

Research Article



A Study of the Effect of the Percentage and the Components of the Aqueous Phase on the Chemical Stability of Vitamin C in its Dermal Preparations

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ABSTRACT

The aim of this research is to study both the effect of the percentage of the aqueous phase, and the effect of the components of that phase on the chemical stability of Vitamin C or Ascorbic Acid (AA) in some dermal preparations (aqueous solution, w/o creams, o/w creams) at a concentration of 2%. The study was conducted in accelerated conditions with the prepared cream being stored at 37°C and 45°C degrees for a period of 28 days. The samples were analyzed weekly for the purpose of determining the concentration of the undegraded AA using the light spectrum scale (Spectrophotometer). The shelf-life of each formulation was calculated according to the Q10 method and the results were evaluated statistically using the t-student test. The results revealed that lowering the percentage of water within the formulation and adding certain substances (mineral salts, sorbitol, ethanol) to the aqueous phase improves the stability of the AA.

Keywords: Ascorbic Acid, Accelerated stability, Q10 method, shelf-life.

INTRODUCTION

Ascorbic Acid works as a topical antioxidant that removes free radicals and as a co-factor in synthesizing collagen which in turn increases the elasticity of the skin.¹⁻⁸

Ascorbic Acid rapidly oxidizes to the ineffective Dehydroascorbic acid, which results in a low stability for dermal preparations containing AA. It takes only a few days for an AA solution (5%) to degrade completely into the oxidized form.⁹

Research efforts are focused on the development of topical dermal preparations containing AA that achieve a good stability, so that they can be used safely and effectively. This mandates the need to conduct chemical and physical stability studies to guarantee that the most stable and effective formulation is chosen.¹⁰ To perform these studies, the prepared formulations are subjected to harsh conditions such as high temperature⁵, and the chemical degradation with time is monitored to predict the shelf-life of the product according to a certain component as well as studying the chemical kinetics of this component.¹⁰

In this study, the chemical stability of dermal preparations containing AA (aqueous solution, o/w cream, w/o cream) will be investigated. The preparations were packaged in tightly sealed containers and stored at temperatures of 37°C and 45°C in closed ovens¹¹ for an entire month.^{12,13} A chemical analysis (quantitative measure) of the Ascorbic Acid was performed periodically every 7 days using the light spectrum scale (Spectrophotometer).^{14,15}

The degradation kinetics of the AA in all of the different formulations was specified and then the shelf-life for the

AA in each formulation was calculated using the Q10 method.

The shelf-life of a product is defined as the time period over which 90% of the initial concentration of the medicine remains stable.^{16,17} The (Q10) is the percentage between two different reaction rate constants when the temperature rises by 10°C.¹⁸ The Q10 method is an approximate method to predict the shelf-life of a medicine and requires certain stability data which are obtained by degrading the medicine at two different temperature levels at least. The Q10 method is independent of the reaction order^{10,12} and is based on the shelf-life of the product at a certain temperature and a calculation of both the activation energy (Ea) and Q10 so as to calculate the shelf-life at the required temperature.¹²

After obtaining the different information, we will mathematically study the effect of the components of the aqueous phase of the emulsion on the stability of the AA in dermal preparations.

MATERIALS AND METHODS

Materials

- Extra pure Ascorbic Acid (Loba chemie, India).
- Distilled water, calcium chloride CaCl₂, magnesium chloride MgCl₂, sorbitol, ethanol 95%, Vaseline, Spermacite, bees wax, paraffin oil, propylene glycol, span 60, span 80, Tween 80. All of these materials were at the laboratorial purity level.

Devices

- Spectrophotometer (PG instruments, T60 U spectrophotometer, England).



- Mechanical shaker (Heidolph, RZR 2012, Germany).
- Centrifuge (Kendro, Lobofuge 200, USA).
- Water bath (Mettler, K.F.T LAB. EQUIPMENT, Germany).
- Oven (Mettler, carbolite 53336RB, Germany).
- Sensitive scale (Precisa, XB220A, Switzerland).

Methods

Preparation of the Pharmaceutical Forms

Two aqueous solutions were prepared, one containing AA only (w/v, 2%) and another containing AA (w/v, 2%) with additional substances (calcium chloride CaCl_2 , magnesium chloride MgCl_2 , sorbitol, ethanol) by dissolving the different substances in distilled water.

Table 1: w/o creams containing AA and prepared according to different formulations

Formulation no.	Ingredient (g)													
	Ascorbic acid	Sorbitol	CaCl ₂	MgCl ₂	Ethanol 95%	Span 80	Vaseline	Bee wax	Spermaceti	Cetyl alcohol	Liquid paraffin	Borax	Water	Aqueous phase%
1	2	-	-	-	-	6	40	4	-	-	15	-	33	35
2	2	1.125	0.175	0.7	1	6	40	4	-	-	15	-	30	35
3	2	1.5	0.1	0.4	1	6	40	4	-	15	15	-	15	20
4	2	1.975	0.125	0.5	5	-	6	-	14	-	55	0.4	15	25
5	2	1.25	0.15	0.6	6	-	-	6	14	-	50	0.4	20	30
6	2	1.25	0.15	0.6	6	-	-	8.5	16.5	-	50	0.4	15	25
7	2	1.975	0.125	0.5	5	-	8.5	-	16.5	-	55	0.4	20	30

Table 2: o/w creams containing AA and prepared according to different formulations

Formulation no.	Ingredient (g)											
	Ascorbic acid	Sorbitol	CaCl ₂	MgCl ₂	Ethanol 95%	Span 60	Tween 60	Propelyn glycol	Vaseline	Cetyl alcohol	Water	
8	2	1	0.25	1	2	-	5	12	25	25	26.75	
9	2	1	0.25	1	2	5	5	12	22	23	26.75	
10	2	5	1	1.5	10	5	5	12	22	23	13.5	

Table 3: Changes in the AA concentration of cream formulation (3) with time at a temperature of 37°C

	Time (day)	Absorption	Concentration C (g/100ml)	In C
Patch (1)	0	0.511	1.652	0.501987
	7	0.507	1.643	0.496524
	14	0.505	1.635	0.491643
	21	0.502	1.626	0.486123
	28	0.499	1.618	0.481191
Patch (2)	0	0.69	2.214	0.794801
	7	0.685	2.199	0.788003
	14	0.682	2.188	0.782988
	21	0.678	2.177	0.777948
	28	0.674	2.164	0.771958
Patch (3)	0	0.588	1.895	0.639219
	7	0.584	1.884	0.633397
	14	0.582	1.875	0.628609
	21	0.578	1.865	0.623261
	28	0.575	1.856	0.618424

The w/o creams or o/w creams were prepared by melting the contents of the oil phase according to the order of melting points up to 75°C and heating the aqueous phase to 75°C, then adding the internal phase to the external while stirring continuously until the preparation has cooled to room temperature.

Tables (1 & 2) show the formulations of the w/o creams and the o/w creams respectively, as these formulations differ from each other either in the percentage of the

aqueous phase within the cream or in the components of both the oil and aqueous phases

Stability Study Protocol

Method of Sample Storage

Different formulations containing 2% Ascorbic Acid were prepared. These prepared pharmaceutical forms were: aqueous solution, w/o cream, o/w cream (tables 1 & 2).



Three batches of every formulation were prepared to ensure the frequency of the results.

After the preparation: the prepared formulations were stored in 250ml clean, tightly sealed, heat resistant, glass containers. Each batch was divided into two parts: one part was stored at a temperature of 37°C and the other part was stored at 45°C for 28 days. All sample containers were covered with aluminum foil to protect them from the effects of light. Samples were drawn periodically (every week) from these containers in order to measure the AA quantitatively using the Spectrophotometer.

Treatment of Samples to Determine the AA Concentration

To measure the effective and remaining amount of Ascorbic Acid in each of the formulations prepared, an amount of 5g from each of the prepared creams was placed in a test tube to which 5ml of distilled water was added and it was centrifuged at a speed of 5300 cycles/minute for 20 minutes.

After centrifuging, 2 ml of the aqueous layer containing Ascorbic Acid was taken from the bottom of the test tube and diluted in distilled water. The Ascorbic Acid absorptions were measured using a Spectrophotometer at a wavelength of 265 nanometers and the corresponding concentrations were obtained by applying the formula:

$$Y=0.0320X-0.018 \quad (1)$$

The Mathematical Study of Stability

After obtaining the AA concentration in each of the prepared formulations every week for 28 days, we studied the degradation kinetics of the AA in each formulation at storage temperatures of 37°C and 45°C with the aim of verifying the order of degradation of the AA in the formulation. The regression lines were plotted according to the orders (0, 1). These two orders were regarded as sufficient because it is well-known that most pharmaceutical degradation reactions follow these two orders and that AA degrades in pharmaceutical and nutritional preparations with kinetics of the first order or pseudo-first order.^{13,19}

The Q10 method was used in calculating the shelf-life of the AA in the formulation. This requires determining the AA degradation at one temperature at least (37°C in our research) in order to calculate the shelf-life of the formulation at this temperature. It is sufficient to know the reaction constant at another temperature (45°C) and use the two reaction constants at the aforementioned temperatures to calculate the activation energy (Ea) (according to Arrhenius equation) from which the Q10 can be calculated. Lastly, the shelf-life of the AA in each formulation was calculated at room temperature making use of its storage life at 37°C and the Q10 calculated previously.

The Q10 Method^{12,20}

The shelf-life of the AA in each formulation (at 37°C for example) was calculated using the following equation:

$$\text{Shelf life} = t_{90(T1)} = 0.105 / k \quad (2)$$

*Where K is the reaction rate constant (first order) at 37°C

The activation energy of the degradation reaction of the Ascorbic Acid was calculated from the following equation, derived from the Arrhenius equation at two different temperatures.

$$\ln k_2 \setminus k_1 = Ea/R (T_2-T_1 \setminus T_2T_1) \quad (3)$$

*Where K1 is the reaction rate constant at temperature T1, K2 is the reaction rate constant at temperature T2, T1 is the first storage temperature at 310 Kelvin and T2 is the second storage temperature at 318 Kelvin.

The Q10 value was calculated from the equation:

$$Q_{10} = e^{[Ea \cdot 10 / R(T+10)T]} \quad (4)$$

$$Q_{\Delta T} = Q_{10}^{\Delta T/10} \quad (5)$$

*The Shelf life of formulation X at room temperature was calculated from the equation:

$$t_{90(T2)} = t_{90(T1)} / Q_{10}^{\Delta T/10} \quad (5)$$

*Where t90 (T2) is the shelf-life at 25° and t90 (T1) is the shelf- life at 37°C.

Statistical Analysis

The statistical analysis was carried out using the Student test via Excel 2007 software. The results were considered statistically significant if the P-value≤0.05.

RESULTS AND DISCUSSION

Calculation of the Shelf-life by Q10 Method

An example on calculating the shelf-life for one of the prepared formulations using the Q10 method is mentioned. Let this formulation be formulation (3) from table (1).

Calculation of the Shelf-life for Formulation (3)

Three batches of the w/o cream were prepared according to formulation (3). The AA absorption was measured in the samples that were drawn periodically at the wavelength 265 nanometers and the corresponding concentration was calculated by applying equation (1). Table (3) shows an example of the changes in the AA concentration with time when stored at room temperature of 37°C.

By plotting the regression lines according to the orders (0, 1) temperatures of 37°C and 45°C (figures 1 & 2) for batch (1) of formulation (3), the degradation order of the AA was determined. This is necessary to determine the reaction order at one temperature at least to calculate the shelf-life according to the Q10 method.



Batch (1) At 37°C:

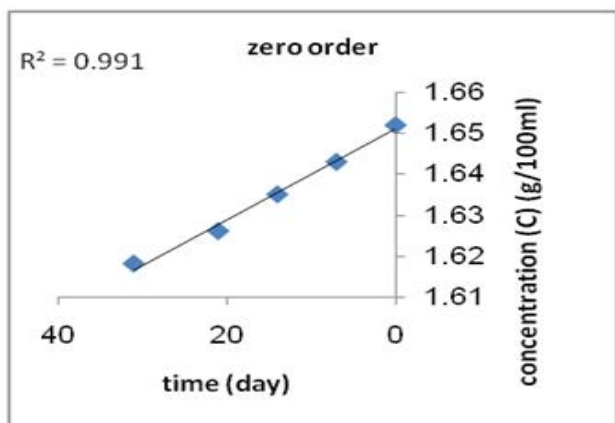


Figure 1: The regression line for the AA concentration against time (order 0)

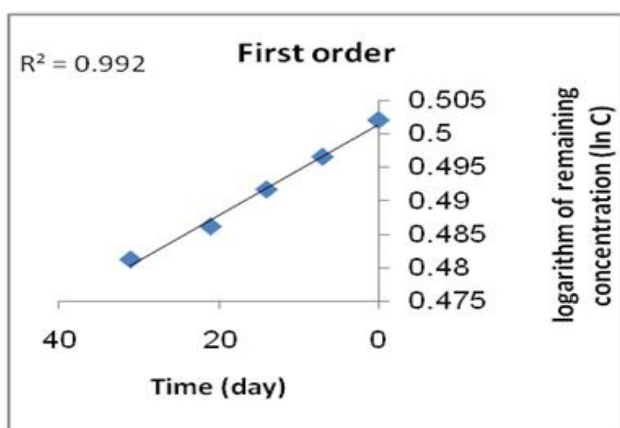


Figure 2: The regression line for the logarithm of the remaining concentration of the AA against time (first order)

The regression plots were obtained for the remaining batches at the two temperatures 37°C and 45°C and all the coefficients of determination resulting from this plot were tabulated in table (4) below.

Table 4: Values of the coefficients of determination r^2 for the equations that show the degradation kinetics of the AA in the prepared w/o cream according to formulation (3) at temperatures of 37°C and 45°C.

	At 37°C	At 45°C
Batch (1)	Zero order: $r^2=0.991$	Zero order: $r^2=0.997$
	First order: $r^2=0.992$	First order: $r^2=0.998$
Batch (2)	Zero order: $r^2=0.996$	Zero order: $r^2=0.997$
	First order: $r^2=0.997$	First order: $r^2=0.998$
Batch (3)	Zero order: $r^2=0.998$	Zero order: $r^2=0.965$
	First order: $r^2=0.999$	First order: $r^2=0.967$

From table (4) we find that r^2 can take the highest value in regression lines of the first order (at both temperatures studied) which verifies this order in the degradation of the AA and has helped us in calculating the activation energy when applying the Arrhenius equation.^{23,25}

Calculating the Shelf-Life of Batch (1)

Referring back to the curve representing the logarithm of the remaining concentration of the AA (lnC) against time at 37°C for batch (1) we found:

$$K_1 = -(y_2 - y_1 / x_2 - x_1)$$

$$K_1 = -(0.491643 - 0.496524) / 14 - 7$$

$$= 0.00488 / 7$$

$$= 0.000697 \text{ day}^{-1}$$

$$\text{Shelf life} [t_{90(T1)}] = 0.105 / k_1$$

$$= 0.105 / 0.000697$$

$$= 150 \text{ days}$$

*Where t_{90} (T1) is the shelf -life at 37°C.

*From the curve representing the logarithm of the remaining concentration of the AA (lnC) against time at 45°C for batch (1) we found:

$$K_2 = -(y_2 - y_1 / x_2 - x_1)$$

$$K_2 = -(0.623047 - 0.628502) / 14 - 7$$

$$= 0.000779 \text{ day}^{-1}$$

*Applying Arrhenius equation (equation 3) we had:

$$\ln 0.000779 / 0.000697 = E_a [318 - 310] / 195878.48$$

$$E_a = 2723.339 \text{ cal/mol}$$

*The value of Q_{10} was calculated by applying equation (4) and we obtained:

$$Q_{10} = 1.148163$$

*Applying equation (5) we obtained:

$$Q_{\Delta T} = 1.148163^{-1.2}$$

*From the above we derived the $t_{90(T2)}$ (the shelf-life at temperature 25°C) by applying equation (6).

$$t_{90(T2)} = 177.8118 \text{ days}$$

Table 5: Shelf-life of AA in cream formulation (3)

Batch number	Shelf life (day)	Mean± SD (day)
(1)	177.8118	1.061± 176.929
(2)	177.2251	
(3)	175.7528	

As in the previous example, the shelf-life was calculated for all of the prepared formulations (table 6):

A Mathematical Study of the Effect of the Different Factors on the Stability of AA

1) Effect of the Aqueous Phase

The effect of the percentage and the components (alcohol, propylene glycol, salts, ...) of the aqueous phase on the stability of AA were studied and the following results were obtained:



A. Effect of the Percentage of the Aqueous Phase

The effect of the percentage of the aqueous phase on the stability of AA in w/o creams was studied, whereby the percentage of the aqueous phase was lowered by lowering the water amount from (35% to 20%) as in formulations (2 & 3) and from (30% to 25%) as in formulations (5 & 6) and from (30% to 25%) as in formulations (4 & 7).

It can be noticed from table (6) that reducing water resulted in an increase in the shelf-life, as the shelf-life increased from 127 days (formulation 2) to 176 days (formulation 3) ($P<0.05$) and from 143 days (formulation 5) to 155 days (formulation 6) ($P<0.05$) and from 176 days (formulation 4) to 188 days (formulation 7) ($P<0.05$). This can be attributed to the decrease in the water amount in the formulation.^{9,21}

Table 6: The shelf-life of AA in all of the prepared formulations

Formulation number	Shelf life (day) (Mean± SD)	Formulation number	Shelf life (day) (Mean± SD)
0	9.73±85.472	6	6.86±155.150
1	2.20±69.449	7	5.11±188.546
2	1.17±127.705	8	2.54±130.179
3	1.06±176.929	9	3.68±163.685
4	2.68±178.326	10	175.086 2.16±
5	2.94±143.151		

B. Effect of the Components of the Aqueous Phase

The components or the percentage of the components of the aqueous phase were altered whether in o/w creams or w/o creams, and the effect of each change on the shelf-life of AA was examined in the corresponding formulation, for example:

- The aqueous phase of formulation (0) (it is a formulation of an aqueous solution) contained a combination of additional substances (mineral salts, sorbitol, ethanol), whereas the aqueous phase of formulation (1), a w/o cream formulation, did not contain any of these additives. The comparison here was between (formulations 0 and 1), that is between an aqueous solution with additional substances and a w/o cream with an aqueous phase that did not contain any additional substances. The purpose of the comparison was to judge whether the additional substances to the aqueous phase or the emulsification within the oil were the cause of the better stability of AA (the comparison was within the limits of our research).
- The same additional substances were added in formulation (0), an aqueous solution formulation, to the aqueous phase of formulation (2), a w/o cream and a comparison was conducted between this last

formulation (formulation 2) with formulation (1), a w/o cream, with an aqueous phase that did not include these additives.

- The percentage of each of the substances added to the aqueous phase was increased in formulation (10), an o/w cream formulation, in comparison with their respective percentages in formulation (9), also an o/w cream formulation. The increments were as followed: the percentage of $MgCl_2=1\%$ in formulation (9) was increased to 1.5% in formulation (10), the percentage of $CaCl_2=0.25\%$ in formulation (9) became 1% in formulation (10), the percentage of sorbitol was 1% in formulation (9) and reached 5% in formulation (10). Finally, the percentage of ethanol was increased from 2% in formulation (9) to 10% in formulation (10).

In comparison with the aqueous solution formulation with a shelf-life of 85 days approximately and formulation (1) with a shelf-life of 69 days approximately, we found that the aqueous solution formulation was more stable than formulation (1) ($P<0.05$). This can be explained by the presence of sorbitol which contributes to reducing the water activity in the formulation. Moreover, as has been mentioned in a study; sorbitol being a reduced sugar oxidizes first thereby delaying the oxidation of AA.²² In addition to the above, the presence of ethanol helps in stabilizing the negatively charged ascorbate ion which slows the decarboxylation process. Thus, adding polyols slows the decarboxylation process of the unstable ascorbate ion and subsequently improves the stability of the AA in aqueous solutions [22]. The presence of calcium chloride and magnesium chloride enhances the stability of the AA as they reduce water activity as has been corroborated by several studies and researches.^{9,22} Moreover, the ionic strength within the aqueous phase is increased by the Mg^{2+} and Ca^{2+} ions that surround the negatively charged oxygen atom in the ascorbate ion creating what is known as ionic shielding, which inhibits the oxidation of AA and subsequently increases its stability.²³

Therefore, we can say that formulating AA within a w/o cream cannot be considered a better way to improve its stability in all conditions, but that it also depends on the components of the aqueous phase. We found that the stability of AA in an aqueous solution containing a reduced sugar, stabilizing salts and ethanol was better than its stability in a w/o cream where the aqueous phase did not include these additives, as has been the case in many studies that have shown the improved stability of AA in w/o creams.²²

Likewise, we find the same manner and with the same explanation in formulations (1 & 2), where the shelf-life of AA was increased from 69 days to 127 days approximately ($P<0.05$).

In comparing the shelf-life of AA in the two formulations (9 & 10), we found that the shelf-life for formulation (10)

was equivalent to 175 days approximately, which is longer than the shelf-life of AA in formulation (9) which was 163 days with a statistically significant difference where ($P < 0.05$). The two formulations were o/w creams; however in formulation (10) we used greater amounts of salts, reduced sugar (sorbitol) and ethanol which led us to conclude that by increasing the amounts of additional substances (within the limits of our study) we can increase the shelf-life of AA by reducing the aqueous activity.

From all of the above, we concluded that any agent that can reduce water activity can increase the stability of AA in its formulations, whether in the form of solutions or creams.

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