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# Glutathione stabilizes ascorbic acid in aqueous solution

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**Abstract**

This report shows that glutathione affects ascorbic acid degradation in aqueous solution resulting in a significant stabilization of the vitamin. For the GSH concentrations used in this work the effect was higher on diluted ascorbic acid solutions. It is also shown that for a fixed ascorbic acid concentration, as glutathione concentration increases the degradation reaction shifts from first to zero order.

*Keywords:* Ascorbic acid; Vitamin C degradation; Glutathione; Order of reaction; Stability in aqueous solution

## 1. Introduction

Beside the wide use of L-ascorbic acid (AA) in the prevention and treatment of vitamin C deficiencies, beneficial effects of this substance have been reported in several pathological conditions (Ovesen, 1984).

AA is also employed as an antioxidant in foodstuffs, cosmetic and pharmaceutical preparations.

Although the AA stability in different environmental conditions was extensively studied (Blaug and Hajratwala, 1972; Sidhu and Sudgen, 1992), its rapid degradation in aqueous solutions is still a major drawback for the design of various dosage forms for AA and its use (Alwood, 1984).

The aim of the present work was to investigate the effect of glutathione (GSH) on AA degradation in aqueous solutions. The endogenous tripeptide GSH, due to its free sulfhydryl group may serve as a nucleophile and as a reducing agent. In this sense glutathione may act as a preservative in ascorbic acid solutions by means of its transformation to the oxidized form (GSSG).

## 2. Materials and methods

GSH and AA were supplied by Sigma (St. Louis, MO, USA). Sterile distilled water was used as solvent. All other substances were of analytical grade. In order to simulate actual conditions of storage and use, experiments on AA solutions were carried out in the presence of air (Sidhu and Sudgen, 1992).

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Table 1
Composition and treatment of solutions containing ascorbic acid (AA) and glutathione (GSH)

| Sample | AA (M) | Vehicle | GSH (M) | Temp. (°C) | Duration |
|---|---|---|---|---|---|
| Control 1 | $3.0 \times 10^{-4}$ | $H_2O$ | — | 5 | 1000 h D |
| Control 2 | $3.0 \times 10^{-4}$ | $HCl/H_2O$ | — | 5 | 1000 h D |
| A | $3.0 \times 10^{-4}$ | $H_2O$ | $3.0 \times 10^{-4}$ | 5 | 250 days D |
| B | $3.0 \times 10^{-4}$ | $H_2O$ | $1.0 \times 10^{-3}$ | 5 | 250 days D |
| C | $3.0 \times 10^{-4}$ | $H_2O$ | $3.0 \times 10^{-3}$ | 5 | 250 days D |
| D | $3.0 \times 10^{-4}$ | $H_2O$ | $4.5 \times 10^{-3}$ | 5 | 250 days D |
| E | $6.0 \times 10^{-2}$ | $H_2O$ | — | 25 | 27 days L |
| F | $6.0 \times 10^{-2}$ | $H_2O$ | $3.3 \times 10^{-2}$ | 25 | 27 days L |
| G | $6.0 \times 10^{-1}$ | $H_2O$ | — | 25 | 27 days L |
| H | $6.0 \times 10^{-1}$ | $H_2O$ | $3.3 \times 10^{-2}$ | 25 | 27 days L |
| I | $6.0 \times 10^{-1}$ | $H_2O$ | — | 121 | 15 min ** |
| J | $6.0 \times 10^{-1}$ | $H_2O$ | $3.3 \times 10^{-2}$ | 121 | 15 min ** |

D, dark; L, light; **, sterilization by autoclave.

AA concentrations were determined by UV spectrophotometry or by HPLC. The UV absorption was measured with a Lambda 3A UV/Vis Perkin Elmer spectrophotometer. Different quartz cells (0.1 and 1.0 cm) were used, according to the AA concentration measured, and appropriate corrections were applied for the bathochromic shift observed during degradation (Touitou et al., 1991). All reported data represent the mean values of three separate experiments (reproducibility ± 3.5%).

HPLC measurements were performed for some of the solutions under investigation in order to confirm that non-altered AA was quantified by the UV method. AA was determined at 265 nm by using a Merck-Hitachi HPLC apparatus equipped with a variable UV detector. The detection was made on a reverse phase C18 column with a mobile phase composed of 12% acetonitrile in water, at a flow of 1 ml/min. L-ascorbic acid was used as external standard.

## 3. Results

Aqueous solutions of AA and different GSH concentrations, as well as control solutions, were prepared and submitted to various environmental conditions. Their compositions are given in Table 1.

Fig. 1 presents the percentage of AA, remaining in the aqueous solution kept in the dark at 5 ± 1°C, as a function of time. The initial AA concentration was $3.0 \times 10^{-4}$ M and the pH of the solution was 5.3. Experiments were also performed, in parallel, in an acidic solution (pH = 3.5) by addition of diluted HCl. As it is possible to observe in this plot, acidification led to a remarkable stabilization of the vitamin in solution. Nevertheless, after 1000 h, when no more vitamin was detected in the distilled water solu-

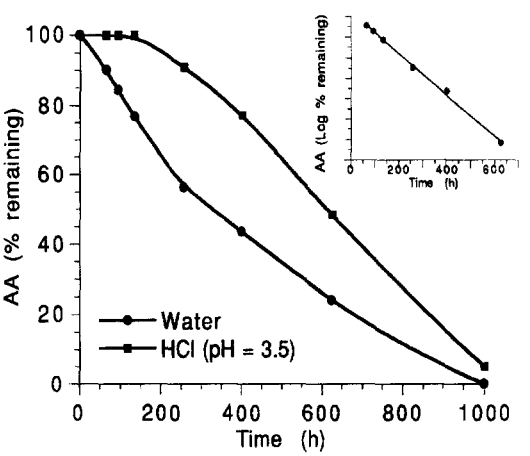


Fig. 1. Degradation with time (h) of L-ascorbic acid (AA) in distilled water and a in a pH 3.5 HCl solution. The initial concentration of AA was $3 \times 10^{-4}$ M and the temperature was kept at 5 ± 1°C throughout the experiment. Inset: semilogarithmic plot of AA degradation in distilled water.

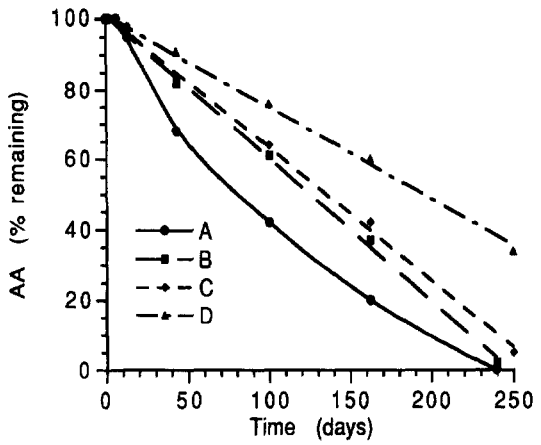


Fig. 2. Degradation with time (days) of L-ascorbic acid (AA) in the presence of glutathione (GSH) in aqueous solutions kept in the dark at a temperature of 5 ± 1°C; GSH concentrations were: $3.0 \times 10^{-4}$ M (A); $1.0 \times 10^{-3}$ M (B); $3.0 \times 10^{-3}$ M (C) and $4.5 \times 10^{-3}$ M (D). The initial concentration of AA was $3.0 \times 10^{-4}$ M.

tion, only a small percentage (5.2) was still present in the acidic solution. Furthermore, when the logarithm of the percentage of remaining AA vs. time was plotted (Fig. 1, inset), a linear trend ($R = 0.9985$) was obtained. This confirms (Sidhu and Sudgen, 1992) that AA degradation rate in distilled water is consistent with a first order reaction.

Fig. 2 shows the effect of increasing molar concentrations of GSH ($3.0 \times 10^{-4}$; $1.0 \times 10^{-3}$; $3.0 \times 10^{-3}$ and $4.5 \times 10^{-3}$ M) on AA degradation in water. It is possible to observe that as GSH concentration was increased a corresponding decrease of AA degradation was measured. It is noteworthy that, in the presence of an excess of GSH with respect to AA (i.e. [GSH] $\geq 10^{-3}$ M), the degradation reaction shifted from first to zero order, as indicated by the linear trends of plots B, C and D reported in Fig. 2 ($R$ range = 0.9989–0.9993).

Measurement of pH of the various compositions indicated that solutions of AA in $3.0 \times 10^{-3}$ M GSH and in dilute HCl (sample C and control 2, respectively, in Table 1) had the same pH value of 3.5; at the same time, the comparison between the plots representing AA degradation in these two solutions at 5°C (Fig. 1, plot HCl and Fig. 2, plot C), clearly indicates that a much more noticeable stabilization of the vitamin was obtained in the presence of glutathione. Consequently, the remarkable reduction of vitamin C degradation rate induced by GSH cannot be simply related to the effect of the acidic tripeptide on the pH of the solution.

As a further step, the effect of GSH on the degradation of more concentrated AA solutions $6.0 \times 10^{-2}$ and $6.0 \times 10^{-1}$ M (corresponding to 1.0 and 10.0% w/v, respectively) was tested at room temperature and after sterilization by autoclaving (E–J, Table 1). Measurements made by HPLC gave the results presented in Table 2.

From these results it can be seen that an aqueous solution of 1% ascorbic acid (sample E), kept at room temperature in the presence of light, lost, in 27 days, 21% of its initial concentration. The presence of glutathione in such a system (sample F) allowed a drug recovery of 98.5%. In the same experimental conditions, a lower degradation (8%) occurred in the 10-times more concentrated AA solution (sample G). The addition of glutathione (sample H) suppressed completely the AA degradation during this experiment. Moreover, glutathione prevented the degradation of ascorbic acid in solution during simulated sterilization conditions. While the recovery of ascorbic acid from a 10% w/v AA aqueous solution heated at 121°C for 15 min in an autoclave was only 75.5% of its initial concentration (sample I), in the same experimental conditions AA was completely recovered from the sample containing glutathione (J).

It must be pointed out that the effect of GSH on AA occurred in vitro and not, as usual, through an enzymatic process.

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Table 2
Effect of $3.3 \times 10^{-2}$ M of glutathione (GSH) on the degradation of ascorbic acid (AA) in aqueous solutions at room temperature and after sterilization

| Sample | AA (M) | GSH | Temp. (°C) | Time | AA recovered (%) |
|---|---|---|---|---|---|
| E | $6.0 \times 10^{-2}$ | – | 25 | 27 days L | 79.1 |
| F | $6.0 \times 10^{-2}$ | + | 25 | 27 days L | 98.5 |
| G | $6.0 \times 10^{-1}$ | – | 25 | 27 days L | 91.9 |
| H | $6.0 \times 10^{-1}$ | + | 25 | 27 days L | 101.9 |
| I | $6.0 \times 10^{-1}$ | – | 121 | 15 min ** | 75.5 |
| J | $6.0 \times 10^{-1}$ | + | 121 | 15 min ** | 101.1 |

L, light; **, sterilization by autoclave.

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