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SPECTRAL STUDY OF PHOTOLYSIS OF AQUEOUS CYANOCOBALAMIN SOLUTIONS IN PRESENCE OF VITAMINS B AND C

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ABSTRACT

The UV and visible absorption characteristics of B and C vitamins have been studied in the pH range 2.0-7.0. The overlapping of the absorption bands of thiamine hydrochloride, nicotinamide, pyridoxine hydrochloride and ascorbic acid with cyanocobalamin in the UV region and those of riboflavin in the UV and visible region may influence the rate of photolysis of cyanocobalamin in aqueous solution due to mutual interaction on exposure to light. The spectral variations in cyanocobalamin solutions at pH 2.0-7.0 containing appropriate amounts of the individual B/C vitamins, during photolysis, have been monitored and the effect of pH on the rates of reaction has been discussed. The rates of photolysis, in general, decrease with an increase in pH probably due to gradual deprotonation of cyanocobalamin cation (B_{12} H^+) as indicated by the magnitude of absorbance loss at the maxima at 361 and 550 nm.

INTRODUCTION

Cyanocobalamin is a light sensitive vitamin (DeRitter, 1982; Connors *et al.*, 1986; British Pharmacopoeia, 1998) and is used as a component of vitamin B-complex/multivitamin preparations and parenteral nutrition solutions. The photolysis of cyanocobalamin in aqueous solutions has been studied by several workers (Baxter *et al.*, 1953; DeMerre and Wilson, 1956; Bayer, 1964; Pratt, 1964, 1972; Vogler *et al.*, 1976; Kirschbaum, 1981; Ahmad, 2001; Ahmad *et al.*, 1992, 1993, 2003; Ansari, 2002) and has been found to produce hydroxocobalamin (vitamin B_{12b}) by photoaquation (Pratt, 1972). The photolysis of cyanocobalamin is accompanied by variations in spectral characteristics of the degraded solutions indicating the transformation of the molecule to hydroxocobalamin (Bayer, 1964; Pratt, 1964; Vogler *et al.*, 1976; Ahmad, 2001; Ahmad *et al.*, 1992; Ansari, 2002). The present work is based on a spectral study of the photolysis of aqueous cyanocobalamin solutions at pH 2.0-7.0 in the presence of vitamins B and C.

MATERIALS AND METHODS

Cyanocobalamin, hydroxocobalamin, thiamine hydrochloride, riboflavin, nicotinamide, pyridoxine hydrochloride and ascorbic acid were obtained from Sigma Chemical Co. and were found to be chromatographically pure. All reagents and solvents used were analytical grade or of the purest form available from BDH/Merck. The following buffer systems were used: KCl-HCl, pH 2.0; citric acid-Na_2HPO_4, pH 2.5-7.0; the ionic strength was 0.05 M in each case.

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Photolysis:

A 5 x 10^{-5} M aqueous solution of cyanocobalamin at the required pH containing appropriate amounts of the individual B/C vitamins was placed in a 100 ml Pyrex flask and irradiated with Philips HPLN 125 W high-pressure mercury vapour fluorescent lamp (emission at 405, 436 and 545 nm) fixed horizontally at a distance of 30 cm from the centre of the flask. The temperature of the solution was maintained at 25±2°C during irradiation. Samples were withdrawn at appropriate intervals for spectral measurements.

Spectral measurements:

The UV and visible absorption spectra of B and C vitamins and photolysed solutions were measured with a Shimadzu UV-240 recording spectrophotometer using silica cells of 10-mm pathlength.

RESULTS AND DISCUSSION

***Spectral characteristics of B and C vitamins*:**

The UV and visible absorption spectra of various B (thiamine hydrochloride, riboflavin, nicotinamide, pyridoxine hydrochloride and cyanocobalamin) and C (ascorbic acid) vitamins were determined in the acid and neutral solutions at pH 2.0, 4.0 and 7.0. A typical set of absorption spectra at pH 7.0 is shown in Fig. 1. An examination of the spectral characteristics of these vitamins in the pH range 2.0-7.0 shows that there is no difference in the position of the absorption maxima of some of the vitamins (riboflavin, nicotinamide, cyanocobalamin) whereas others appear to be influenced by a change in pH in this region due to ionisation of the molecule (thiamine hydrochloride, pyridoxine hydrochloride, ascorbic acid).

Cyanocobalamin exhibits absorption maxima at 278, 361 and 550 nm (pH 2.0-7.0) while thiamine hydrochloride exhibits absorption maxima at 246 nm (pH 2.0-4.0) and 234 and 266 nm (pH 7.0), riboflavin at 223, 267, 374 and 444 nm (pH 2.0-7.0), nicotinamide at 262 nm (pH 2.0-7.0), pyridoxine hydrochloride at 291 nm (pH 2.0-4.0) and 254 and 325 nm (pH 7.0) and ascorbic acid at 242 nm (pH 2.0) and 265 nm (pH 4.0-7.0). These values are in agreement with the spectral data of these vitamins reported by British Pharmacopoeia (1998), Budavari (1989), Moffat (1986), Dowson *et al.* (1986), Sunshine (1981) and Hashmi (1973).

It may be concluded from the nature of the spectral characteristics of these vitamins that there is considerable overlapping of the absorption bands of cyanocobalamin and other vitamins in the UV (thiamine hydrochloride, riboflavin, nicotinamide, pyridoxine hydrochloride and ascorbic acid) and visible (riboflavin) region at pH 2.0-7.0 and that there is a probability of mutual interaction (e.g. complex formation, energy transfer, deactivation) on exposure to light. Cyanocobalamin and riboflavin both are capable of absorbing the visible bands (405, 436 and 545 nm) emitted by the radiation source. Thiamine hydrochloride, nicotinamide, pyridoxine hydrochloride and ascorbic acid absorb only in the UV region and would not be affected by the emission of the radiation source. Any change in the photochemical behaviour of cyanocobalamin in the presence of other vitamins individually would probably result from their interaction during irradiation.

***Spectral characteristics of photolysed solutions*:**

The UV and visible absorption spectra of cyanocobalamin solutions photolysed at pH 2.0, 4.0 and 7.0 were recorded at various time intervals to observe spectral variations during photolysis. The spectra of the aqueous solutions of cyanocobalamin (pKa 3.3, Kirschbaum, 1981) containing thiamine hydrochloride at pH 2.0, on photolysis for 6 hours, showed a gradual decrease at 550 nm

with concomitant increase at 525 nm, along with a hypsochromic shift from 361 to 351 nm and a hypochromic effect (Fig. 2). Isosbestic points (spectral crossover points) are observed at 295, 325, 355, 447 and 533 nm which indicated the presence of two absorbing species. Isosbestic points are a means of drug identification because absorbance of two species at these positions is independent of wavelength and pH (Smith and Stewart, 1981). It may be concluded from the spectral variations of cyanocobalamin solutions during photolysis at pH 2.0 (absorption maxima at 361 and 550 nm) that the molecule is undergoing transformation to hydroxocobalamin (absorption maxima at 351 and 525 nm). The spectral characteristics of the solution on irradiation for 6 hours are very similar to those of hydroxocobalamin which is the only photoproduct identified by spectral method (Bayer, 1964). Thus the spectral variations of cyanocobalamin solutions at pH 2.0 indicate the formation of hydroxocobalamin. Similar variations in the spectra are observed on the photolysis of cyanocobalamin solutions in presence of thiamine hydrochloride at pH 4.0 and 7.0 except that the magnitude of these variations is smaller than that observed at pH 2.0 for the protonated molecule (B_{12} H^+). The relatively slow rates of photolysis at pH 4.0 and 7.0 may be due to gradual deprotonation of cyanocobalamin cation which is more susceptible to degradation than the neutral molecule (Ahmad *et al.*, 1992).

The UV and visible absorption spectra of the photolysed solutions of cyanocobalamin (pH 2.0) in the presence of riboflavin are shown in Fig. 3. The cyanocobalamin absorption in the 250-500 nm region is dominated by the absorption of riboflavin which is also degraded during the photolysis of cyanocobalamin as evident from the loss of absorbance at 444 nm. The 361 nm absorption maximum of cyanocobalamin is overlapped by the 374 nm absorption maximum of riboflavin, therefore, the only variations observed in the spectra of cyanocobalamin during photolysis are due to 550 nm absorption maximum which shows a gradual decrease, with time, due to degradation of cyanocobalamin. The reaction at pH 4.0, compared to that at pH 2.0, appears to be slightly faster probably due to the nonionic nature of riboflavin at that pH (pKa 1.7; Moffat, 1986) and its influence on partially ionized (16.6%) cyanocobalamin. The rate is slowest at pH 7.0 at which both molecules exist in the non-ionized state and may have low interaction.

The spectral variations of photolysed solutions of cyanocobalamin (pH 2.0, 4.0, 7.0) in the presence of nicotinamide and those in the presence of pyridoxine hydrochloride (not shown) are very similar to those observed in the presence of thiamine hydrochloride as described above and are also indicative of the formation of hydroxocobalamin at variable rates in these solutions.

The spectral variations in cyanocobalamin solutions, on photolysis at pH 2.0 in the presence of ascorbic acid (pKa 4.2; Moffat, 1986), are shown in Fig. 4. The decrease in absorption at 361 and 550 nm maxima, with time, is greatest at pH 2.0, followed by that at pH 4.0 and 7.0. This suggests that the interaction between the predominantly protonated form of cyanocobalamin (B_{12} H^+) and the non-ionized form of ascorbic acid is highest at pH 2.0 and that between the largely non-ionized form of cyanocobalamin and ionized form of ascorbic acid (~ 50%) at pH 4.0 is relatively low. The magnitude of spectral changes indicates that the rate of photolysis is slowest at pH 7.0 at which cyanocobalamin is non-ionized and ascorbic acid is fully ionized, suggesting a low probability of interaction.

The spectral changes observed in cyanocobalamin solutions photolysed at pH 2.0-7.0 in the presence of vitamins B and C are greater than those of the cyanocobalamin solutions photolysed alone under the same conditions, indicating that vitamins B and C influence photolysis of cyanocobalamin by enhancing the rates of reaction.

The effect of an individual vitamin on the rates of photolysis of cyanocobalamin depends on the extent of their mutual interaction in the ground or excited state and susceptibility to undergo

degradation. The nature of the species (ionized/non-ionized) involved in the interaction would be determined by the pH at which the reaction is carried out. The photophysical and photochemical aspects of drug stability have been discussed by Moore (1996) in detail.

It may be pointed out that a careful study of the spectral variations of photolysed solutions of drugs may lead to the development of specific analytical methods for the assay of these substances and to follow the kinetics of degradation reactions. This has been illustrated with reference to riboflavin, cyanocobalamin and their degradation products (Ahmad and Rapson, 1990; Ahmad *et al*., 1973, 1980, 1992, 2003).

CONCLUSION

It may be concluded from the present study that vitamins B and C influence the photolysis of cyanocobalamin at pH 2.0-7.0 by accelerating the rate of degradation. The magnitude of spectral variations during photolysis may be considered as a measure of the rate of degradation which decreases with an increase in pH. Cyanocobalamin solutions in the presence of individual vitamins B and C appear to be most stable to photodegradation at pH 7.0. This pH is suitable for vitamin formulations provided it does not adversely affect the stability of other vitamins.

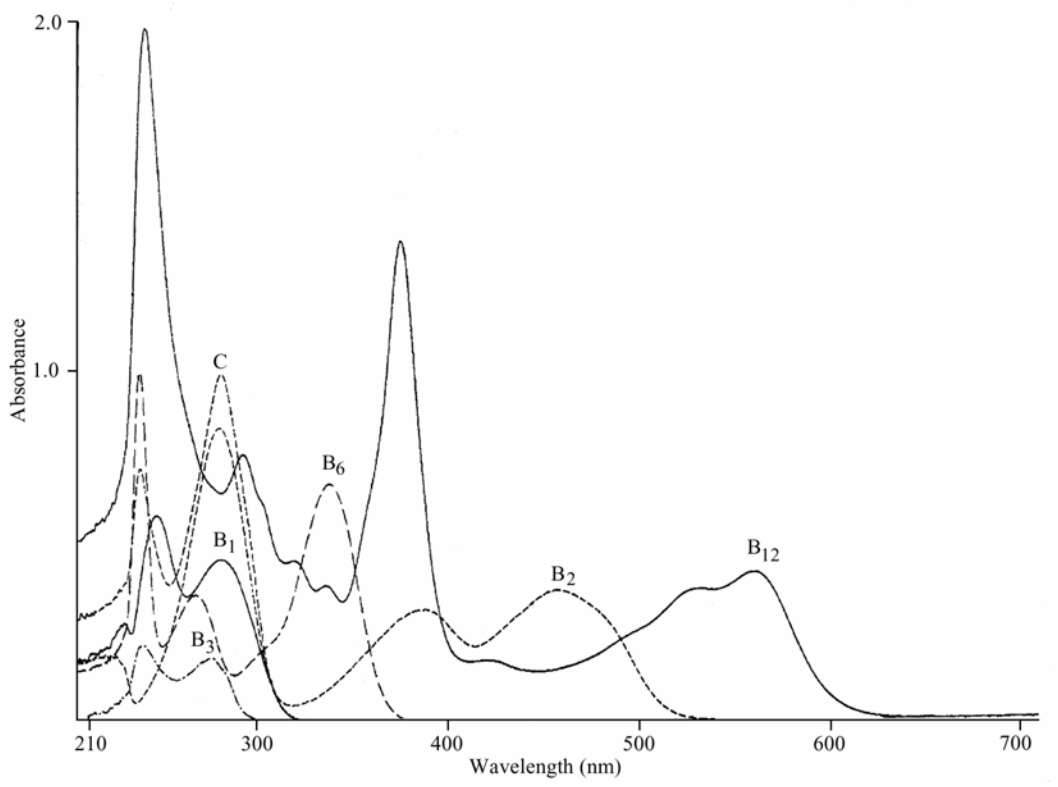

Fig. 1: UV and visible absorption spectra of vitamins B and C at pH 7.0: thiamine hydrochloride (B_1), riboflavin (B_2), nicotinamide (B_3), pyridoxine hydrochloride (B_6), cyanocobalamin (B_{12}) and ascorbic acid (C).

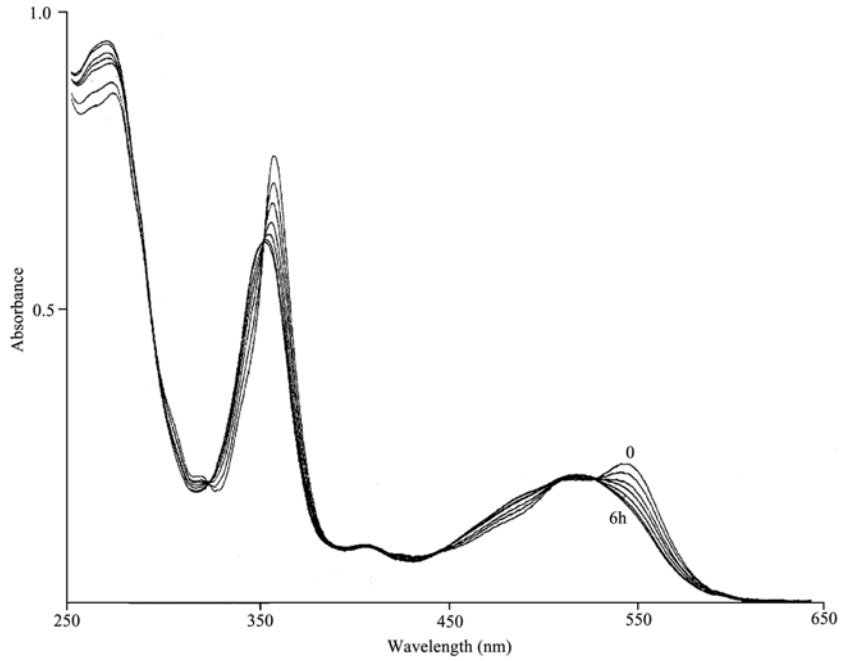

Fig. 2: Spectral variations of cyanocobalamin solution ($5x10^{-5}$ M) in presence of thiamine hydrochloride ($1x10^{-4}$ M) during photolysis at pH 2.0. Irradiation time: 6 hours.

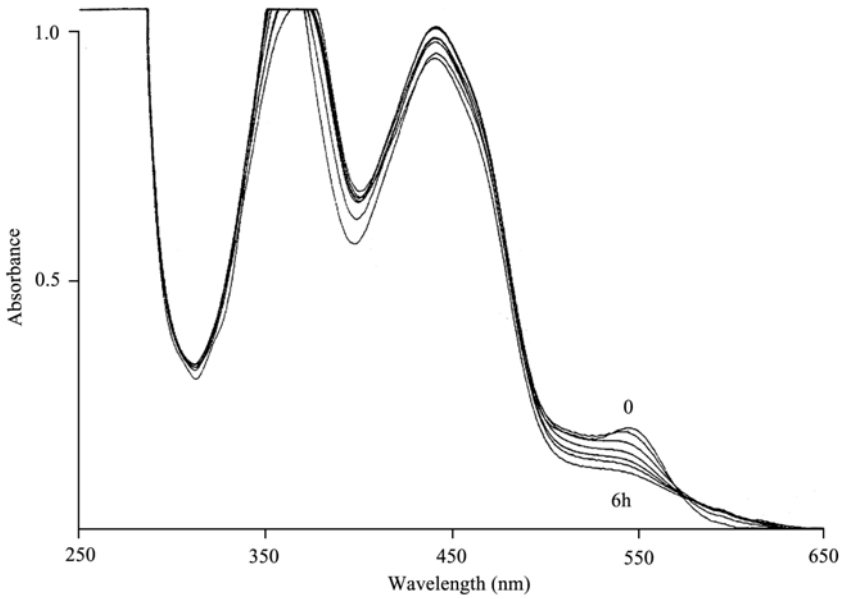

Fig. 3: Spectral variations of cyanocobalamin solution ($5x10^{-5}$ M) in presence of riboflavin ($1x10^{-4}$ M) during photolysis at pH 2.0. Irradiation time: 6 hours.

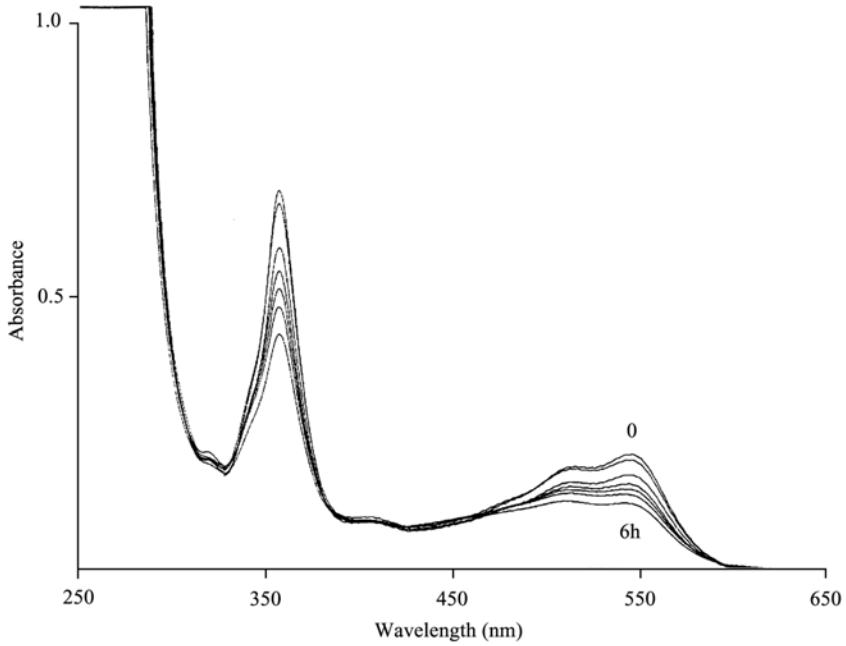

Fig. 4: Spectral variations of cyanocobalamin solution ($5x10^{-5}$ M) in presence of ascorbic acid ($1x10^{-3}$ M) during photolysis at pH 2.0. Irradiation time: 6 hours.

REFERENCES

Ahmad, I. and Hussain, W. (1993). Stability of cyanocobalamin solutions in sunlight and artificial light. *Pak. J. Pharm. Sci.* **6**(1): 23-28.

Ahmad, I. and Rapson, H.D.C. (1990). Multicomponent spectrophotometric assay of riboflavin and photoproducts. *J. Pharm. Biomed. Anal.* **8**: 217-223.

Ahmad, I., Ansari, I.A. and Ismail, T. (2003). Effect of nicotinamide on the photolysis of cyanocobalamin in aqueous solution. *J. Pharm. Biomed. Anal.* **31**: 369-374.

Ahmad, I., Beg, A.E. and Zoha, S.M.S. (1973). Studies on degradation of riboflavin and related compounds. II. Multicomponent spectrophotometric determination of thermal degradation products of riboflavin. *J. Sci. Univ. Kar.* **2**(1): 84-91.

Ahmad, I., Hussain, W. and Fareed, A.A. (1992). Photolysis of cyanocobalamin in aqueous solution. *J. Pharm. Biomed. Anal.* **10**: 9-15.

Ahmad, I., Rapson, H.D.C., Heelis, P.F. and Phillips, G.O. (1980). Alkaline hydrolysis of 7,8-dimethyl-10-(formylmethyl) isoalloxazine. A kinetic study. *J. Org. Chem.* **45**: 731-733.

Ahmad, M. (2001). A study of photochemical interaction of cyanocobalamin with thiamine and pyridoxine, Ph. D. Thesis, University of Karachi, Karachi.

Ansari, I.A. (2002). Photolysis and interaction of cyanocobalamin with some B-vitamins and ascorbic acid in parenteral solutions, Ph. D. Thesis, University of Karachi, Karachi.

Baxter, N., Horsford, J., Wokes, F., Norris, F.W. and Fernandes, S.J.G. (1953). Cyanocobalamin and hydroxocobalamin in vitamin B_{12} injections. *J. Pharm. Pharmacol.* **5**: 723-736.

Bayer, J. (1964). Zur spektrophotometrischen untersuchung von cyano-und hydroxocobalamin. *Pharmazie.* **19**: 602-605.

British Pharmacopoeia (1998). Her Majesty's Stationary Office, London, p.413.
Budavari, S., Ed. (1989). The Merck Index, 11th Edn., Merck & Co., Inc., Rahway, NJ, pp.130, 1304, 1569, 1577.
Connors, K.A., Amidon, G.L. and Stella, V.J. (1986). Chemical Stability of Pharmaceuticals, 2nd Edn., Wiley, New York, pp.377-384.
DeMerre, L.J. and Wilson, C. (1956). Photolysis of vitamin B_{12}. *J. Am. Pharm. Assoc. Sci. Ed.* **45**: 129-134.
DeRitter, E. (1982). Vitamins in pharmaceutical formulations. *J. Pharm. Sci.* **71**: 1073-1096.
Dowson, R.M.C., Elliott, D.C., Elliott, W.H. and Jones, K.M. (1986). Data for Biochemical Research, 3rd Edn., Clarendon Press, Oxford, pp.116-138.
Hashmi, M.H. (1973). Assay of Vitamins in Pharmaceutical Preparations, Wiley, New York.
Kirschbaum, J. (1981). Cyanocobalamin. In: Analytical Profiles of Drug Substances (Florey, K., Ed.), Vol. 10, Academic Press, New York, pp.183-288.
Moffat, A.C., Ed. (1986). Clarke's Isolation and Identification of Drugs, Pharmaceutical Press, London, pp.360, 496, 807, 959.
Moore, D.E. (1996). Photophysical and photochemical aspects of drug stability. In: Photostability of Drugs and Drug Formulations (Tonnesen, H.H., Ed.), Tayler & Francis, London, Chapter 2.
Pratt, J.M. (1964). The chemistry of vitamin B_{12}. Part II. Photochemical reactions. *J. Chem. Soc.* 5154-5160.
Pratt, J.M. (1972). Inorganic Chemistry of Vitamin B_{12}, Academic Press, New York, p.258.
Smith, R.V. and Stewart, J.T. (1981). Textbook of Biopharmaceutic Analysis, Lea & Febiger, Philadelphia, PA, p.129.
Sunshine, I. (1981). CRC Handbook of Spectrophotometric Data of Drugs, CRC Press, Boca Raton, FL, pp.24, 80.
Vogler, A., Hirschmann, R., Otto, H. and Kunkely, H. (1976). Photochemistry of biologically important metal complexes. 1. Cyanocobalamin and related corrin complexes of rhodium (III). *Ber. Bunsenges Physik Chem.* **80**: 420-424.