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Short communication

Effect of nicotinamide on the photolysis of cyanocobalamin in aqueous solution

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Abstract

The photolysis of cyanocobalamin by visible light in the presence of nicotinamide at pH 1.0–7.0 has been studied. The second-order rate constants for the bimolecular interaction of these vitamins have been determined which vary from 9.50×10^{-3} (pH 1.0) to 1.25×10^{-3} M^{-1} min^{-1} (pH 7.0). The rate–pH profile indicates a gradual slow decrease in rate in the pH range 1–3 followed by a relatively fast decrease in the pH range 3–7. The protonated form of cyanocobalamin appears to be more susceptible to photolysis than the non-ionised form. Cyanocobalamin photolysis leads to the formation of hydroxocobalamin at pH 1.0–7.0.

Keywords: Cyanocobalamin; Hydroxocobalamin; Nicotinamide; Photolysis; Rate–pH profile

1. Introduction

Cyanocobalamin (vitamin B_{12a}) is sensitive to light [1–3] and is degraded to hydroxocobalamin (vitamin B_{12b}) in aqueous solution [4,5]. The kinetics of photolysis reaction has been studied at pH 1.0–12.0 [6]. The photolysis of cyanocobalamin is accelerated by nicotinamide [7]. Thiamine and nicotinamide or their degradation products destroy cyanocobalamin activity [8,9].

Cyanocobalamin and nicotinamide both are components of liquid vitamin preparations and are liable to interaction on exposure to light. So far, no quantitative information is available on the extent of their interaction in aqueous solution. The present work is based on a kinetic study of the effect of nicotinamide on the photolysis of cyanocobalamin at pH 1.0–7.0 using a previously reported two-component spectrophotometric method [6] and assessment of its optimum stability from the rate–pH profile. A similar work on the effect of riboflavin on the photolysis of folic acid in aqueous solution has recently been reported [10]. Such information may assist the formulator in the choice of appropriate pH for multivitamin preparations.

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2. Experimental

In view of the photosensitivity of cyanocobalamin, the experimental work was carried out in a dark chamber under subdued light. The solutions of cyanocobalamin containing nicotinamide were protected from light before irradiation. Freshly prepared solutions of the vitamins were used for each experiment to avoid any chemical or photochemical effects.

2.1. Materials

Cyanocobalamin, hydroxocobalamin and nicotinamide (Ph.Eur.) were obtained from Fluka (Switzerland) and their purity was confirmed by thin-layer chromatography (TLC). All reagents and solvents were analytical grade or of the purest form available from Merck/BDH. The following buffer systems were employed: KCl–HCl, pH 1.0–2.0; citric acid–Na_2HPO_4, pH 2.5–7.0; the ionic strength was 0.05 M in each case to minimise any salt effect.

2.2. Assay method

The assay of cyanocobalamin and its photoproduct hydroxocobalamin was carried out by a two-component spectrophotometric method at 525 and 550 nm (pH 4.0, acetate buffer), developed by Ahmad et al. [6]. The method was validated in the presence of nicotinamide to ensure its specificity under the present experimental conditions. The reproducibility of the method was examined by preparing several synthetic mixtures of cyanocobalamin and hydroxocobalamin in the presence of the highest concentration of nicotinamide (2.0×10^{-3} M) used in this work.

2.3. Photolysis

2.3.1. Radiation source

The study of the photolysis of cyanocobalamin in the presence of nicotinamide necessitates the choice of a radiation source with strong emission in the visible region. A Philips HPLN 125 W high-pressure mercury-vapour fluorescent lamp was, therefore, considered suitable for the present work, with emission at approximately 405, 436, 540 and 577 nm, the range absorbed by cyanocobalamin only. The number of quanta emitted by the radiation source was calculated as $1.12 \pm 0.10 \times 10^{17}$ quanta s^{-1} [11].

2.3.2. Method

A series of 5×10^{-5} M aqueous solutions of cyanocobalamin (100 ml) was prepared at the appropriate pH (0.05 M buffer) and sufficient amount of nicotinamide was added to each solution to produce several dilutions in the concentration range $0.2–2.0 \times 10^{-3}$ M. The pyrex flasks containing the solutions were placed in a radiation chamber and irradiated with the Philips lamp fixed at a distance of 30 cm from the centre of the flasks. The temperature of the solutions during irradiation was maintained at 25 ± 2 °C. Samples were withdrawn at appropriate intervals for spectrophotometric assay and TLC. Controlled solutions wrapped in aluminium foil were placed in the dark and assayed for cyanocobalamin content at the end of the reaction.

2.3.3. Thin-layer chromatography

Cyanocobalamin solutions photolysed in the presence of nicotinamide were examined by TLC for the degradation products using 250-μm silica gel GF254 plates and the following solvent systems: (A), 1-butanol-acetic acid-0.066 M potassium dihydrogen phosphate-methanol (36:18:36:10, v/v) [12] and (B), methanol-water (95:5, v/v) [13]. The spots were located visually (red colour) or under UV light.

3. Results and discussion

3.1. Composition of photolysed solutions

Chromatographic examination of the photolysed solutions of cyanocobalamin in the presence and absence of nicotinamide by TLC using solvent systems (A) and (B) revealed the formation of hydroxocobalamin as a photoproduct at pH 1.0–7.0. The identity of this product was confirmed by a comparison of its Rf values with that of the authentic compound in both solvent systems.

3.2. Assay of photolysed solutions

Since the photolysed solutions of cyanocobalamin (absorption maxima 361 and 550 nm) contain hydroxocobalamin (absorption maxima 351 and 525 nm) as a photoproduct, an interference may be expected in the assay of vitamin B_{12a}. This has been overcome by the use of a specific two-component spectrophotometric method [6] validated in the presence of nicotinamide. The results of the assay of varying concentrations of cyanocobalamin and hydroxocobalamin in the synthetic mixtures are given in Table 1. It has been observed that the values of R.S.D. fall within $\pm 3\%$ indicating that the method is reliable for the assay of the two vitamins in photolysed solutions and no interference is caused at the analytical wavelengths by nicotinamide (absorption maximum, 260–261 nm) [14,15] which absorbs in the UV region. The assay values of cyanocobalamin and hydroxocobalamin in photolysed solutions show an almost constant molar balance, with time, with respect to the initial concentration of cyanocobalamin and, thus, indicate the validity of the analytical data. The assay of control solutions stored in the dark showed negligible change in B_{12a} content during the reaction.

The assay method has been found to be convenient and specific to follow the kinetics of the photolysis reactions. An application of the method to generate kinetic data for a typical photolysis reaction at pH 4.0 has been made and the results are given in Table 2. The regression analysis of the kinetic data is given in Table 3. The correlation coefficient values (0.997–0.999) indicate a good linear relationship over the concentra-

Table 1
Analysis of synthetic mixtures of cyanocobalamin and hydroxycobalamin in presence of nicotinamide

Cyanocobalamin				Hydroxocobalamin			
Added ($M \times 10^5$)	Found ($M \times 10^5$)	Recovery (%)	R.S.D. (%)	Added ($M \times 10^5$)	Found ($M \times 10^5$)	Recovery (%)	R.S.D. (%)
5.00	4.83	96.60	1.86	0.99	0.96	96.96	0.02
4.47	4.41	100.44	0.30	1.76	1.80	102.27	2.63
4.03	4.07	100.99	0.87	2.03	2.12	104.43	1.73
3.60	3.57	99.16	0.92	2.43	2.50	102.88	2.02
3.02	3.09	102.31	1.53	3.00	2.93	97.66	0.85
2.47	2.41	97.57	2.08	3.43	3.52	102.62	1.08
2.04	2.08	101.96	1.48	4.04	4.04	100.00	1.14
1.54	1.53	99.35	2.53	4.58	4.60	100.43	1.28
1.34	1.29	96.26	1.87	5.12	5.32	103.90	0.80
1.02	1.02	100.00	2.15	5.50	5.48	99.63	0.96

Values expressed as a mean of three to five determinations.

Table 2
Photolysis of 5.0×10^{-5} M solution of cyanocobalamin in presence of nicotinamide (2.0×10^{-3} M) at pH 4.0 (citro-phosphate buffer)

Time (min)	Cyanocobalamin ($M \times 10^5$)	Hydroxycobalamin ($M \times 10^5$)	Total ($M \times 10^5$)
00	5.03	0.00	5.03
40	4.41	0.53	4.94
80	3.86	1.10	4.96
120	3.42	1.55	4.96
160	3.01	1.94	4.95
200	2.65	2.29	4.94

Table 3
Regression analysis of the kinetic data for first-order plots at pH 4.0

Irradiation time (min)	Cyanocobalamin ($M \times 10^5$)	Nicotinamide ($M \times 10^3$)	Range for Cyanocobalamin ($M \times 10^5$)	Slope	Intercept	Correlation coefficient
00–200	5.00	0.20	2.53–5.00	0.00139	−4.3139	0.9998
00–200	5.00	0.60	2.54–5.00	0.00122	−4.3409	0.9885
00–200	5.00	0.90	2.65–5.00	0.00139	−4.2996	0.9998

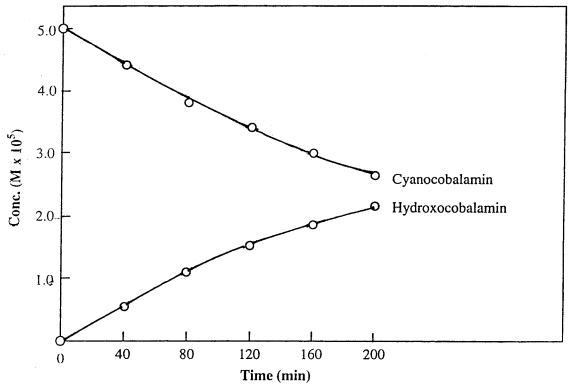

Fig. 1. Photolysis of cyanocobalamin solution in presence of 2.0×10^{-3} M nicotinamide (pH 4.0).

Table 4
Apparent first-order rate constants ($k_{obs} \times 10^3$ (min^{-1})) for the photolysis of cyanocobalamin at pH 1.0–7.0 in presence of nicotinamide ($0.2–2.0 \times 10^{-3}$ M)

pH	Nicotinamide ($M \times 10^3$)					
	0.20	0.60	0.90	1.20	1.50	2.00
1.00	5.18	5.39	5.57	5.76	5.96	6.18
1.25	5.07	5.30	5.43	5.63	5.80	6.10
1.50	4.95	5.16	5.30	5.48	5.64	5.93
1.75	4.19	4.40	4.56	4.72	4.88	5.16
2.00	4.14	4.37	4.50	4.66	4.79	5.07
2.25	3.89	4.09	4.24	4.37	4.54	4.77
2.50	3.68	3.87	4.03	4.16	4.19	4.53
2.75	3.40	3.87	4.01	4.14	4.14	4.49
3.00	3.39	3.57	3.68	3.80	3.94	4.24
3.25	3.26	3.42	3.53	3.65	3.78	3.98
3.50	3.11	3.25	3.34	3.45	3.57	3.74
3.75	3.05	3.18	3.27	3.36	3.45	3.61
4.00	2.99	3.11	3.19	3.28	3.36	3.49
4.25	2.76	2.85	2.92	3.02	3.06	3.18
4.50	2.51	2.58	2.65	2.72	2.76	2.87
4.75	1.85	1.92	1.98	2.05	2.09	2.18
5.00	1.55	1.61	1.66	1.70	1.76	1.80
5.25	1.38	1.44	1.47	1.52	1.56	1.64
5.50	1.27	1.31	1.34	1.39	1.43	1.49
5.75	1.15	1.20	1.24	1.27	1.31	1.37
6.00	0.92	0.97	0.99	1.02	1.06	1.09
6.25	0.53	0.54	0.57	0.62	0.64	0.69
6.50	0.48	0.52	0.53	0.56	0.58	0.64
6.75	0.45	0.47	0.48	0.52	0.54	0.57
7.00	0.41	0.46	0.47	0.49	0.51	0.55

Table 5
Second-order rate constants (k_2 (M^{-1} min^{-1})) for the photochemical interaction of cyanocobalamin and nicotinamide at pH 1.0–7.0

pH	k_2 (M^{-1} min^{-1})	Correlation coefficient
1.00	0.570	0.99802
1.25	0.559	0.99906
1.50	0.550	0.99825
1.75	0.535	0.99992
2.00	0.519	0.99921
2.25	0.485	0.99968
2.50	0.475	0.98841
2.75	0.450	0.98992
3.00	0.420	0.99520
3.25	0.385	0.99975
3.50	0.349	0.99945
3.75	0.305	0.99305
4.00	0.275	0.99960
4.25	0.235	0.99975
4.50	0.205	0.99859
4.75	0.190	0.99850
5.00	0.155	0.99342
5.25	0.140	0.97857
5.50	0.130	0.98885
5.75	0.115	0.97857
6.00	0.099	0.99378
6.25	0.097	0.99587
6.50	0.080	0.98791
6.75	0.077	0.99963
7.00	0.075	0.98978

tion range employed and the assay method is sufficiently precise to enable kinetic treatment of the analytical data.

3.3. Kinetics of photolysis

The kinetics of the photolysis of cyanocobalamin in the presence of nicotinamide has been studied at pH 1.0–7.0 [16]. A kinetic plot for the reaction at pH 4.0 (Fig. 1) indicates a gradual loss of cyanocobalamin with concomitant formation of hydroxocobalamin as a function of time. The apparent first-order rate constants (k_{obs}) for the photolysis reactions at pH 1.0–7.0 are given in Table 4. The kinetic data indicate a definite increase in k_{obs} with an increase in nicotinamide concentration at all pH values. Thus, the photolysis of cyanocobalamin is accelerated by nicotinamide as observed by Patel and Soni [7]. In order

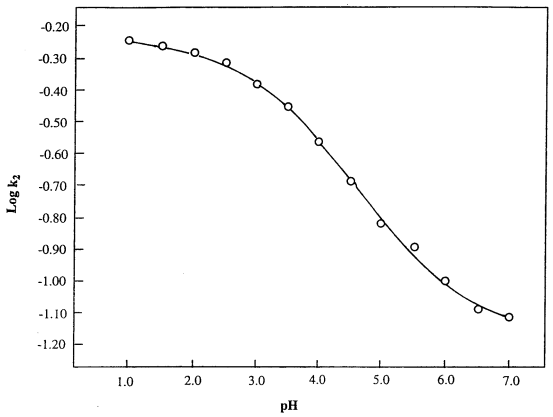

Fig. 2. log k_2–pH profile for the photolysis of cyanocobalamin in presence of nicotinamide.

to develop a correlation between the rate constants and the nicotinamide concentrations, k_{obs} values were plotted against the respective molar concentrations of nicotinamide and the second-order rate constants (k_2) for the bimolecular interaction of cyanocobalamin and nicotinamide were determined from the slopes of the straight lines (Table 5).

3.4. Rate–pH profile

The log k_2–pH profile for the photolysis of cyanocobalamin in the presence of nicotinamide (Fig. 2) shows a gradual slow decrease in rate in the pH range 1–3 followed by a relatively fast decrease in the pH range 3–7 and is in general agreement with the profile of cyanocobalamin alone in this region [6].

Cyanocobalamin (pK_a 3.3) [17] and nicotinamide (pK_a 3.3) [18] both have the same pK_a values and, therefore, predominantly exist in the protonated form at pH values below 3.0 (i.e. more than 66%). The log k_2–pH profile indicates that the rate of photolysis at pH 1.0 is about two-fold compared with that of pH 4.0 and about eight-fold compared with that of pH 7.0 (Table 5). Thus, the protonated form of cyanocobalamin is more susceptible to photolysis in the presence of nicotinamide than that of the non-ionised form and the rate is affected by the ionisation of the molecule. Both cyanocobalamin and nicotinamide undergo

deprotonation on a change in pH from 1.0 to 7.0, resulting in a gradual decrease in the rate. This might be due to a relatively greater photostability of the former vitamin in the non-ionised form. Nicotinamide accelerates the photolysis of cyanocobalamin but does not appear to alter the shape of the $\log k$–pH profile compared with that obtained in its absence [6].

3.5. Pharmaceutical implications

On the basis of the photodegradation behaviour of cyanocobalamin in the presence of nicotinamide, it may be suggested that liquid vitamin preparations containing both the vitamins are more stable to visible light at pH 6.0–7.0, however, other factors (i.e. light intensity, solvent, concentration, oxygen content, buffers etc.) should be given due consideration to achieve optimum stability of these preparations.

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