PHOTODEGRADATION OF CYANOCOBALAMIN IN THE PRESENCE OF ASCORBIC ACID

Kiran Qadeer, Tania Mirza, Zufi Shad and Iqbal Ahmed

ABSTRACT:
The photodegradation of cyanocobalamin at pH 4.0 in the presence of ascorbic acid has been studied. Hydroxocobalamin has been identified as a major degradation product of the reaction. Cyanocobalamin and hydroxocobalamin in degraded solutions have been assayed at 550 and 525nm by a two-component spectrophotometric method in which ascorbic acid does not interfere. The apparent first-order rate constants for the photodegradation of cyanocobalamin at pH 4.0 are in the range of 2.21-2.70 x 10^{-3} min^{-1}. These values indicate that the photodegradation of cyanocobalamin is enhanced in the presence of ascorbic acid by mutual interaction and needs protective measures to control the degradation of the vitamin.

INTRODUCTION:
Cyanocobalamin (vitamin B_{12}) occurs in nature as a cofactor that was originally isolated as cyanocobalamin and hydroxocobalamin (vitamin B_{12b}) from liver. It is a cobalt containing compound with a corrin ring and is sensitive to light. The chemistry and biochemical aspects of the vitamin have extensively been reviewed. Several workers have presented information on the chemical and photo stability of cyanocobalamin in aqueous solution and pharmaceutical preparations. The photodegradation of cyanocobalamin to hydroxocobalamin in aqueous solution is well known and the kinetics of the reaction has been studied. A recent kinetic study of the effect of riboflavin on the photodegradation of cyanocobalamin in aqueous solution has been reported.

The present work has been undertaken to study the photodegradation of cyanocobalamin in the presence of ascorbic acid since both vitamins are present in pharmaceutical preparations and ascorbic acid may lead to the destruction of cyanocobalamin.

MATERIALS AND METHODS
Cyanocobalamin, hydroxocobalamin and ascorbic acid were obtained from Merck & Co.

The following buffer system was used: acetate buffer, pH 4.0 (ionic strength 0.02M). All reagents and solvents were of the purest form available from Merck & Co.

PHOTODEGRADATION OF CYANOCOBALAMIN
Aqueous solution of cyanocobalamin (5x10^{-5}M, pH 4.0) containing several concentrations of ascorbic acid (1.0-5.0x10^{-4}M) were prepared (100 ml) and irradiated with a Philips HPLN 125W-high pressure mercury vapour fluorescent lamp (visible emission) from a distance of 30 cm. The assay of cyanocobalamin and hydroxocobalamin in the photodegraded solutions was carried out at suitable intervals.

THIN-LAYER CHROMATOGRAPHY (TLC)
TLC was carried out on 20-um silica gel GF 254 plates employing the mobile phase: A methanol-water (95:5, v/v); and B) 1-butanol-acetic acid-0.066 M KH_{2}PO_{4}-methanol (36:18:36:10, v/v). The spots of vitamin B_{12} and B_{12b} were detected under UV light (Uvitec lamp, UK).

ABSORBANCE MEASUREMENT
The absorbances of the pure and photodegraded solutions of cyanocobalamin were measured with a Shimadzu UV-1601 spectrophotometer using 10 mm...
silica cells and appropriate control solutions.

ASSAY OF CYANOCOBALAMIN AND HYDROXOCOBALAMIN

The assay of cyanocobalamin and hydroxocobalamin in photodegraded solutions was carried out by a specific two-components spectrophotometric procedure at 550 nm (pH 4.0) by the method of Ahmed et al. The method was validated with respect to accuracy, precision, specificity, detection limit, quantitation limit and linearity and range prior to its application of the present study.

RESULTS AND DISCUSSION

CHARACTERIZATION OF PHOTODEGRADATION PRODUCTS

In order to determine cyanocobalamin in photodegraded solution, it is necessary to ascertain the nature of photodegradation products present in the solution so as to apply an analytical method for the accurate assay of cyanocobalamin without any interference. A TLC examination of the solution using solvent system A and B showed the presence of cyanocobalamin and its photodegradation product, hydroxocobalamin. This product has previously been detected in the photodegraded solutions of cyanocobalamin. Hydroxocobalamin may further undergo degradation to irreversible oxidation products resulting in the deactivation of cyanocobalamin as follows.

\[
\begin{align*}
[\text{Co}^{+3}\text{CN}] & \xrightarrow{\text{hy}} [\text{Co}^{+3}\text{OH}] & \xrightarrow{\text{irreversible oxidation products}} \\
\text{Cyanocobalamin} & & \text{Hydroxocobalamin}
\end{align*}
\]

DETERMINATION OF CYANOCOBALAMIN

A specific two-component spectrophotometric method has been developed for the assay of cyanocobalamin and hydroxocobalamin in aqueous solution. It has been applied to determine cyanocobalamin and hydroxocobalamin in photodegraded solutions and parental preparations. The method has a reproducibility of +3% and is reliable for kinetics studies. It was validated under the present conditions in the presence of ascorbic acid before application to the assay of the two vitamins in photodegraded solutions. The results of the assay of cyanocobalamin and hydroxocobalamin in the solutions of cyanocobalamin photolysed in the presence of ascorbic acid at pH 4.0 are presented in Table 1. These results indicate that in the presence of increasing concentrations of ascorbic acid, the molar balance of cyanocobalamin and hydroxocobalamin is changed and the values of total moles/1 of the two vitamins are gradually decreased. This could probably be due to the fact that, in addition to cyanocobalamin, ascorbic acid may also cause the degradation of hydroxocobalamin to irreversible oxidation products. This has previously been observed in the degradation of cyanocobalamin in parenteral preparations.

KINETICS OF PHOTODEGRADATION

In the present study the values obtained for the concentrations of cyanocobalamin in the photodegradation reaction at pH 4.0 in the presence of ascorbic acid were subjected to kinetic studies. It was observed that cyanocobalamin follows an apparent first-order kinetics (kobs) which is in agreement with the kinetics of photodegradation of cyanocobalamin in the presence of ruboflan. The increase in kobs (Table 2) an increase in the concentration of ascorbic acid suggests that ascorbic acid is exerting a greater effect on the molecule resulting in enhanced degradation of cyanocobalamin as well as hydroxocobalamin. This effect may probable be due to the involvement of a redox reaction between ascorbic acid and cyanocobalamin causing degradation. On the basis of the results obtained in this study it may be concluded that in multivitamin preparations one of the factors involved in the loss of cyanocobalamin may be the presence of ascorbic acid. This may be overcome by protecting the preparations from light, changing the pH of the preparations and adding suitable stabilizers.
Table 1
Assay of cyanocobalamin and hydroxocobalamin in photodegraded solution of cyanocobalamin (5x10^{-5} M) in the presence of ascorbic acid (1x10^{-4} M) at pH 4.0

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Cyanocobalamin (Mx10^3)</th>
<th>Hydroxocobalamin (Mx10^3)</th>
<th>Total (Mx10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.426</td>
<td>0.108</td>
<td>4.534</td>
</tr>
<tr>
<td>30</td>
<td>4.141</td>
<td>0.203</td>
<td>4.344</td>
</tr>
<tr>
<td>60</td>
<td>3.886</td>
<td>0.272</td>
<td>4.158</td>
</tr>
<tr>
<td>90</td>
<td>3.668</td>
<td>0.362</td>
<td>3.970</td>
</tr>
<tr>
<td>120</td>
<td>3.398</td>
<td>0.483</td>
<td>3.881</td>
</tr>
</tbody>
</table>

* The loss in total molar balance in due to the oxidation of hydroxocobalamin by ascorbic acid

Table 2
Apparent first-order rate constant ($k_{obs}$) for the photodegradation of cyanocobalamin (5x10^{-5} M) in the presence of ascorbic acid (1.0 - 5.0 x 10^{-4} M) at pH 4.0

<table>
<thead>
<tr>
<th>Ascorbic acid concentration (Mx10^5)</th>
<th>$k_{obs}$ (min^{-1})</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>2.207</td>
<td>0.998</td>
</tr>
<tr>
<td>2.0</td>
<td>2.312</td>
<td>0.999</td>
</tr>
<tr>
<td>3.0</td>
<td>2.443</td>
<td>0.998</td>
</tr>
<tr>
<td>4.0</td>
<td>2.583</td>
<td>0.999</td>
</tr>
<tr>
<td>5.0</td>
<td>2.706</td>
<td>0.999</td>
</tr>
</tbody>
</table>

* The values of $k_{obs}$ are dependent on ascorbic acid concentration.
REFERENCES


