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Assessment of the Influence of Some Formulation Excipients on Vitamin C Stability

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Abstract: Formulating Ascorbic acid (Vitamin C, Vit C) presents many challenges due to its sensibility to oxidation. This study aimed to enhance the stability of Vit C in aqueous solutions through the addition of different excipients, including polyols (eg. Sorbitol, Glycerin and Propylene Glycol PG), sugars (eg. Xylose), viscosity modifiers (eg. Gum Arabic and Carbopol), acidity regulators (Citric and Tartaric Acids). The kinetics of Vit C degradation were evaluated by determining the reaction order, calculating rate constants and estimating shelf lives (t_{90}). The results demonstrated that the shelf life of Vit C was enhanced when Sorbitol, Glycerin, Propylene glycol, Xylose, Gum Arabic and Tartaric Acid were used (the shelf lives ranged from 12 to 37 days compared to 8 days (in Control). However, lower shelf lives were reported when Carbopol and Citric Acid were used ($t_{90} \approx 5$ days).

Keywords: *Vitamin C, Stability, Shelf Life, Additives.*

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I. INTRODUCTION

The bioactive form of vitamin C (L-ascorbic acid) offers multiple benefits to the body [1], including beneficial effects on the skin [2]. Vitamin C (Vit C) is essential for collagen synthesis [3], playing a crucial role in both proline hydroxylation and fibroblast activation, which are vital for the formation of new collagen [4,5,6,7]. Additionally, vitamin C inhibits melanin production [8], making it an effective depigmenting agent [9,10]. It also has the ability to neutralize free radicals [11], acting as a potent antioxidant by donating electrons, thereby protecting other compounds from oxidation [12,13,14,15,16,17].

However, Vit C freely oxidized in aqueous solutions and loses its positive properties. It degrades rapidly in the presence of oxygen, light, and free-radical mediated oxidative processes, with these reactions being strongly catalyzed by transition metal ions, leading to the rapid destruction of Vit C [18]. Oxidation is further accelerated at neutral and alkaline pH levels [19].

The chemical structure of vitamin C allows it to convert into dehydroascorbic acid in aqueous environments after electron donation [20]. Upon exposure to heat, temperature, and alkaline conditions, it is reversibly oxidized into dehydroascorbic acid (Fig. 1), which can then irreversibly hydrolyze into 2,3-diketogulonic acid [21]. The formation of diketogulonic acid represents the complete loss of Vit C's bioactivity [22]. This oxidation process is often accompanied by a yellowish discoloration.

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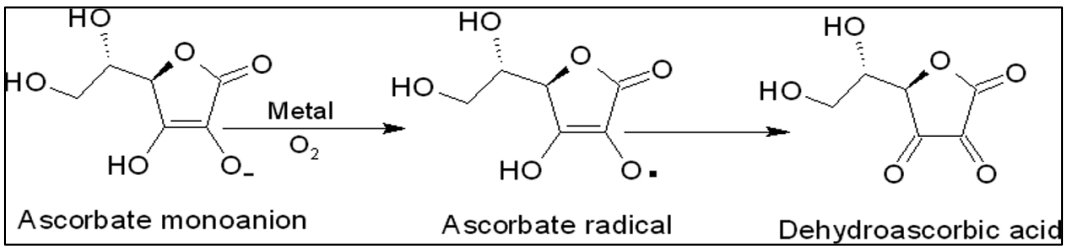

Fig. 1. Oxidation of Vit C in Aqueous Solution

Several strategies have been reported in the literature to reduce the oxidation and degradation of vitamin C. These include reducing oxygen uptake, adjusting pH, and controlling water content [23]. The use of anti-chelating agents and antioxidants [24,25,26] can help prevent vitamin C degradation. Both the acidity and concentration of topical vitamin C formulations significantly influence its activity and absorption. The formulations of topical Vit C are typically developed in concentrations ranging from 1% to 20% [27,28,29].

The aim of this study is to quantitatively assess the stability of Vit C through an accelerated stability test using the Arrehenius equation. The effect of various ingredients, such as Sorbitol, Glycerin, and Propylene Glycol. Xylose, Gum Arabic, Carbopol, Citric acid and Tartaric Acid will be mathematically analyzed. These additives are known to influence viscosity or water activity or pH levels. The accelerated stability test will allow the determination of rate constants and the calculation of the shelf life of Vit C in different aqueous solutions.

II. MATERIAL AND METHODS

A. Material

Vit C was purchased from Loba chemie, India. 2,6-dichorophenol: Indophenol Sodium was supplied from Tmmedia, India. Sorbitol, Glycerin, Propylene Glycol, Xylose, Gum Arabic, Carbopol, Citric and Tartaric Acids were of analytical grade.

B. Methods

➢ *Preparation of Vit C solutions*

Vit C (5%) and different additives: Sorbitol, Glycerin, Propylene Glycol, Xylose, Gum Arabic, Carbopol, Citric and Tartaric Acids (5%) were dissolved in acidic water (pH=3). In acidic pH, Vit C is unionized (pKa=4.2) [20]. Low pH levels (< 3) are required for stability and optimal percutaneous absorption [30,31]. The compositions of prepared solutions were presented in TABLE 1.

Table 1. Composition of Vit C Solutions

	(Control)	***F1***	***F2***	***F3***	***F4***	***F5***	***F6***	***F7***	***F8***
Vit C (g)	5	5	5	5	5	5	5	5	5
Soritol (g)	5	5	-	-	-	-	-	-	
Glycein (g)	5	-	5	-	-	-	-	-	-
Propylene glycol (g)	5	-	-	5	-	-	-	-	-
Xylose (ml)	5	-	-	-	5	-	-	-	-
Carbopol (g)	5	-	-	-	-	5	-	-	-
Citric acid (g)	5	-	-	-	-	-	5	-	-
Tartaric acid (g)	5	-	-	-	-	-	-	5	-
Water (pH=3) up to	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100ml	100ml	100ml

➢ *Vit C Solutions Characterization*

- *Visual Inspection*

Vitamin C Solutions were Visually Examined for color changes [32].

- *Determination of Vit C*

The active form of Vitamin C was quantified using the 2,6-dichlorophenolindophenol (DCPIP) titration method, following the AOAC official method (1984) [33] with minor modifications. In this modification, metaphosphoric acid was replaced by 0.2% hydrochloric acid (HCl). Diluted Vitamin C solutions from different formulations were titrated with a standardized DCPIP solution.

- *Stability Analysis of Vit C Using Accelerated test*

Nine sealed tubes containing 5% ascorbic acid were stored in two ovens (A&E Lab, UK) at temperatures of 37°C and 50°C. As expected, the degradation rate of Vitamin C increased with temperature [34]. All samples were stored in the dark, and aliquots were taken at predetermined intervals: 0 (100% Vit C), 7, 14, 21, and 30 days. An aqueous solution of 5% Vitamin C without additives served as the control for comparison.

✓ *Kinetic Modeling*

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According to the literature, both zero-order and first-order kinetic models are commonly applied to describe linear and exponential relationships between concentration and time [35].

✓ *Shelf Life Determination*

The shelf life (t_{90}) is defined as the time required for the Vit C concentration to decrease by 10% [36]. Using degradation kinetics data and the Arrhenius equation [37], various parameters were calculated, including the degradation rate constants of Vit C (k) at 37°C and 50°C (K_{37} and K_{50}). The Arrhenius equation (K = A·e^(-Ea/RT)) was then used to calculate activation energies (Ea) [38]. The rate constant at room temperature (K_{20}) was estimated, allowing the shelf life (t_{90}) of Vit C in different solutions to be determined.

III. RESULTS AND DISCUSSION

Vit C solutions became more yellowish as the time passes du to Vit C decomposition. After the second week all Vit C solutions (at 50 °C) were dark and no possible visible differences could be noticed.

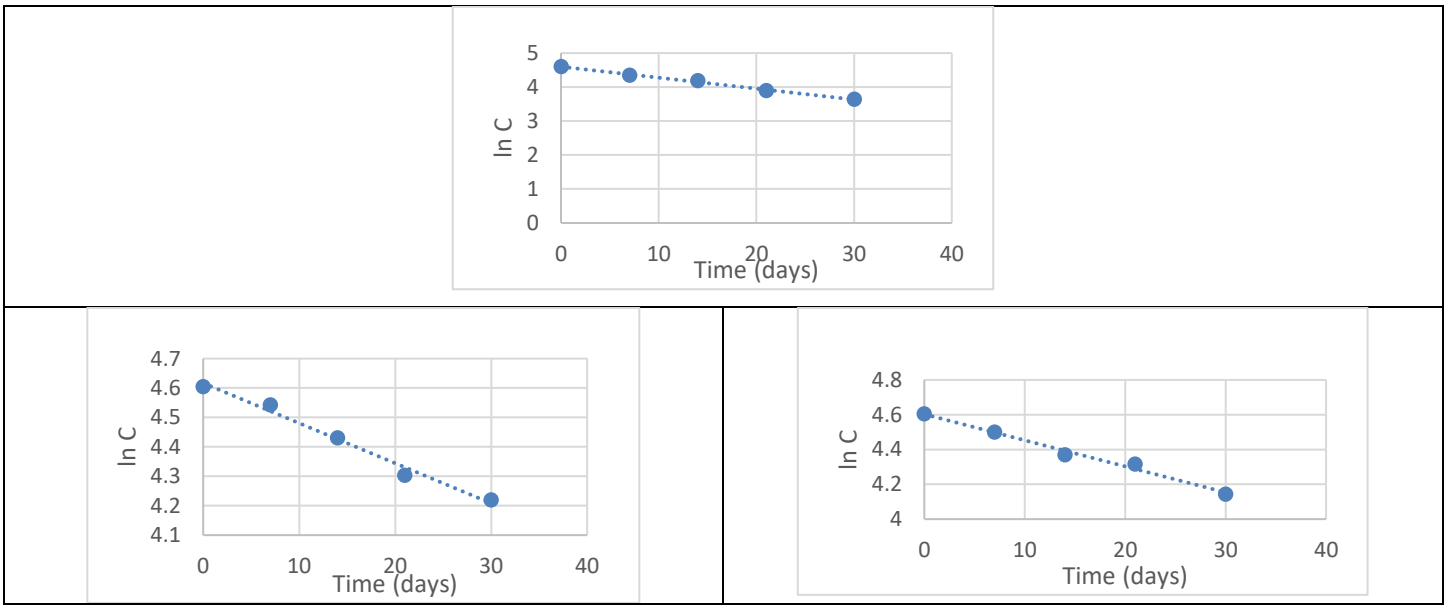
➢ *Determination of Vit C Shelf Life in Different Solutions*

TABLE 2. presents the R^2 values of Vit C degradation rates in various solutions at two temperatures: 37˚ and 50˚ C.

Table 2. Determination Coefficients: (R^2_1 for First Order and R^2o for Zero Order) of Vit C Degradation Kinetics at 37˚ and 50˚C

		Control	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*	*F8*
37˚ C	**R^2_1**	0.994	0986	0.988	0.998	0.993	0.999	0.967	0.978	0.98
	R^2o	0.972	0.983	0.984	0.985	0.99	0.994	0.965	0.968	0.979
50˚ C	**R^2_1**	0.984	0.973	0.991	0.991	0.989	0.951	0.942	0.991	0.957
	R^2o	0.949	0.961	0.968	0.937	0.925	0.86	0.857	0.93	0.917

As shown in TABLE 2, Vit C degradation follows the equation for first-order which is consistent with previous studies [39,40]. Fig. 2. illustrates the degradation profiles of Vit C at 37˚C according to first order kinetics. The calculated shelf lives of Vit C in different formulations ranged from 5 to ≈ 37 days as summarized in TABLE 3.



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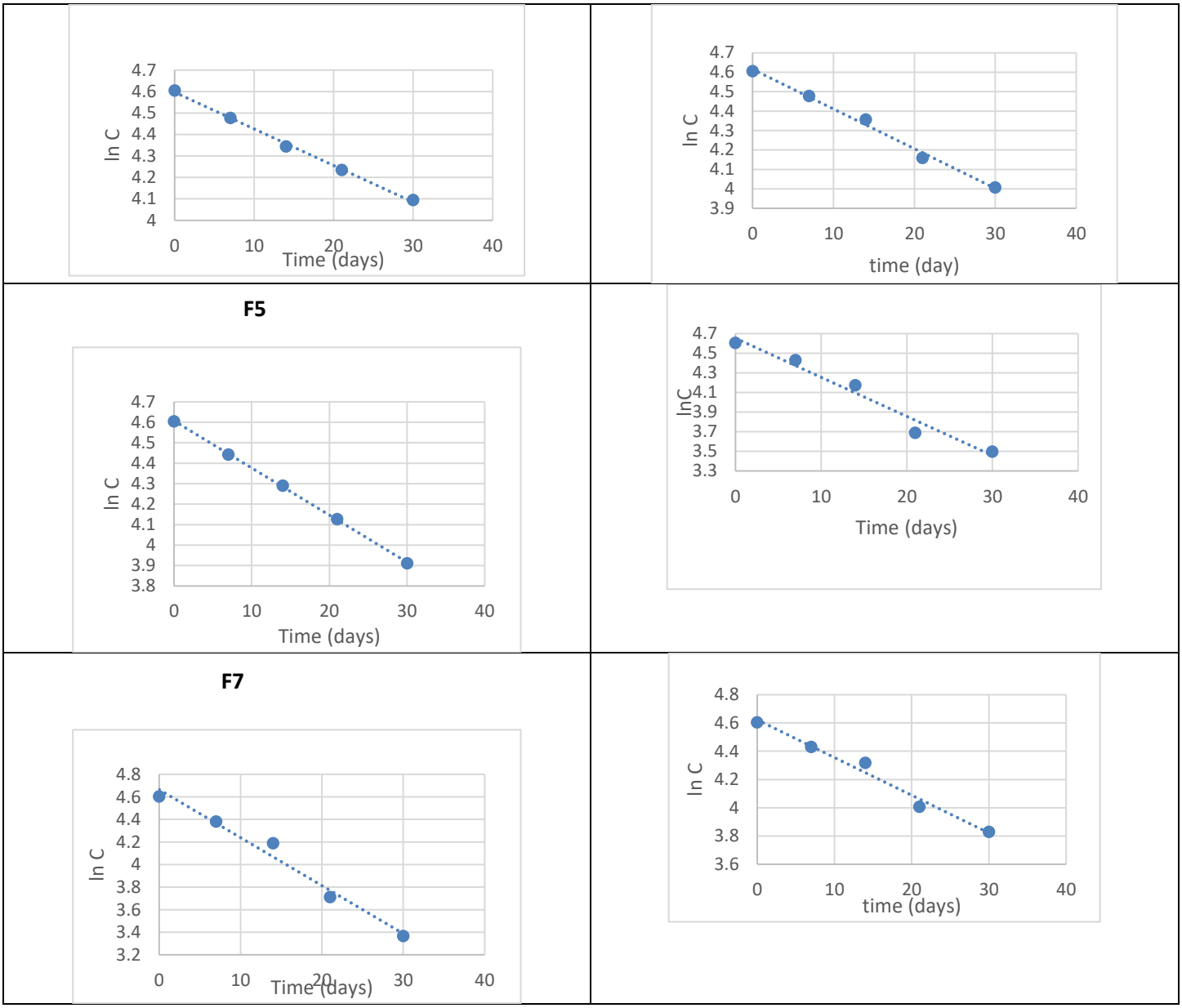

Fig. 2. Profiles of Vit C Degradation (first order) at 37 C

Table 3. Shelf Lives (t_{90}) of Vit C in Different Formulations

	Control	***F1***	***F2***	***F3***	***F4***	***F5***	***F6***	***F7***	***F8***
t_{90} (day)	7.7	37.2	33.5	30	18.6	13.9	5	4.9	11.8

According to data from TABLE 3 and degradation kinetics of Vit C, Sorbitol (F1) has a lower degradation rate constant in comparison with Control (≈37 vs. ≈8 days). Sorbitol increases solution viscosity [41] reducing in consequence oxygen diffusion [38]. additionally, Sorbitol can reduce water activity permitting better Vit C stabilization [42].

TABLE 3 shows that Sorbitol has the lower degradation rate constant in comparison with other formulations. These findings agree

with other previous study [43] in which the authors explained that Sorbitol exhibited the strongest stabilizing effect because it reduces water activity and limits oxygen-mediated oxidation. Sorbitol is a polyol containing six hydroxyl groups,

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which enables high hydrogen bonding with water molecules and Vit C. This interaction restricts water molecules mobility and activity. Moreover, Sorbitol has higher molecular weight than the other polyols used in this study (eg. Glycerin and PG), this means that Sorbitol can enhance viscosity more than Glycerin and PG, contributing to higher shelf life (≈37 vs ≈34 and 30 days respectively) [43].

Similarly, Glycerin has a significant effect on Vit C stability in comparison with Control (≈34 vs. ≈8 days). Glycerin enhances viscosity and prevents diffusion of molecules (eg. Oxygen) responsible of Vit C destabilization [38,44, 45].

PG binds to water reducing its activity and enhancing its viscosity [41]. PG showed lower stabilizing efficacy than Glycerin because it contains only two hydroxyl groups (Glycerin has 3), reducing its capacity to bind water and form hydrogen-bonding networks. PG has also a hydrophobic character disrupting solvent structure, resulting in weaker suppression of oxidation compared to Glycerin [43]

The presence of sugars showed a positive effect on Vit C stability compared to pure water [46]. Xylose reduces water activity lowering the number of free water molecules necessary for oxidation [46].

Being a reducing sugar (promoting side reactions) rather than a sugar alcohol, Xylose exhibited weaker stabilizing effect compared to polyols. This might be explained by the fact that Polyols are inert molecules providing a cleaner stabilizing medium [47].

The shelf life of Vit C in solution containing Gum Arabic was almost 14 days versus 8 days in pure water. Gum Arabic is a hydrophilic polysaccharide, it contains multiple OH groups that may bind to water molecules reducing its activity. These OH bonds may also protect Vit C from oxidation, additionally, Gum Arabic increases water viscosity [41].

However, the stabilizing effect of Gum Arabic is minimal when used as a simple excipient in solution when compared to its capacity to form a wall material in microencapsulation systems. It was evident that its positive effects on Vit C stability are not pronounced like polyols and sugars. [48].

For the two used acids (Citric and Tartaric), the stabilization effect was different. Citric acid can stabilize Vit C through pH reduction and metal chelation. However, citrate complexes with metals may remain redox-active, potentially catalyzing oxidative degradation. Consequently, Citric Acid may demonstrate a neutral or even pro-oxidant effect. [49]. However, Tartaric Acid exerts better stabilization than Citric Acid due to its weaker chelating ability and simpler ionic medium. Unlike Citric Acid, Tartaric Acid results in a more chemically inert acidic environment and reduced catalytic oxidation of Vit C [50].

Although acidic conditions favor Vit C stability, once the pH is adjusted within the range (≈3–4), further pH adjustment does not significantly reduce degradation rates. Therefore, excipients that can acidify the medium demonstrate limited stabilizing effect compared to polyols [51].

Polymers such as Carbopol increase viscosity but do not necessarily reduce water activity [52]. Carboxyl groups may accelerate oxidation rate [53, 54], Carbopol isn`t a true chelating agent so it doesn`t protect Vit C from metal-mediated oxidation [55, 41]. The increased viscosity observed with Carbopol was insufficient to improve Vit C stability [56] because there are many other factors related to Carbopol enhancing degradation rate (discussed above).

IV. CONCLUSION

The observed stability order (Sorbitol > Glycerin > Propylene Glycol > Xylose > Gum Arabic > Tartaric Acid > Citric Acid≈ Carbopol) reflected the significant role of water activity reduction, hydrogen bonding ability and chemical inertness. Polyols (eg. Sorbitol, Glycerin and PG) demonstrated better stabilization by limiting oxygen transfer and suppressing oxidative pathways, whereas acids (eg, Citric Acid and Tartaric Acid) influenced stability mainly through pH control and metal interactions. pH control and viscosity weren`t the main factors for Vit C stabilization.

In this study, the shelf-lives weren`t sufficient but they may be a base for further studies when other variables are considered.

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