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STABILITY OF CYANOCOBALAMIN SOLUTIONS IN SUNLIGHT AND ARTIFICIAL LIGHT

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ABSTRACT

A comparison of the degradation of cyanocobalamin (vitamin Bu) solutions on exposure to sunlight and artificial light has been made using two concentrations, i.e., 950 µg/ml (corresponding to parentcral solutions) and 95 pgiml (1:10 dilution) at pH 4.0, 5.5 and 7.0. Cyanocobalamin and the pholopmduct, hydroxocobalamin (vitamin Bub), have been determined by a two component spectrophotometric method at 550 and 525 nm. Cyanocobalamin loss (950 µg/ml and 95 pg/ml) on exposure to sunlight for 2 hours amounts to 14.7-21.0% and 56.3-81-5% respectively indicating that light intensity is the rate determining factor in the reaction. In artificial light for 2 hours B_{12} (950 µg/ml and 95 µg/ml) loses 2.7-19.4% and 15-27.7% respectively. Thus B_{12} degradation in sunlight is greater compared to that of the artificial light, with the formation of some oxidation products, in addition to B_{12b}.

INTRODUCTION

Cyanocobalamin (vitamin $B_{12)}$ on exposure to daylight (Veer *et al.,* 1950; Baxter *et al,* 1953), sunlight (Baxter *et al.,* 1953; DeMerre and Wilson, 1956), and artificial light (Hogenkamp, 1975; Vogler *et al,* 1976) is degraded to hydroxocobalamin (vitamin B_{12b}) in weak acid solutions as indicated by the spectral variations of degraded solutions (Bayer, 1964; Ahmad et *al.,* 1992). The magnitude of the photochemical change depends upon the intensity and wavelength of light (Anderson *et al,* 1991). The stability (Hashmi, 1973; DeRitter, 1982; Connors *et al,* 1986) and photochemistry (Pratt, 1972; Brown, 1973; Kirschbaum, 1981) of cyanocobalamin and derivatives have been reviewed in detail.

Recently some studies on the photolysis of cyanocobalamin alone (Ahmad *et al,* 1992), in the presence of riboflavin (Ahmad and Hussain, 1992), and its stability in parenteral solutions (Ahmad and Hussain, 1993) have been reported. The present work deals with a study of the degradation of cyanocobalamin in presence of sunlight and artificial light in concentrations employed for parenteral solutions.

MATERIALS AND METHODS

Cyanocobalamin and hydroxocobalamin (Eur. P.) were obtained from Fluka (Switzerland) and their purity was confirmed by thin layer chromatography (TLC). All solvent and reagents were analytical grade or of the purest form available from BDH/Merck. Citric acid-disodium hydrogen phosphate, pH 4.0-7.0 (ionic strength 0.05 M) was employed as buffer system.

Photolysis

950 µg/ml and 95 µg/ml of cyanocobalamin were dissolved in the appropriate buffer solution (pH 4.0, 5.5 and 7.0) in a 100 ml volumetric flask (pyrex) and exposed directly to sunlight and to artificial light using Philips HPLN 125 W high pressure mercury vapour fluorescent lamp (emission at 405, 436, 454 and 577 nm) fixed at a distance of 30 cm in a radiation chamber.

Assay

The concentrations of cyanocobalamin and hydroxocobalamin in photolysed solutions were determined by a two-component spectrophotometric method at 550 and 525 nm (Ahmad et al, 1992).

RESULTS AND DISCUSSION

Cyanocobalamin is a photosensitive compound and is converted to hydroxocobalamin in aqueous solution under the influence of light (Ahmad *et al*, 1992). In order to study the photostability of cyanocobalamin, B_{12} solutions were exposed to sunlight and to artificial light at pH 4.0, 5.5 and 7.0 (pH requ[1]rements of B_{12} injections 3.8- 7.0; BP, 1988; USP, 1990) and B12 and B_{12b}, content of the degraded solutions was determined.

Cyanocobalamin solutions [950 ug/ml (7.0×10^{-4} M) and 95 ug/ml (7.0×10^{-5} M)] at pH 4.0 5.5 and 7.0 on exposure to sunlight (emission at 295-899 nix) degrade 21.0, 15.8, 14.7% and 81.5, 69.2, 56.3% respectively. The formation of hydroxocobalamin for the two solutions at these pH values was 15.2, 8.9, 8.7% and 78.9, 67.9; 55.2% respectively and the formation of irreversible oxidation products under the same conditions was 5.8, 6.9, 6.0% and 2.6, 1.3, 1.1% respectively (Table 1). The formation of the oxidation products was evident from slight fading of the $B_1$2 solutions and appears to take place through BIM. Exposure of B12e solutions (950 ug/ml and 95 ug/ml) at pH 4.0 to sunlight for two hours resulted in a loss amounting to 22.5 and 18.7%, with the solutions turning yellowish red and yellowish pink respectively.

Under the artificial light B_{12} solutions at the two concentrations at pH 4.0, 55 and 7.0 lose 19.4, 3.5,1.5% and 27.7, 6.9, 2.7% respectively. The formation of B_{12b} under the same conditions was 193, 3.4, 13% and 27.8, 7.1, 2.8% respectively (Table 2). There is a good agreement between the % loss of B_{12} and the % formation of B_{12b} indicating that artificial light (visible radiation) does not cause the formation of oxidation products. Thus sunlight (uv, visible and heat radiations) is more effective in degrading B_{12} solutions and the formation of irreversible oxidation products might have resulted by the accompanied thermal reaction as observed in the photolysis of methylcobalamin (Pratt, 1964).

Table 1

Assay of cyanocobalamin and hydroxocobalamin in vitamin B_{12} solutions exposed to sunlight* (25°-28°C)

Vit. B_{12} Concentration µg/ml	pH	Exposure time hours	Cyanocobalamin content*** µg/ml B_{12}	% initial concn.	% overall loss	% loss in terms of B_{12b}	% loss in terms** of oxidation products	Hydroxocobalamin (B_{12b}) µg/ml
950	4.0	2.0	750.7	79.0	21.0	15.2	5.8	144.9
95	4.0	2.0	17.5	18.5	81.5	78.9	2.6	75.0
950	5.5	2.0	799.8	84.2	15.8	8.9	6.9	84.8
95	5.5	2.0	29.3	30.8	69.2	67.9	1.3	64.5
950	7.0	2.0	810.2	85.3	14.7	8.7	6.0	82.7
95	7.0	2.0	41.5	43.7	56.3	55.2	1.1	52.4

*Exposure to sunlight between 8 a.m. and 10 a.m. in the month of December.
**Values obtained from % overall loss of B_{12}- % loss in terms of B_{12b}.
***No change was observed in control samples stored in the dark for 2 hours.

Table 2

Assay of cyanocobalamin and hydroxocobalamin in vitamin B_{12} solutions exposed to artificial light* (25°-27°C)

Vit. B_{12} Concentration µg/ml	pH	Exposure time hours	Cyanocobalamin content*** µg/ml B_{12}	% initial concn.	% overall loss	% loss in terms of B_{12b}	% loss in terms** of oxidation products	Hydroxocobalamin (B_{12b}) µg/ml
950	4.0	2.0	765.7	80.6	19.4	19.5	-	185.0
95	4.0	2.0	68.7	72.3	27.7	27.8	-	26.4
950	5.5	2.0	916.5	96.5	3.5	3.4	-	32.7
95	5.5	2.0	88.4	93.1	6.9	7.1	-	6.7
950	7.0	2.0	936.2	98.5	1.5	1.5	-	14.2
95	7.0	2.0	92.5	97.3	2.7	2.8	-	2.7

*Philips HPLN 125 W high-pressure mercury vapour fluorescent lamp (emission at 405, 436, 545 and 577 nm); light intensity $1.14 \pm 0.10 \times 10^{17}$ quanta s^{-1} determined by potassium ferrioxalate actinometry (Hatchard and Parker, 1956).

**Values obtained from % overall loss of B_{12} -% loss in terms of B_{12b}.

***No change was observed in control samples stored in the dark for 2 hours.

The aerobic photolysis of cyanocobalamin follows zero-order reaction (Vogler *et al.,* 1976; Ahmad *et al.,* 1992), in which the limiting factor is absorption of light. This is evident from the difference in the extent of photolysis (% loss) for 950 µg/ml and 95 µg/ml concentrations at all pH values under both the sunlight and artificial light and depends on the number of quanta absorbed to cause photochemical change in the solutions. Under the same quanta of light a dilute solution may degrade faster than the concentrated solution of the same species. This is an agreement with the observations of Baxter *et al* (1953) for the photolysis of low and high potency cyanocobalmin injections.

The photodegradation of B_{12} is greater at pH 4.0 compared to that at pH 7 as reported by Baxter et al. (1953). This appears to be due to greater susceptibility of the protonated form of B_{12} i.e., the 5,6-dimethylbenzimidazole moiety (pKa 33, Hill et al., 1962), to photolysis than the neutral form of the molecule.

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