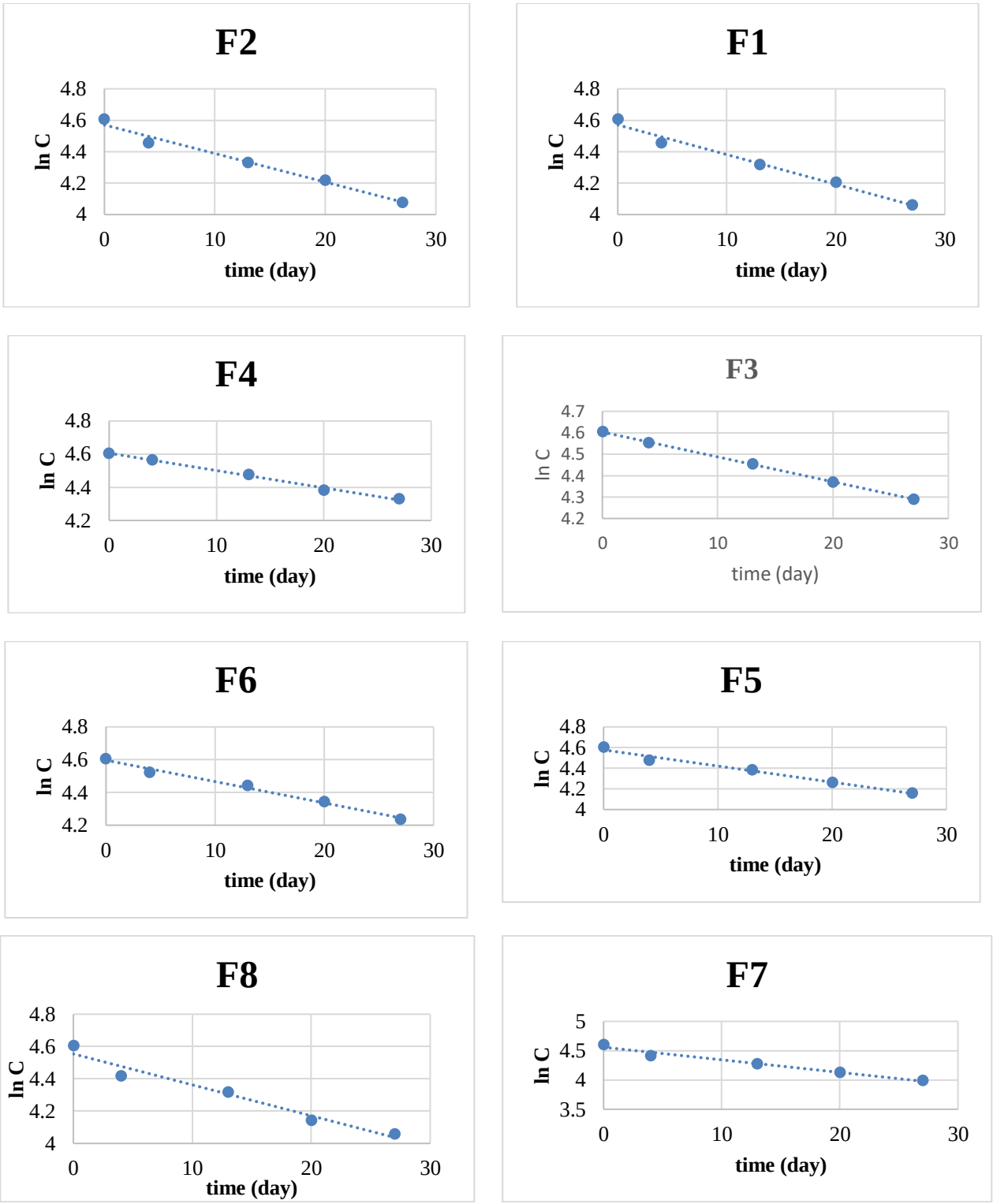

Fig. 2: Profiles of Vit C degradation (first order) at 37 C

Table 5: The shelf lives (t_{90}) of Vit C in different formulations

	F1	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*	*F8*
t_{90} (day)	10.28	14.93	20.5	29	17.72	19.85	8.4	10.21

According to data from TABLE 5. and the degradation kinetics of Vit C, a higher concentration of Vit C (F2) has a lower degradation rate constant in comparison with F1 (14.93 days vs 10.28 days). These findings agree with other previous studies[52,53]. This might be explained by the depletion of radicals by high concentration of vitamin. However, some studies have shown that high concentration of Vit C is auto-oxidized to produce dehydroascorbic acid anions[54]. Furthermore, the autoxidation of ascorbic acid occurs more importantly in alkaline medium which was not the case here (TABLE 2. shows that pH values are acidic throughout the test period and at all tested temperatures). By

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calculating the amount of degraded Vit C in both formulations (F1 and F2) at the end of tested period, it was 2.1g in F1 against 4.1g in F2 (at 37 C) and 3.2g in F1 against 6.5g in F2 (at 45C). These results agreed with other research works[55] which may explain the little intensity of color in F2 in comparison with F1 (figures no shown).

SMB is used in acidic preparations and it releases sulphur dioxide gas which prevents oxidation[56]. The antioxidant effect of SMB is usually enforced by different synergetic agents (eg. EDTA, salicyclic acid, citric acid, tartaric acid and malic acid[57]. SMB is widely used as anioxidant in Vit C formulations[58,59]. The t_{90} of Vit C in F3 containing MBS was higher than that of F2. This could be attributed to the capacity of metabisulfite Na to scavenge free radicals more rapidly than Vit C did. In addition, MBS could prevent the oxidation of many compounds with phenolic groups[60] like Vit C.Hence, it is evident from the TABLE 5. that the higher percentage of sodium metabisulphite is more effective for the stability of ascorbic acid[61, 62].

Another antioxidant can be used in Vit C formulations is hydroquinone. Similarly to hydroquinone derivative (eg. tert-butyl hydroquinone), hydroquinone might protect Vit C in solution by conversions dehydroascorbic acid into ascorbic acid thereby ascorbic acid is protected from degradation[63]. Satoh et al. suggested that semiquinones generated by hydroquinone were scavenged by Vit C[64]. However, if an organic solvent is present (eg. alcohol), the quenching effect of alcohol against semiquinones must be consided[51]. Thus, a determined amount of Vit C stayed under the effective reduced form more than that of F2 and the shelf life thereby increased in formulations F5 and F6 in comparison with F2 non-containing hydroquinone.

In the literature, for preserving the “antioxidative” property of Vit C, it is mostly used in combination with another antioxidant like vitamin E (alpha-tocopherol)[65, 66]. When both vitamins C and E are combined, Vit C quenches reactive species, thereby preventing the oxidation of vitamin E[67]. Analysis of the reaction details, Alpha-tocopherol first functions as the primary antioxidant that reacts with an organic free radical[68, 69, 70]. Ascorbate has a lower redox potential than tocopherol (+0.08 vs +0.37)[71]. As antioxidants of higher electronegativity will regenerate those of lower electronegativity[71], Vit C will be an effective co-antioxidant for the regeneration of α-tocopherol from the tocopheroxyl radical[72]. This analysis agrees with our results, TABLE 5. shows that t_{90} of Vit C in F7 and F8 (both containing vitamin E) is smaller than that of F2 non-containing antioxidants. This could be furthermore explained by the regeneration of vitamin E by Vit C and possible consumption of some amount of Vit C by this action decreasing thereby the effective reduced form of Vit C.

The shelf life of Vit C in F7 is less than that of F8 (8.39 vs 10.21 days), this might be due to the presence of an effective ratio between the two vitamins: E and C for working synergistically. Above this ratio, both vitamins might work separately and Vit E may help Vit C in quenching radicals increasing thereby the shelf life to 10 days in F8.

IV. CONCLUSION

Aqueous solution containing Vit C were formulated and the effects of different factors were studies. The shelf life of Vit C increased when the concentrations of Vit C. Metabisulfite Na seems to be the first candidate to formulate Vit C aqueous solutions followed by hydroquinone. In contrast, formulations with vitamin E must be optimized to increase the shelf life of Vit C.

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