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MULTICOMPONENT SPECTROPHOTOMETRIC ASSAY OF CYANOCOBALAMIN, If YDROXOCOBALAMIN AND RIBOFLAVIN

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ABSTRACT

The photolysis of cyanocobalamin to hydroxocobalamin in aqueous solution is influenced by riboflavin. A multicnmponent spectmphotometric method has been developed for the simultaneous determination of cyanocobalamin, hydroxocobalamin and riboflavin in degraded solutions by absorbance measurements at 550, 525 and 445 nm. The relative standard deviation of the method is 25% and the recoveries range from 97.3-103.3%. The method has been applied to study the photolysis of cyanocobalamin in presence of riboflavin.

Introduction

The official methods (B.P., 1988) for the assay of riboflavin (vitamin B_2), cyanocobalamin (vitamin B12), and hydroxocobalamin (vitamin B_{12b}) in aqueous solution are based on the measurement of absorbance at 444, 361 (U.S.P., 1990), and 351 nm, respectively. However, in vitamin mixtures and their degraded solutions problems may arise in the assay of individual vitamins due to mutual interference or from the absorbance contribution of degradation products at the analytical wavelengths. Multicomponent spectrophotometric methods have been developed for the assay of riboflavin (Ahmad *et al.*, 1973; Fasihullah, 1988; Ahmad and Lipson, 1990), formylmethyl0avin (Ahmad *et al.*, 1980; Heelis *et al*, 1980; Ahmad and Fasihullah, 1991), cyanocobalamin (Ahmad *et al.*, 1992) and degradation products and applied to the hydrolytic, thermal and photodegradation studies of vitamin B_2 and B_{12}.

Interactions between various components of B-vitamins may occur in all types of dosage forms, particularly the liquid preparations. In certain cases, even inert formulation additives and diluents may be involved in these interactions as they alter pH, supply moisture, or introduce trace metals or other reactive contaminants to the preparation (Macek, 1960). Thermal, photochemical or redox reactions may also enhance vitamin interactions and an understanding of the mode of these reactions may be helpful in achieving the stabilization of Biz preparations (Kirschbaum, 1981).

Riboflavin is known to influence the photodegradation of cyanocobalamin to hydroxocobalamin (Baxter *et al*, 1953) and further oxidation products (Patel and Soni, 1964), however, no quantitative information is available on this reaction. In the present work a multicomponent spectrophotometric method has been developed for the assay of cyanocobalamin, hydroxocobalamin and riboflavin in photolysed solutions. Determination of the concentrations of the three compounds in the mixture requires the solution of three simultaneous equations. If Beer's law is followed by each component, these equations are linear and can be solved using matrix method (Bauman, 1962). The assay method has been applied to study the riboflavin induced photodegradation of vitamin B_{12}.

Materials and Methods

Cyanocobalamin, hydroxocobalamin and riboflavin (Ear. P.) were obtained from Fluka (Switzerland) and the[i]r purity was confirmed by thin-layer chromatography (TLC). All solvents and reagents were analytical grade or of the purest form available from BDH/Merck. The following buffers were used for the photolysis reactions: potassium chloride - hydrochloric acid, pH 1.0-2.0; citric acid-disodium phosphate, pH 23-8.0; the ionic strength was 0.05 M in each case.

The methods of photolysis and assay of cyanocobalamin solutions have previously been described (Ahmad et at, 1992), except that suitable quantities of riboflavin were added to cyanocobalamin solutions before photolysis and the absorbance measurements were carried out at 550, 525 and 445 nm for the hree-component assay.

Results and Discussion

Choice of wavelengths

The most important consideration in multicomponent spectrophotometric analysis is the choice of appropriate analytical wavelengths. The essential requirements for this choice include the possession of high sensitivity of measured absorbance and minimum interference from instrumental factors (Willard *et al*, 1981; Knowles and Burgess, 1984). The wavelengths selected for the multicomponent assay of cyanocobalamin, hydroxocobalamin and riboflavin correspond to the visible absorption mamma of the three components (i.e. 550, 525 and 445 nm), are quite distinct from each other and, therefore, most suitable for the analytical work. There is no interference from riboflavin at the absorption maxima of cyanocobalamin and hydroxocobalamin in this region as depicted by the respective absorption spectra of these compounds (Ahmad *et al*, 1990; 1992). Thus a mixture of vitamin Biz, Btu and B2 as such or in degraded solutions can be conveniently analysed by a three-component assay using the wavelengths 550, 525 and 445 nm.

The molar absorptivities of cyanocobalamin, hydroxocobalamin and riboflavin determined at pH 4.0 (acetate buffer) at 550, 525 and 445 nm are 8660, 7640, 3130 $M^{-1}cm^{-1}$, 4920, 8460, 3530 $M^{-1}cm^{-1}$, and 0,0, 12160 $M^{-1}cm^{-1}$ respectively, each value is a mean of three to five determinations (Hussain, 1987). These values have been used to calculate the concentrations of the individual compounds by substituting in the simultaneous equations that are solved by matrix method. The validity of Beer's law relation for all the compounds in the concentration range 1-10x10^4M, alone and in mixtures, was confirmed at the analytical wavelengths and the calibration data for cyanocobalamin and hydroxocobalamin have recently been reported (Ahmad *et al*, 1992).

Choice of assay pH

The absorption spectra of cyanocobalamin, hydroxocobalamin and riboflavin are affected by changes in pH (Bayer, 1964; Beckett and Stenlake, 1986). It is, therefore, necessary to carry out the absorbance measurements at a fixed pH to achieve maximum accuracy and reproducibility in the assay. This is particularly applicable to the study of cyanocobalamin degradation as a function of pH over a wide range since the absorption characteristics of Biz may considerably vary in the acidic and alkaline media. Hydroxocobalamin and riboflavin are assayed at pH 4-45 (acetate buffer) according to the B.P. (1988) method. Thus pH 4.0 (acetate buffer) was considered appropriate for the multicomponent assay of these compounds. Moreover, these compounds are quite stable in acidic media in the dark (Connors *et al*, 1986; Fasihullah, 1988).

Reproducibility of assay

The reproducibility of the assay method was tested by preparing a number of synthetic mixtures of cyanocobalamin, hydroxocobalamin and riboflavin in different proportions and then by assaying these compounds accord'ng to the newly developed method. The results of the three-component assays are reported in Table 1. The reproducibility of the method, as judged from the relative standard deviation (RSD), is 25% and the recoveries range from 973-1033%. The method is specific and appears to be satisfactory for the study of the photolysis of cyanocobalamin in presence of riboflavin.

In order to observe the effect of riboflavin on the photolysis of cyanocobalamin, reactions were carried out at pH 1,4 and 7 and the concentrations of vitamin B_{12} and B_2 in degraded solutions were determined by the proposed method. The assay results in terms of percent loss of the two vitamins are given in Table 2. The relatively greater loss of Biz in Presence of B_2 with increasing pH as compared to that of $_{B12}$ alone indicates that B_2 is influencing the photolysis of B_{12} and is being it self protected. This is evident from the loss of B_2 alone which is 4-5 times higher than that present in B_{12} solutions except at pH 1.0 where both species exist predominantly in the protonated forms (Biz pKa 33, Hill a *at*, 1962; B2 pKa 1.7, Windholz, 1983), in which there appears to be little interaction on photolysis. In view of the overlapping absorption spectra of B_{12} and B_2 it maybe suggested that B2 is probably acting as a photosensitizer in this reaction and it may enhance the destruction of Bit in vitamin B-complex preparations. Further work is in progress and the assay method has been applied to study the kinetics of photolysis to explain the behaviour of riboflavin in this reaction.

Table 1

Analysis of synthetic mixtures of cyanocobalamin, hydroxocobalamin and riboflavin*

Cyanocobalamin				Hydroxocobalamin				Riboflavin			
Added ($Mx10^5$)	Found ($Mx10^5$)	Recovery (%)	RSD (%)	Added ($Mx10^5$)	Found ($Mx10^5$)	Recovery (%)	RSD (%)	Added ($Mx10^5$)	Found ($Mx10^5$)	Recovery (%)	RSD (%)
0.326	0.321	98.5	2.16	0.769	0.779	100.2	0.26	6.555	6.771	103.2	0.30
0.480	0.481	100.2	0.75	1.116	1.152	103.2	0.17	5.907	5.899	99.9	0.19
1.016	0.998	98.2	1.09	0.628	0.629	100.2	1.05	5.786	5.980	103.3	1.76
1.303	1.320	101.3	2.42	0.846	0.856	101.2	1.66	5.058	5.063	100.1	0.09
1.564	1.527	97.6	1.89	1.148	1.144	99.6	0.41	4.248	4.310	101.5	1.82
2.059	2.043	99.2	0.60	1.180	1.208	102.4	1.58	3.399	3.370	99.1	0.08
2.619	2.623	100.2	1.39	1.064	1.062	99.8	2.15	2.549	2.571	100.9	1.81
2.606	2.579	99.0	0.27	1.539	1.555	101.0	0.16	1.699	1.685	99.2	0.21
3.127	3.021	96.6	0.41	1.128	1.165	103.3	0.91	1.097	1.089	99.3	0.72

*Values expressed as a mean of three to five determinations.

Table 2

Photolysis of cyanocobamin solution ($5x10^{-5}M$) in presence of equimolar concentration of riboflavin using Philips HPLN 125W lamp*

Solution	pH	Percent loss B_{12}	Percent loss B_2
B_{12}	1.0	37.1	
$B_{12} + B_2$	(0.05 M KCl-	38.6	8.2
B_2	HCl buffer)		9.1
B_{12}	4.0	30.7	
$B_{12} + B_2$	(0.05 M citric acid-	48.0	11.5
B_2	phosphate buffer)		48.3
B_{12}	7.0	6.5	
$B_{12} + B_2$	(0.05 M citric acid-	100.0	19.8
B_2	phosphate buffer)		86.5

*Emission at 405, 436,545 and 578 nm; exposure time: 3 hours.

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