

## Research Article



## The Shelf Life of Vitamin C in a w/o Emulsion According to the Q10 Method

Natali Moussa\*<sup>1</sup>, Lama Al Haushey<sup>2</sup>

<sup>1</sup>Masters degree in Pharmaceutical Chemistry and Quality Control, Faculty of Pharmacy, Tishreen University, Latakia, Syria.

<sup>2</sup>Instructor, Department of Pharmaceutics and Pharmaceutical Technologies, Faculty of Pharmacy, Tishreen University, Latakia, Syria.

\*Corresponding author's E-mail: [dr.nathaloo@hotmail.com](mailto:dr.nathaloo@hotmail.com)

Accepted on: 28-10-2014; Finalized on: 31-01-2015.

### ABSTRACT

This aim of this research is to calculate the shelf-life of Vitamin C or Ascorbic Acid (AA) in a dermal preparation, namely w/o cream at a concentration of 2%. Vitamin C w/o Cream was prepared taking into consideration the effects of the percentage of the aqueous phase and the effects of its components on the stability of AA, since lowering the water content within the formulation and adding certain substances (minerals, sorbitol, ethanol) to the aqueous phase improves the stability of the AA. 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 the formulation was calculated according to the Q<sub>10</sub> method. Stability data was analyzed and a time-concentration curve was plotted. Vitamin C follows degradation kinetics of the first order. The stability constants were derived from the curve and the activation energy was calculated using Arrhenius equation. Next, the activation energy was used according to the Q<sub>10</sub> method so as to calculate the Q<sub>10</sub> and later the shelf-life of Vitamin C which reached 208 days approximately at room temperature.

**Keywords:** Ascorbic Acid, accelerated stability, shelf-life, Q<sub>10</sub> method.

### INTRODUCTION

Many people have been increasingly interested in protecting their skin from sunlight induced aging or photoaging, and keeping it in a radiant and healthy condition.

Among the effective substances that affect the radiance and safety of the skin and aid in its protection from wrinkles is Ascorbic Acid (AA) or Vitamin C. Likewise, there has been an increasing interest in dermal preparations containing AA since it 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<sup>1-8</sup>.

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<sup>9</sup>.

Research efforts are focused on the development of topical dermal preparations containing AA that achieve a good stability, so that they can be used and 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<sup>10</sup>.

To perform these studies, the prepared formulation is subjected to harsh conditions such as high temperature<sup>5</sup>, 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<sup>10</sup>.

The aim of performing a stability test is to determine the shelf-life of the pharmaceutical product, which was previously tested to determine its stability before it was marketed. These tests involved subjecting the product to the same conditions as those of its storage<sup>11</sup>. These tests are known as long-term stability testing, as they require a long time and are expensive<sup>12</sup>. Therefore, different methods known as accelerated stability are used to evaluate the stability at various stages during the development of the medicine. These tests enable us predict the stability of medicinal products over the long run. The ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) defines the protocols according to which long term and accelerated stability testing is conducted in terms of temperature, humidity (depending on the climatic zone), required storage period, number and type of batches examined<sup>13,14</sup>.

According to the ICH (Q1A (R2), 2003) "Stability Testing of New Drug Substances and Products", the storage temperature during the accelerated stability testing should be at least 15 °C above the actual storage temperature with a relatively adequate humidity. As for the storage period, the ICH states that it should not be less than 6 months for a new medicinal preparation. However it excludes chemical substances that degrade rapidly or that are subject to significant chemical degradation within 3 months. Thus, the ICH stipulates that it is not necessary to test the medicinal substance over a period of six months but that a period of less than



3 months would suffice for a single batch only, provided the test is repeated more than is usual during the chosen storage period<sup>13,14</sup>. For example, we find that Vitamin C is highly degradable and highly sensitive to oxidation, which is why we find that several global studies on accelerated stability testing that aim to predict its stability are conducted within 28 days as a storage period<sup>15,16</sup>.

In addition, this exception (that is the possibility of conducting the accelerated testing in a period of less than 3 months) includes products that undergo a significant change within 3 months of testing; the ICH defines such a significant change as<sup>13</sup>:

- A 5% change in the concentration of the medicine from its initial concentration, such that the medicine no longer achieves its required efficacy, that is a degradation in the product that exceeds its acceptance criteria.
- The inability of the product to satisfy its acceptance criteria in terms of appearance, physical characteristics, color and phase separation.

In any case, the ICH mentions that the occurrences of some changes in the physical characteristics during the accelerated tests are to be expected such as the melting of creams and the softening of suppositories.

In this study, the chemical stability of a dermal product containing AA (w/o cream) will be investigated. The cream was packaged in tightly sealed containers and stored at temperatures of 37 °C and 45 °C in closed ovens<sup>13</sup> for an entire month<sup>15,17</sup>.

A chemical analysis (quantitative measure) was performed periodically every 7 days using the light spectrum scale (Spectrophotometer)<sup>14,20</sup>.

The degradation kinetics of the AA in the aqueous solution and in the w/o cream were specified and then the shelf-life for the AA in the cream was calculated using the Q<sub>10</sub> method (the aqueous solution was stored in tightly sealed glass containers at 25 °C for 140 days so as to study the kinetics of the AA in its aqueous solution).

The shelf-life of a product is defined as the time period over which 90% of the initial concentration of the medicine remains stable<sup>14,20</sup>.

The (Q<sub>10</sub>) is the percentage between two different reaction rate constants when the temperature rises by 10 °C<sup>21</sup>. The Q<sub>10</sub> 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 Q<sub>10</sub> method is independent of the reaction order<sup>10,17</sup> and is based on the shelf-life of the product at a certain temperature and a calculation of both the activation energy (E<sub>a</sub>) and Q<sub>10</sub> so as to calculate the shelf-life at the required temperature<sup>17</sup>.

## MATERIALS AND METHODS

### Materials

- Extra pure Ascorbic Acid.
- Distilled water, calcium chloride CaCl<sub>2</sub>, magnesium chloride MgCl<sub>2</sub>, sorbitol, ethanol 95%, Vaseline, Spermacite, bee wax, paraffin oil, cetyl alcohol, propylene glycol, span 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<sub>2</sub> 0.5g, magnesium chloride MgCl<sub>2</sub> 2g, sorbitol 5g, ethanol) that were dissolved in distilled water.

w/o cream was prepared by melting the contents of the oil phase according to the order of melting points up to 75 °C (Vaseline 40g, liquid paraffin 15g, bee wax 9g, spermaceti 5g, span 80 6g) and heating the aqueous phase to 75 °C (cetyl alcohol 5g, propylene glycol 5g, sorbitol 1.5g, ethanol 4g, AA 2g, CaCl<sub>2</sub> 0.1g, MgCl<sub>2</sub> 0.4g, water 7g) then adding the internal phase to the external while stirring continuously until the preparation has cooled to room temperature.

An experiment that highlights the rate of AA degradation within its aqueous solution was carried out, in which the AA aqueous solution at a concentration of 0.016g/100ml was placed in a Spectrophotometer and left in it.

The changes in absorption and hence in the concentration of the AA within it were observed.

The absorption spectrum of the AA of the UV within the range (230-320 nanometers) was drawn every hour for five hours and the occurring change in this spectrum was monitored, Figure (A:1).

This experiment was performed on the other AA solution at the same concentration, Figure (B:1), but with the addition of certain substances to this solution (calcium chloride CaCl<sub>2</sub>, magnesium chloride MgCl<sub>2</sub>, sorbitol, ethanol) at specific concentrations.



### Preparation of the Standard curve and Measuring the Absorption of the AA within the UV

The standard solution was prepared at a concentration of 100.64mg/100ml.

Following that, the solutions of the standard curve starting from the standard solution were prepared by taking a certain volume from it in each time and diluting it up to 100ml in distilled water using a calibration balloon of a volume 100ml.

The absorption of the AA in every solution of the standard curve were measured using the spectrophotometer at a wavelength of 265 nanometers, and a straight line was drawn by plotting the absorption readings against the concentrations, Figure (1) from which the following equation was derived:

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

Where: Y is the absorption; X is the concentration (mg/ml).

This equation will be used in determining the concentration of the AA during the stability study.

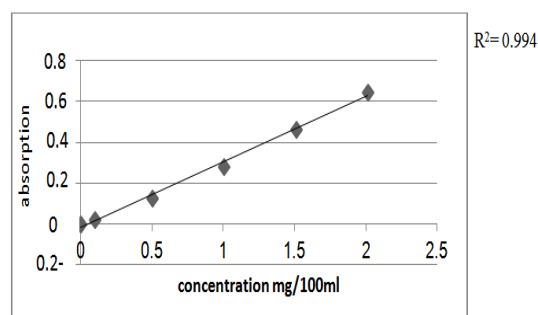


Figure 1: Standard curve for AA in distilled water

### Stability Study Protocol

#### The Degradation Kinetics of the AA in the Aqueous Solution

It was imperative before conducting the stability tests that the kinetics of Vitamin C in its aqueous solution were verified, which is why these kinetics were studied in an aqueous solution containing AA only (formulation 1).

The following plots were drawn to determine the order of degradation of the Ascorbic Acid in its aqueous solution:

1. The regression line that represents the concentration changes C against time t, to judge if the Ascorbic Acid degradation follows a kinetic of the zero order.
2. The regression line that represents a change in the logarithm of the remaining concentration of the AA (lnC) against time to discover if the degradation follows a kinetic of the first order.

#### Method of Sample Storage

The w/o cream containing 2% Ascorbic Acid was prepared. Three samples were prepared from the studied formulation to ensure the frequency of the results.

After the preparation: the prepared w/o cream was stored in 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.

Note: The aqueous solution prepared from the AA was stored for 140 days only because it is preferable to increase the number of storage days when studying accelerated stability if the formulation examined allows for it (Asean Stability Guideline, ICH Harmonized Tripartite Guideline). Thus, we stored the cream for a period of 28 days only because the heat affects the stability of the cream itself and results in an emulsion phase separation dissolution which in turn affects the results of the study.

#### Treatment of Samples to Determine the AA Concentration

To measure the effective and remaining amount of Ascorbic Acid in the formulation, an amount of 5g from the prepared cream 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 were taken from the bottom of the test tube and diluted in distilled water. The AA 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 the prepared cream every week for 28 days, we studied the degradation kinetics of the AA in the 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<sup>15,22</sup>.

The Q<sub>10</sub> 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 ( $E_a$ ) (according to Arrhenius equation) from which the  $Q_{10}$  can be calculated. Lastly, the shelf-life of the AA in the formulation is calculated at room temperature making use of its storage life at 37 °C and the  $Q_{10}$  calculated previously.

### The $Q_{10}$ Method<sup>17,23</sup>

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

$$\text{Shelf life} = t_{90(T_1)} = 0.105/k \quad (2)$$

\*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:

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

\*Where  $K_1$  is the reaction rate constant at temperature  $T_1$ ,  $K_2$  is the reaction rate constant at temperature  $T_2$ ,  $T_1$  is the first storage temperature at 310 Kelvin and  $T_2$  is the second storage temperature at 318 Kelvin.

The  $Q_{10}$  value was calculated from the equation:

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

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

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

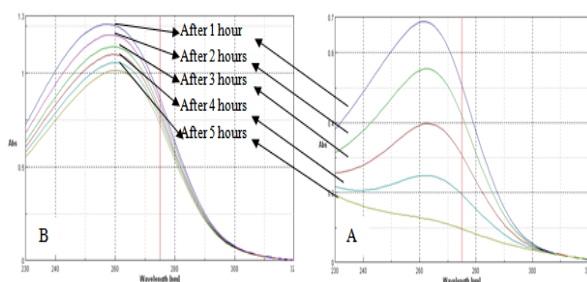
$$t_{90(T_2)} = t_{90(T_1)} / Q_{10}^{\Delta T/10} \quad (6)$$

\*Where  $t_{90}$  ( $T_2$ ) is the shelf-life at 25 °C and  $t_{90}$  ( $T_1$ ) is the shelf- life at 37 °C.

## RESULTS AND DISCUSSION

### The Importance of AA Stability Studies

From what was previously mentioned we observed the complete and rapid degradation of AA in its aqueous solutions and thus we considered monitoring the change in the concentration of the AA that is stable in its aqueous solution. This was done by leaving it in the Spectrophotometer.



**Figure 2:** Resulting change in the absorption of AA with time (rapid degradation). **Figure 2A:** Aqueous solution for AA only. **Figure 2B:** Aqueous solution for AA with certain additives.

We can notice from the figure (A: 2) a change in the absorption of the AA within the UV, where the absorption decreases (and so does the concentration of the AA) with time to disappear completely after 5 hours. This confirms the very rapid degradation of AA which necessitates changing the AA chemically or changing its pharmaceutical formulation to attain better stability for it.

The AA remained relatively stable in the other aqueous solution, where its quantity did not disappear after 5 hours, Figure (B: 2). This proves the importance of relying on additives such as (calcium chloride  $CaCl_2$ , magnesium chloride  $MgCl_2$ , sorbitol, ethanol) in formulating the cream prepared in this research.

This method which revealed the reduction of the amount of AA in its compounds is widely known, as the decrease in absorption with time is considered an important proof and a significant indicator of the decreased concentration and efficacy of the AA<sup>24</sup>.

### Degradation Kinetics of the AA in its Aqueous Solution

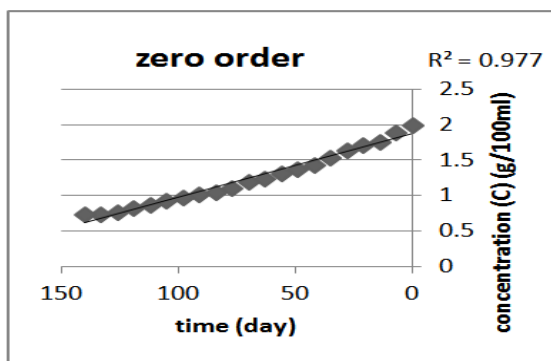
An aqueous solution for the AA (2%) was stored at room temperature for 140 days and we obtained the results shown in Table 1 and which depicts the degradation kinetics of the AA in this solution.

**Table 1:** Concentration changes of the AA in its aqueous solution with time at room temperature

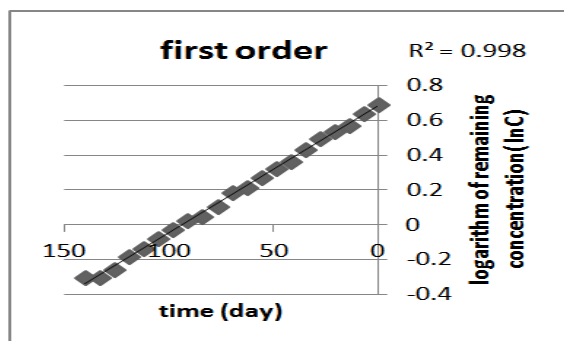
Time (day)	Concentration(C) (g/100ml)	Logarithm of the remaining concentration (ln C)
0	2	0.693147
7	1.896	0.639746
14	1.77	0.57098
21	1.716	0.539996
28	1.65	0.500775
35	1.541	0.432432
42	1.441	0.365337
49	1.384	0.324978
56	1.316	0.274597
63	1.246	0.219938
70	1.209	0.189794
77	1.116	0.109751
84	1.053	0.051643
91	1.028	0.027615
98	0.978	-0.02225
105	0.93	-0.07257
112	0.876	-0.13239
119	0.835	-0.18032
126	0.778	-0.25103
133	0.741	-0.29975
140	0.74	-0.30111

The regression lines were drawn according to orders (0, 1), Figures (3, 4):





**Figure 3:** The regression line for the AA concentration against time (zero order).



**Figure 4:** The regression line of the logarithm of the remaining concentration of the AA against time (first order).

Comparing the coefficients of determination resulting from these different regression lines according to order (0, 1),  $R^2=0.977$  (zero order) &  $R^2= 0.998$  (first order). We find that the degradation of AA within its aqueous solution exhibits first order kinetics since it corresponds to the highest coefficient of determination  $R^2$ .

A simple application of the equation: Shelf-life  $[t_{90} (T)] = 0.105/k (2)$ , we find that the shelf-life of Vitamin C in this aqueous solution is 15 days only. This also indicates of the rapid degradation of Vitamin C and the need to work on enhancing its stability in dermal preparations to guarantee their efficacy.

**Calculation of the Shelf-life by Q10 Method**

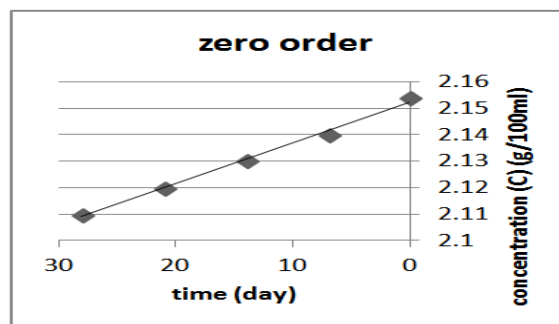
Three batches of the w/o cream were prepared. The AA absorption was measured in the samples that were drawn periodically at the wavelength 265 nanometers and the corresponding concentration was calculated by applying equation (1). Table 2 shows an example of the changes in the AA concentration with time when stored at room temperature of 37 °C.

By plotting the regression lines according to the orders (0, 1) at temperatures of 37°C and 45°C (figures 5, 6) for batch 1 from the prepared formulation, 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.

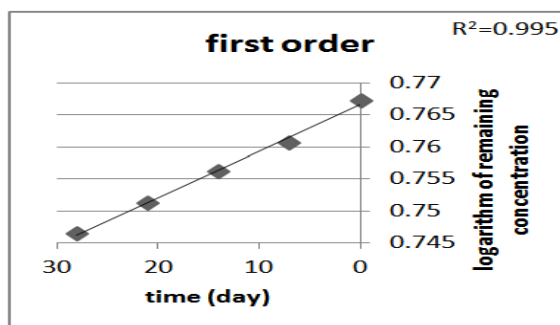
**Table 2:** Changes in the AA concentration in the prepared w/o cream with time at a temperature of 37°C

	Time (day)	Absorption	Concentration (C) (g/100ml)	ln C
Batch (1)	0	2,154	0.767327	
	7	2,1399	0.760759	
	14	2,1302	0.756216	
	21	2,1198	0.751322	
Batch (2)	0	1,9542	0.669981	
	7	1,9431	0.664285	
	14	1,9344	0.659797	
	21	1,926	0.655445	
Batch (3)	0	1,9584	0.672128	
	7	1,9429	0.664182	
	14	1,9342	0.659694	
	21	1,925	0.654926	
	28	1,916	0.65024	

**Batch (1) At 37 °C**



**Figure 5:** The regression line for the AA concentration against time (zero order).



**Figure 6:** The regression line of 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.

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 which helped us in calculating the activation energy when applying the Arrhenius equation<sup>23,25</sup>.

### Calculating the Shelf-Life of Batch (1)

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

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

$$K_1 = -(0.756216 - 0.760759) / 14 - 7$$

$$= 0.004543227 / 7$$

$$= 0.000649032 \text{ day}^{-1}$$

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

$$= 0.105 / 0.000649032$$

$$= 161.7874 \text{ days}$$

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

\*From the curve representing the logarithm of the remaining concentration of the AA (lnC) with the significance of time at 45 °C for batch (1) we find:

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

$$K_2 = -(0.675645 - 0.681024) / 14 - 7$$

$$= 0.000768441 \text{ day}^{-1}$$

\*Applying Arrhenius equation (equation 3) we had:

$$E_a = 3897.997 \text{ cal/mol}$$

$$\ln 0.000768441 / 0.000649032 = E_a [318 - 310] / 1.987 * 310 * 298$$

\*The value of Q<sub>10</sub> was calculated by applying equation (4) and we obtained:

$$Q_{10} = 1.218666$$

\*Applying equation (5) we obtained:

$$Q_{\Delta T} = 1.218666^{-1.2}$$

\*From the above we derived the t90(T2) (the shelf-life at temperature 25 °C) by applying equation (6)

$$t_{90(T2)} = 205.1192 \text{ days}$$

The values of the shelf-life of AA in the three batches of the prepared cream are calculated by the same method above (205.1192 day for batch (1), 210.7264 day for batch (2), 208.4557 day for batch (3), Mean ± SD = 208.1004 ± 2.820)

### The Effect of the Percentage and Contents of the Aqueous Phase on the Stability of AA

#### Effect of the Percentage of the Aqueous Phase

Lowering the percentage of the aqueous phase in the formulation by reducing the quantity of water leads to an improvement in the stability of Vitamin C<sup>9,21</sup>, whereby a reduction in the amount of free water lessens the undesirable chemical changes or the changes that occur during the storage period.

### Effect of the Components of the Aqueous Phase

The presence of sorbitol contributes to lowering the aqueous activity in the formulation, and many results have mentioned that it is believed that sorbitol as a reduced sugar oxidizes first and delays the onset of the oxidation of AA<sup>26</sup>.

Ethanol contributes in stabilizing the Ascorbic ion and slows the decarboxylation, accordingly adding polyols lowers the decarboxylation of the unstable Ascorbic and in turn improves the stability of AA in aqueous solutions<sup>26</sup>.

Calcium Chloride and Magnesium Chloride increase the stability of the AA because they reduce the aqueous activity which is confirmed by many studies<sup>9,22</sup>.

Moreover, the ionic strength increases in the aqueous phase as a result of the presence of Ca<sup>2+</sup> and Mg<sup>2+</sup> electrolytes which surround the negatively charged oxygen ion in the Ascorbic electrolyte, forming what is known as ionic shielding that hinders the oxidation of the AA and thereby increases its stability<sup>27</sup>.

Many studies are available which show that the AA had better stability in w/o emulsions versus its stability in its aqueous solution.

### CONCLUSION

To determine the shelf-life of the w/o cream containing Ascorbic Acid, an accelerated stability test was conducted at two temperatures, 37 °C and 45 °C for 28 days. The degradation kinetics of the Acid was of the first order in the aqueous solution and in the prepared cream. The storage life of the prepared cream was calculated using the Q<sub>10</sub> method which is regarded as a corner stone in predicting the storage life for medicinal substances with heat-sensitive degradation. The storage life of the cream reached 208 days approximately whereas it previously used to be 15 days in the aqueous solution of the Ascorbic Acid of the same concentration.

### REFERENCES

1. Silva GM, Campos PM, Study of the rheologic behaviour and HPLC determination of a skin care formulation containing Ascorbic Acid or Magnesium Ascorbyl Phosphate, *Cosmetic Science For The New Century Berlin*, 21(b), 2000, 11-14.
2. Nusgens BV, *Topically applied Vitamin C enhances the mRNA level of collagens I and II, their processing enzymes and tissue inhibitor of matrix metalloproteinase in the human dermis*, *J Invest Dermatol*, 116(6), 2001, 853-859.
3. Jentzsch A, Streicher H, Engelhart K, *The synergistic antioxidative effect of ascorbyl phosphate and alfatocopheryl acetate*, *Cosmet Toiletries*, 116(6), 2001, 55-64.
4. Pinnel SR, *Topical L-ascorbic acid: percutaneous absorption studies*, *Dermatol Surg*, 27(2), 2001, 137-142.



5. Gasper LR, Campos PM, *Rheological behavior and the SPF of sunscreens*, Int J Pharm, 250(1), 2003, 35-44.
6. Campos PM, Goncalves GMS, Gasper LR, *In vitro antioxidant activity and in vivo efficacy of topical formulations containing vitamin c and its derivatives studied by non-invasive methods*, Skin Res Technol, 14(3), 2008, 376-380.
7. Yilmaz S, Sadikoglu M, Saglikoglu G, Yagmur S, Askin G, *Determination of Ascorbic Acid in Tablet Dosage Forms and some fruit juices by DPV*, Int J Electrochem Sci, 3, 2008, 1534-1542.
8. Campos FM, Ribeiro SMR, Della Lucia CM, Santana HMP, Stringheta PC, *Optimization of methodology to analyze ascorbic acid and dehydroascorbic acid in vegetables*, Quim Nova, 32(1), 2009, 87-91.
9. Parr J, Capistrano SJ, Sun Z, Valley F, Chang Y, Uyen HO U, Grove G, George D, Caza CD, *Stabilized Ascorbic compositions*, Patent Application Publication US, 0034548A, 2002, 1-3.
10. Silva GM, Campos PM, *Histopathological studies of ascorbic acid and magnesium ascorbyl phosphate in a skin care formulation*, Int J Cosmet Sc, 22(3), 2000, 169-179.
11. Rawlin EA, *Bentleys Text Book of Pharmaceutics*, 8<sup>th</sup> edition, Cassell Ltd. 1979, 164-165.
12. Yoshioka S, Stella VG, *Stability of drugs and dosage forms*, Kluwer Academic/ Plenum Publishers, New York, 2000.
13. ICH Harmonized tripartite guidelines for stability testing of new drug substances and products - Q1A (R2), 4 version, 2003.
14. World Health Organization Guidelines for stability testing of pharmaceutical products containing established drug substances in conventional dosage forms. WHO Technical Report Services, WHO, Geneva, 1996, 863.
15. Goncalves GMS, Campos PM, *Shelf life and rheology of emulsions containing vitamin c and its derivatives*, Revista de Cienciac Farmaceuticas Basica e Aplicada, 30(2), 2009, 89-94.
16. Akhtar N, Ahmad M, Khan HMS, Akram J, Gulfishan, Mahmood A, Uzair M, *Formulation and characterization of multiple emulsion containing 1% L- Ascorbic Acid*. Chemical Society of Ethiopia, 24(1), 2010, 1-10.
17. Shaheen MS, *Accelerated study of Metronidazole Infusion 100 ml*, The Journal of Teachers Association RMC, 18(2), 2005, 118-121.
18. Arayne MS, Sultana N, Bibi Z, *Rapid and Specific Spectrophotometric and RP-HPLC Methods for the Determination of Ascorbic Acid in Fruits Juices and in Human Plasma*, J. Chem. Soc. Pak, 31(3), 2009.
19. Selimovic A, Salkic M, Selimovic A, *Direct spectrophotometric determination of L-Ascorbic Acid in pharmaceutical preparations using Sodium oxalate as a stabilizer*, International of basic and applied sciences IJBAS-IJENS, 11(02), 2011.
20. U.S. Food and Drug Administration (FDA), *Stability testing of new substances and products*, ICH Q1A, FDA, Washington, DC, 2001.
21. Ansel HC, Allen JLV, Popovich NG, *Pharmaceutical Dosage Forms and Drug Delivery Systems*, 7<sup>th</sup> Ed., Lippincott Williams & Wilkuns, U.S.A, 1999, 102-167.
22. Connors Ka, Amidon GL, Stella VJ, *Chemical stability of pharmaceuticals*, 2<sup>nd</sup>. Ed., John Wiley & Sons, Canada, 1986, 3-31, 208-220.
23. Sinko Pj, *Martin's physical pharmacy and pharmaceutical sciences*, 6<sup>th</sup>. ed., Wolters Kluwer, Lippincott Williams &Wilkins, U.S.A, 2011, 318-354.
24. Cioroi M, Moisi M, Cretv R, *Spectrophotometric evaluation for the stability of the Ascorbic Acid from the sweet briar extract (Rosa Canina) and white sea buckthorn (Hypophae Rhamnoides)*, The annals of the University Dunarea de jos of Galat, 2009.
25. Aulton ME, *The Design And Manufacture Of Medicines*, 3<sup>rd</sup>. ed., Churchill Livingstone Elseovier, U.S.A, 650-665.
26. Ramires JE, Judy Hattendorf J, Del Ray M, *Stabilized Ascorbic composition*. Patent Application Publication US, 0077220A1, 2007, 1-7.
27. Suk Lee J, Woong Han J, Hoon Han S, *The Stabilization of L-ascorbic acid in aqueous solution and water-in-oil-in water double emulsion by controlling pH and electrolyte concentration*, J. Cosmet. Sci., 55, 2004, 1-12.

Source of Support: Nil, Conflict of Interest: None.

