Optimization of Disodium Edetate and Few Potent Antioxidants Requirement for the Stabilization of Vitamin C in Solution

S. A. AGNIHOTRI* AND G. M. PANPALIA

Department of Pharmaceutical Sciences, Birla Institute of Technology, Ranchi-835215.

The present investigation is a preformulation study on stabilization of vitamin C with disodium edetate and some antioxidants. The concentration of disodium edetate for the stabilization of vitamin C in aqueous solution at pH 4.0 was found to be critical; less than 0.05% w/v was insufficient and more than 0.05% w/v was detrimental. The first order kinetics was the prevailing mechanism in this case and kinetic rate constants were calculated by regression analysis. Differential scanning calorimetric study suggested the effect of disodium edetate concentration on the thermal behavior of vitamin C and results are reciprocal of kinetic study. The study was further extended to understand the effect of some antioxidants such as cysteine, thiourea and nicotinic acid on the stabilization of vitamin C. In all the cases the effect of stabilization increased with increased concentration. Cysteine was found to be more protective to the aqueous stability of vitamin C. In total both disodium edetate and antioxidants were found to improve the stability of vitamin C. However, required stability was not achieved with permissible concentration of both the stabilizers suggesting the need of further investigation.

Vitamin C or L-ascorbic acid is an optically active strong monobasic reducing agent in acidic and neutral solutions. The degradation of vitamin C occurs under both aerobic and anaerobic conditions with different decomposition products. The emergence of vitamin C as a skin-fairing, nourishing and rejuvenating agent increased research in the field of cosmetics containing vitamin C. Rovesti was the first to document the use of creams containing 3% and 5% ascorbyl oleate for bleaching freckles in human. In recent years, numerous patents have explored the utility of vitamin C, vitamin C fatty acid esters, vitamin C salts and its other derivatives in the cosmetic formulations. Vitamin C has also been reported to control skin roughness, ageing and skin damage from ultra violet (UV) light. However, less importance is given in the patent literature for the stability aspects of vitamin C in topical formulations.

*For correspondence
E-mail: sunil_agnihotri@yahoo.com
Present address: Center of Excellence in Polymer Science, Karnataka University, Dharwad-580 003.

Vitamin C is highly oxidation prone, which can be understood from the fact that it is the antioxidant of choice for pharmaceutical preparations. Definitely, it would be a difficult task to stabilize it in cosmetic products. Although a large amount of data is available on the stability aspects of vitamin C, the available data is not sufficient to really help such a formulation. The decomposition kinetics of vitamin C probably had been studied more thoroughly than that of any other drug, we are only now beginning to understand the mechanism of auto oxidation. For example—the role of pH in the stability of the vitamin C is still utterly confusing.

Chelating agents and antioxidants are the major class of stabilizing agents for oxidation-prone drugs. Though disodium edetate (Na₂EDTA) remains to be the most popular chelating agent, not much attention has been directed towards the optimum concentration required for the stabilization of drugs. Various published reports suggest the use of thiourea, cysteine, cystine, tyrosine, sarcosine, pyridine, quinoline, nicotinic acid, cysteine hydrochloride, glutathione and thioacetic acid for the stabilization of...
vitamin C. But in most of the cases, the effective concentration is not mentioned and more important aspect being that the relative stabilizing efficiency of most of them have not been estimated. It was with this objective the current investigation was undertaken to study the stabilization of vitamin C aqueous solution in presence of Na₂EDTA and some antioxidants.

MATERIALS AND METHODS

Vitamin C was procured from Loba Chemicals, Mumbai and Na₂EDTA was procured from BDH Chemicals, Mumbai. L-cysteine, nicotinic acid and thiourea were all purchased from S. D. Fine Chemicals Ltd., Mumbai. All other chemicals were of laboratory grade and used without further purification.

Effect of Na₂EDTA concentration on stability of 2% w/v vitamin C solution at pH 4.0:

A series of solutions were prepared with 2% w/v vitamin C containing varying concentration of Na₂EDTA over a large concentration range from 0.005 to 0.2% w/v in boiled and cooled double distilled water. pH of the solution was adjusted to 4.0 using 1 N KOH. Control sample is also prepared without Na₂EDTA.

These solutions were analyzed for the initial vitamin C content at zero time with suitable dilution in 0.1 N HCl using UV/Vis spectrophotometer (Systronic, Mumbai, India) at λ max of 243 nm. A systemic validation of the above method was performed and was found to be accurate and specific for the analyses of vitamin C. For the stability study the samples were stored at 40±1°C using thermosatically controlled ovens (Scientific Instrument Services, Mumbai, India). At predetermined time intervals, 2.5 ml of samples were withdrawn from each flask for the analysis of the drug content after suitable dilution as explained for zero time samples.

Effect of concentration of Na₂EDTA on the thermal behavior of vitamin C:

The possible influence of Na₂EDTA on the thermal behavior of vitamin C was examined at different levels of the chelating agents as those used for the conventional solution stability studies. For example, equivalent to the solution stability evaluation system of 2% w/v vitamin C containing 0.2% w/v (the maximum concentration of the study) of Na₂EDTA had been the solid-state admixture containing 2 g of vitamin C and 0.2 g of Na₂EDTA.

The thermal analysis was carried out on DSC-50 (Shimadzu, Tokyo, Japan) using extended thermal analysis software. Prior to analysis, about 5 mg of the samples equivalent to 5 mg of the drug were weighed accurately in aluminium crucible. A programmed 3 step heating at the rate of 25°min from ambient to 150°, 5°/min from 150-275° and final step at the rate of 20°min from 275-360° was adopted in an attempt to obtain separation of the endothermic and the exothermic peaks, sufficient enough to enable to calculate enthalpy of fusion and enthalpy of decomposition much precisely. The signals were processed by the supplementary data processing system and plotted on chromatopac C-R4A. The heats absorbed or evolved corresponding to the peak temperature was recorded on the plots with the accuracy of ±0.1 mcal/mole.

Evaluation of stability of vitamin C in presence of various antioxidants:

The general concentration range suggested for antioxidants is 0.001-0.1%. Thus the study was conducted in presence of antioxidants within this concentration range. The solution stability of 2% w/v aqueous solution of vitamin C was studied with a constant level of 0.05% w/v of Na₂EDTA in the presence of varying concentration of thiourea, nicotinic acid or cysteine. The pH of the solution was adjusted to 4.0 as above and stored at 40±1°C. The samples were analyzed at zero and at predetermined time intervals as explained previously.

Statistical analyses were accomplished using SPSS statistical package. The statistical parameters of the regression equations of the kinetics studies (correlation coefficient, variance ratio and standard error of estimate of the response variable) were used to determine goodness of fit. Analysis of variance followed by least significant difference procedure was used for the comparison of stabilization effects of different antioxidants.

RESULTS AND DISCUSSION

Vitamin C in aqueous solution degrades by oxidative pathway catalyzed by heavy metal ions especially the Cu²⁺ ions. A quite good amount of information is available on the stabilization of vitamin C in aqueous medium by Na₂EDTA. However, still there is a scarcity of the data on the specific requirement of this chelating stabilizer even for the simple system containing vitamin C. Normally, Na₂EDTA is mentioned to be used over a wide concentration ranging between 0.01-0.075% w/v. Since the action of chelating stabilizers is indirect, dependent on the removal of free heavy metal ions from the solution in the form of soluble complexes (chelates), the amount of Na₂EDTA should be sufficient
enough to take the free metal-ions out of play. Consequently vitamin C content of the system is expected to be the limiting factor governing the stoichiometric requirement for Na₂EDTA. The stability constant of the chelate, that is likely to be influenced by the pH of the medium, would indeed influence its actual requirement. With the impression that less than enough is insufficient and more than sufficient is in no way detrimental, to be on the safer side, the normal inclination is to use towards the higher side.

Vitamin C was found to be more stable at pH 4.0 as observed in our previous study. The present investigation is the extension of our ongoing research in the stabilization of vitamin C and to optimize the Na₂EDTA concentration necessary for the maximum solution stability. Fig. 1 represents the quantitative effect of Na₂EDTA on the degree of protection it could afford to vitamin C in aqueous medium adjusted to pH 4.0. As the degradation of drug is concentration dependent¹⁹ the study was carried out on the constant strength of vitamin C solution of 2% w/v and at temperature of 40±1°. The degree of stabilization afforded by various concentration levels of Na₂EDTA are expressed quantitatively in terms of percent drug remaining at the corresponding time periods from as low as 4 d to as high as 75 d. All the experiments were conducted in triplicate and only the mean values are graphically presented, the standard deviations are less than 5%. It may be seen from this figure that the effectiveness of Na₂EDTA in the stabilization of vitamin C presented as aqueous solution varied with the concentrations for example, the amount of drug remaining at the end of two and half months period varied between 45.3-59.8%. This means that Na₂EDTA provided the maximum of 25% protection to vitamin C in an aqueous solution of 2% w/v system. The statistical parameters of the regression equations are presented in Table 1.

![Graph showing stability of vitamin C at pH 4.0](image)

**Fig. 1: Stability of 2% w/v solution of vitamin C at pH 4.0.**

Stability of vitamin C (2% w/v aqueous solution adjusted to pH 4.0) during storage as influenced by concentration of Na₂EDTA, control (-○-), 0.005% (-□-), 0.01% (-△-), 0.02% (-◇-), 0.05% (-◆-), 0.075% (-△-), 0.1% (-■-) and 0.2% (-▲-).

<table>
<thead>
<tr>
<th>Na₂EDTA concentration</th>
<th>Correlation coefficient</th>
<th>Variance ratio</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.992</td>
<td>308.461</td>
<td>1.701x10²</td>
</tr>
<tr>
<td>0.005</td>
<td>0.986</td>
<td>178.995</td>
<td>1.920x10²</td>
</tr>
<tr>
<td>0.01</td>
<td>0.987</td>
<td>192.518</td>
<td>1.708x10²</td>
</tr>
<tr>
<td>0.02</td>
<td>0.982</td>
<td>136.488</td>
<td>1.825x10²</td>
</tr>
<tr>
<td>0.05</td>
<td>0.988</td>
<td>196.283</td>
<td>1.414x10²</td>
</tr>
<tr>
<td>0.075</td>
<td>0.982</td>
<td>134.334</td>
<td>1.803x10²</td>
</tr>
<tr>
<td>0.1</td>
<td>0.983</td>
<td>142.513</td>
<td>2.012x10²</td>
</tr>
<tr>
<td>0.2</td>
<td>0.987</td>
<td>186.089</td>
<td>1.933x10²</td>
</tr>
</tbody>
</table>

Influence of varying concentration of Na₂EDTA at pH 4.0 on the stability of vitamin C. *df= 6, p<0.05.*

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The data was analyzed for first order rate kinetics by using regression analysis and the first order rate constant $K$ was calculated. The fig. 1, symbolizes the first order degradation of vitamin C taking place in the aqueous solution containing different amounts of Na$_2$EDTA. In almost all the systems, regardless of the absence or presence of Na$_2$EDTA and its concentration, log percent drug remaining decreased linearly with time. The initial fast degradation was subsequently followed by the significantly less rate of degradation. As a result first order degradation pattern of each system could be signified by two slopes, $K_1$ and $K_2$ representative of initial and later phase of the storage, respectively. It is interesting to note that the change in slope was deferred with the increasing stabilization of vitamin C by Na$_2$EDTA. Since more than 10% drug decomposition (maximum permissible limit) occurred on the first slope not withstanding the presence or absence of the stabilizer or its concentration. It is mandatory to examine the influence of variation in the concentration of Na$_2$EDTA on the stability of vitamin C measured in terms of $K_1$. Such a quantitative effect of Na$_2$EDTA on the stability of vitamin C (in terms of $K_1$) is presented in fig. 2. This drug stability indicates that less than 0.05% w/v of Na$_2$EDTA appears to be insufficient and the amount in excess of 0.05% w/v also exhibited the derogatory effect on the stability of vitamin C. At the present moment, no specific reason could be assigned to the increased decomposition of vitamin C at the concentration of Na$_2$EDTA in excess of that required for the optimum stabilization of vitamin C.

The DSC technique has been suggested to predict drug-excipient interaction. In recent years, interpretation of thermogram underwent sea changes. However, in absence of supplementary conventional stability studies, it is normally concluded that any change in the characteristic thermal behavior of drug and also excipient exhibits incompatibility. In other words, if the system is fully compatible, thermogram should be the summation of thermal behaviors of individual components, expected on the basis of their relative amounts. Thus any shifting in the peak or the appearance of the new peak apart from their obliteration is believed to be the sign of incompatibility. Shattaway et al. suggested the use of

![Graph of Na$_2$EDTA dependency of stability-profile of vitamin C at pH 4.0.](image1)

**Fig. 2:** Na$_2$EDTA dependency of stability-profile of vitamin C at pH 4.0.
The $K$ values as a function of Na$_2$EDTA concentration in the range of 0 to 0.2% w/v.

![DSC thermal behavior of vitamin C and vitamin C-Na$_2$EDTA admixture.](image2)

**Fig. 3:** DSC thermal behavior of vitamin C and vitamin C-Na$_2$EDTA admixture. Vitamin C-Na$_2$EDTA admixture in the ratio of (1) 2:0, (2) 2:0.01, (3) 2:0.02, (4) 2:0.05, (5) 2:0.075, (6) 2:0.1, (7) 2:0.2.
enthalpy change as the sign of such an interaction in the sense that loss of enthalpy is suggestive of incompatibility. Enthalpy of the mixture was considered as the additive property so that if no interaction takes place the enthalpy of the mixture should be the weighted average of enthalpies of pure components.

Fig. 3 represents the progressive changes in thermal behavior of the drug in presence of gradually increasing amounts of Na₂EDTA. It was observed that the sharp endotherm characteristic of the drug has been increasingly broadened as the relative amount of the additive increased. This is rather expected because of the added material acting as an impurity consequently such a change could not be used as the measure of incompatibility. It was further observed that the endotherm of melting of vitamin C decreased progressively. Since the drug was increasingly stable up to 0.05% w/v Na₂EDTA, so significance could be assigned to this change. However, it was observed that the enthalpy of the system increased from 296 mJ/mg to 409.02 mJ/mg with increase in the concentration of Na₂EDTA from 0 to 0.05% w/v and further increase in concentration has shown somewhat decrease in ΔHf values. Thus it is clear that up to 0.05% w/v Na₂EDTA would produce stabilization of vitamin C. Above 0.05% w/v, Na₂EDTA brought about some lowering of ΔHf. This indicates that above 0.05% w/v Na₂EDTA will not produce beneficial effect at the same time on the basis of somewhat decrease in ΔHf, above 0.05% w/v Na₂EDTA, one cannot anticipate such a drastic drug destabilization.

In addition to this, at 0.075% w/v of Na₂EDTA the destabilizing effect starts and the thermograms are irregular. Abnormal thermal behavior has been considered as a mark of instability²⁰-²². Moreover, after this point the nature of drug fusion endotherm is found to undergo the drastic change in appearance.

The presence of antioxidants further improved the stabilization of vitamin C 2% w/v solution containing 0.05% w/v Na₂EDTA. The quantitative effects of different concentrations of antioxidants are represented in fig. 4. The

**TABLE 2: STATISTICAL PARAMETERS* OF VITAMIN C IN PRESENCE OF Na₂EDTA**

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Concentration</th>
<th>Correlation coefficient</th>
<th>Variance ratio</th>
<th>Standard error</th>
</tr>
</thead>
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<tr>
<td>Cysteine</td>
<td>0.001</td>
<td>0.993</td>
<td>277.262</td>
<td>9.225x10⁻³</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.993</td>
<td>251.108</td>
<td>8.003x10⁻³</td>
</tr>
<tr>
<td>Thiourea</td>
<td>0.001</td>
<td>0.992</td>
<td>251.108</td>
<td>9.968x10⁻³</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.992</td>
<td>254.136</td>
<td>9.090x10⁻³</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>0.001</td>
<td>0.989</td>
<td>186.001</td>
<td>1.149x10⁻²</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.994</td>
<td>322.090</td>
<td>8.164x10⁻³</td>
</tr>
</tbody>
</table>

Influence of different concentrations of antioxidants at pH 4.0 on the stability of vitamin C. *df=5, p<0.05.

**TABLE 3: EFFECT OF CONCENTRATION OF VARIOUS ANTIOXIDANTS ON THE STABILISATION OF VITAMIN C**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Cysteine</th>
<th>Thiourea</th>
<th>Nicotinic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>8.4</td>
<td>8.9</td>
<td>8.9</td>
</tr>
<tr>
<td>0.005</td>
<td>8.2</td>
<td>8.6</td>
<td>8.6</td>
</tr>
<tr>
<td>0.010</td>
<td>7.8</td>
<td>8.3</td>
<td>8.3</td>
</tr>
<tr>
<td>0.050</td>
<td>6.3</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>0.100</td>
<td>5.4</td>
<td>7.6</td>
<td>7.6</td>
</tr>
</tbody>
</table>

*Experiment is not performed. *The K values for vitamin C and vitamin C with Na₂EDTA are 15.1 and 9.5 respectively.
degradation kinetics was analyzed as before and the statistical parameters are presented in Table 2. The first order kinetic rate constant K was calculated from slope of initial phase of degradation, which are presented in Table 3. All the experiments were conducted in triplicate and only the mean values are presented in fig. 4 and Table 3.

It may be seen that the efficacy of cysteine, thiourea and nicotinic acid in protecting vitamin C continuously increased with its concentration even up to 0.1% w/v. This suggests that in the concentration range of 0.001–0.1% w/v, which in fact is considerably wide range under investigation, optimum stabilization was not achieved. The concentration range of all the antioxidants of the study was taken on the basis of the available literature. It may thus be concluded that the optimum concentration of cysteine, for 2% w/v solution of vitamin C adequately protected by Na$_2$EDTA at least, exceeds the general maximum of 0.1%.

Comparison of stabilization effects of different antioxidants was statistically evaluated by analysis of variance (ANOVA). The F value was found to be 4.714 (df=38, P<0.05), which indicates the significant difference between the three antioxidants studied. ANOVA was further extended by least significant difference (LSD) procedure. The results indicate, cysteine is significantly different in stabilizing vitamin C as compared to thiourea and nicotinic acid and there is no significant difference between thiourea and nicotinic acid.

Results obtained in the present investigation suggest that cysteine is a better stabilizer for vitamin C in aqueous solution as compared to either thiourea or nicotinic acid, at least in the concentration range of 0.001-0.1% w/v. The available results further suggest very clearly that the study should be extended covering certain concentration range in excess of a minimum of 0.1%. Depending upon the results of this extending investigation, the consideration may be given in terms of expressing the optimum effectiveness in terms of molar concentration, which undoubtedly would be the best method for such a comparison.

In conclusion, the concentration of Na$_2$EDTA for the stabilization of 2% w/v vitamin C in aqueous solution at pH 4.0, was found to be critical; less than 0.05% w/v is insufficient to tag the available heavy metal catalysts and more than 0.05% w/v deteriorates the drug stability in proportion to the added sequesterant. The differential thermal features strongly support in substantiating the possibility of an interaction between vitamin C and Na$_2$EDTA above the critical ratio of 2:0.05. The criticalness about the concentration of Na$_2$EDTA has not been suggested so far. The concentration range generally recommended is 0.01 to 0.075%. The results of present study strongly indicate that it is not safe to workout with the generalized value; the optimum concentration for each formulation needs to be worked out before any formulation development is undertaken. All the antioxidants used in this investigation improved the stability of vitamin C (optimally stabilized with Na$_2$EDTA) in aqueous solution. As against the generally accepted norms of using a maximum of 0.1% w/v of antioxidant, the present study suggests that none of the studied antioxidants provide optimum stabilization to vitamin C even at 0.1% w/v.

**ACKNOWLEDGEMENTS**

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REFERENCES