Comparative Evaluation of Photostability of Commercially Available Intravenous Preparations of Cobalamins: A Kinetic Study

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Abstract

The photochemical degradation of cyanocobalamin (B_{12}) in various single ingredient and multi-ingredient B_{12} preparations are available in the market. Cyanocobalamin has been found to degrade to hydroxocobalamin (B_{12b}) as detected by thin layer chromatography and spectral changes. Cyanocobalamin and hydroxocobalamin have been analyzed by a two-component spectrophotometric method at their $ë_{max}$ i.e.550 and 525nm, respectively, with a precision of ± 3%. The photodegradation of B_{12} in these formulaions follows apparent first order rate constant (k_{obs}) from 0.44 – 5.05 x 10^{-3} min^{-1}.

The rate constants increase with a decrease in pH probably as a result of gradual protonation of the molecule which are highly prone to photodegradation compared to the neutral form. The degradation of B_{12} is greater in multi-ingredient preparations compared to that of single ingredient preparations. This may result from the interaction of other vitamins present with B_{12} leading to its degradation in multi-ingredient preparations. The rate-pH profile gives an indication of the greater loss of vitamin pH4.0 than that of pH7.0. Cyanocobalamin may be stabilized by maintaining the pH of the preparation near the neutral range where it shows the lowest range of photodegradation.

Keywords

Cyanocobalamin, Hydroxocobalamin, Photolysis, Kinetics, Stabilization

1. INRODUCTION

Vitamin B_{12} is related to the cobalamins; a class of octahedral Co(III) organometallic complexes. Its discovery was originated just about two centuries back when a fatal anemia caused by some abnormalities of digestive and assimilative organs later termed as pernicious anemia. It was treated successfully in 1926 by liver extract (raw) as treatment option. Patients with pernicious anemia lose the capacity to absorb

vitamin B_{12} via the physiological route which requires an extrinsic factor and prior binding to intrinsic factor secreted by stomach. In 1948, Merk isolated vitamin B_{12} as red needle like crystals.The vitamin was later named as cyanocobalamin (Jagerstad *et al*.,2003).

On the basis of corrinoid's nomenclature, cobalamin is a cobinamide in which 5,6-dimethylbenzimidazole is the aglycon linked by aglycosyl bond. The name of cobalamin is Coá-[á-(5,6- dimethylbenzimedazolyl)]- Coâ-'R' cobamide designated by the International Union of Nutritional Science (IUNS) and International Union of Pure and Analytical Chemistry (IUPAC). This ligand (R) can be replaced by R= -CN (Cyanocobalamin), -OH (Hydoroxocobalamin), -H_2O(Aquacobalamin), -NO_2(Nitritocobalamin), -SO_3(Sulfitocobalamin), -CH_3(Methylcobalamin) and -5'-deoxy-adenosyl (Adenosylcobalamin).The Class of Cobalamins having very complex structure containing central corrin nucleus. The corrin ring is comprised of 04 pyrrole rings. These four rings connected with each other through pyrrole nitrogens. The structure of cobalamin is a macrocyclic ring system and assigned into two parts. A planar structure and nucleotide part which are at right angle position. Corrinnucleus is the principal planar part which contain the central cobalt (Fig.1) (Jagerstad *et al*., 2003). The fifth ligand of the cobalt may be occupied covalently by different anionic groups. The normal requirement of vitamin B_{12} for males and females older than 13 years is 2.2 µg/day and for children aged 1-13 years is 0.9-1.8µg/ day (Zempleni *et al*., 2007).

The stability of vitamin B_{12} differs markedly with the variable factors affecting on it such as solvents, ionic strength, pH, light, enzymatic and trace metallic catalyst, and other reactants such as oxygen species etc. Destabilization of vitamin B_{12} may occur due to interactions between various components present in the formulation particularly in the liquid dosage form. In certain cases, even inert additives and diluents may be involved in these interactions as they alter pH, moisture content or introduce trace metals or other reactive species to the preparation. Thermal, photochemical or red-ox reactions may also enhance these interactions and an understanding of the mode of these reactions may be helpful in achieving the stabilization of B_{12} preparations (Ahmad and Hussain,1992).

It has been observed that majority of vitamins in any form (i.e. solid or liquid formulations) may be degraded by the exposure of radiations. The degradation of B_{12} in solution (aqueous) is directly related to the amount of the radiation dose. B_{12}in aqueous solution when undergoes photolysis showed the color change from light the pink to yellow, which is due to the formation of its photo product, B_{12b} (Baxter *et al*., 1953; Juanchi *et al*., 2000). Light splits the organometallic bond giving a 5'–deoxyadenosyl radical capable of further reaction, and cob(II)alamin (Hogenkamp *et al*., 1963). The absorbance of aqueous solution of B_{12} at 361 nm is decreased due to the formation of B_{12b} (Bayer, 1964). The cyanide group (-CN) in B_{12} can be removed by photolysis or reduction to give aquocobalamin (B_{12a}), which in turn can react with various acids to form the bromide, chloride, cyanate, nitrate, sulphate, and thiocyanate (Dolphin,1971). In acidic medium, B_{12b} protonated and converted into aquocobalamin (B_{12a}). Thus in solution it exists as an equilibrium mixture with B_{12} and more stable at acidic pH, commercial products are likely to be mainly in the aquocobalamin form (Smith *et al*.,

1962). Ahmad *et al.* (1992) have studied the photodegradation of cyanocobalamin solution and have determined the log *k*-pH profile. The vitamin B_{12} is most suitable in the pH range 6.0-7.0 and this range is suitable for retaining the pH of the vitamin formulations.

B_{12b} is susceptible to further degradation and may give rise to irreversible oxidation products (Macek, 1960; Bonnet, 1963; Connors *et al*., 1986; Ahmad and Hussain, 1992; Ahmad, 2001; Ansari., 2002; Ahmad *et al*., 2003; Ansari *et al*., 2004; Ahamd*et al*., 2017; Vaid*et al*., 2018; Qadeer*et al*., 2021). The aerobic photolysis of B_{12} follows first-order reactions (Ahamd*et a*l., 2017; Vaid*et al*., 2018; Qadeer *et al*., 2021) in which the limiting factor is absorption of light and depends upon the number of quanta absorbed to cause photochemical change in the B_{12} solution (Baxter *et al*., 1953; Ahmad and Wahid, 1993).

2. MATERIALS AND METHODS

B_{12} and B_{12b} were procured from Fluka (Switzerland). Solvents and reagents of analytical grade were purchased from Merck. Employed buffer systems were: (Clark and Lub'sKCl-HCl, pH 1.0-2.0; Theorell and Stenhagen's citric acid-disodium phosphate buffer (pH 4.5–7.0); Sodium Acetate-acetic acid buffer (pH 4.0), used for the assay of cyanocobalamin and hydroxocobalamin in photolysed solutions).

The pharmaceutical and chemical equivalence of four different brands of Cyanocobalamin injections were investigated after collecting samples from the local market of Karachi, Pakistan. These samples were then coded as brand I, II, III and IV. Pure Cyanocobalamin was taken as standard in this study.

2.1. Storage of Commercial Samples

The commercial preparation of cyanocobalamin injections (Formulation I-IV) were used for the irradiation purpose to analyze the rates of photodegradation in the pH range 4.0 – 7.0.

2.2. Photolysis of Cyanocobalamin

A 3 x 10^{-5} M solution of B_{12} injection at pH 4.0, 4.5, 5.0, 6.0 and 7.0 was prepared. The buffer concentration was maintained at 0.02 M to minimize the salt effect on the reaction. A 25 ml Pyrex volumetric flask containing cyanocobalamin solutions was kept in the radiation chamber at specified place and illuminated with HPLN 125 W Philips lamp (405, 436, 545 and 578 nm) fixed at distance of 25 cm outside the chamber. Samples were withdrawn at interval of 15 min. up to 1 hour for spectrophotometric assay and chromatographic examination. The temperature of the solution was found to be within range 25–27^{o}C during photolysis. Appropriate control solutions in Pyrex flask covered in aluminum foil were placed in dark area during the irradiation period.

2.3. Thin–layer chromatography (TLC)

TLC of photodegraded samples was done with the following:

Silica gel G plates, solvent systems, (a) 1–butanol/ acetic acid–0.006 M KH_2PO_4/methanol (36:18:36:10, v/v) (Cima*et al.* 1962) and (b) methanol–water (95:5, v/v) (Covello *et al.* 1964). Red spots of $B_{12}$and B_{12b} appeared on plates.

2.4. Absorbance measurements

The absorbance of photolysed samples of cyanocobalaminand hydroxocobalamin were determined by using the Shimadzu UV–1601 spe-

ctrophotometer.

2.5. Light intensity measurement

In order to calculate the intensity of Philips HPLN 125 W, high pressure mercury lamp, Ferrioxalateactinometrymethod was applied (Hatchard and Parker, 1956). The calculated value is $1.18 \pm 11 \times 10^{17}$ quanta s^{-1}.

2.6. Two-Component Spectrophotometric Assay of B_{12} and B_{12b}

Cyanocobalamin and hydroxocobalamin contents of the irradiated solution were determined by the two-component spectrophotometric method. The analytical wavelength of 525nm and 550 nm and the respective values of the molar absorptivity were used as reported by Ahmad *et al*. (1992).

A sample (5 ml) containing the 2.5 ml of photodegraded solution and the remaining volume was fulfilled with the buffer (acetate buffer at pH 4.0). The absorbance was observed at the above mentioned wavelengths.

The concentration of the cyanocobalamin and hydroxocobalamin were calculated by resolving the simultaneous equation given as follows:

$$A_1 = {}_1K_{1\cdot 1}C + {}_2K_{1\cdot 2}C \quad \text{——————} \quad (1a)$$

$$A_2 = {}_1K_{2\cdot 1}C + {}_2K_{2\cdot 2}C \quad \text{——————} \quad (1b)$$

Where;

A_1= mixtureabsorbance at $ë_1$: 550 nm

A_2= mixture absorbance at $ë_{2:}$ 525 nm

${}_1C$ = concentration of component 1(B_{12})

${}_2C$ = concentration of the component 2 (B_{12b})

The solution of the equation (1a) and (1b) for ${}_1C$ and ${}_2C$ is as,

$${}_1C = ({}_2K_2.A_1 - {}_2K_1.A_2) \,/\, ({}_1K_{1\cdot 2}K_2 - {}_2K_{1\cdot 1}K_2) \text{——————————(2a)}$$

$${}_2C = ({}_1K_1.A_2 - {}_1K_2.A_1) \,/\, ({}_1K_{1\cdot 2}K_2 - {}_2K_1.K_2) \text{——————————(2b)}$$

The values of the molar absorptivities at 525nm and 550nm used in this study were reported by Ahmed *et al*., 2014 and at 351nm and 361 nm are reported in Table 1.

2.7. Calculation of a Two-component Spectrophotometric Assay

The B_{12} and B_{12b} concentrations were determined using the above equations (i.e. 2a and 2b) in the photodegraded solutions. The reactions were performed at pH 4.0 - 7.0 (Table 2).

Table 1. Molar Absorptivities ($M^{-1}cm^{-1}$) of Cyanocobalamin and Hydroxocobalamin at pH 4.0

Compound	351 nm	361 nm	445 nm	525 nm	550 nm
Cyanocobalamin	17300	28050	3130	7640	8700
Hydroxocobalamin	25580	17440	3530	8640	4920

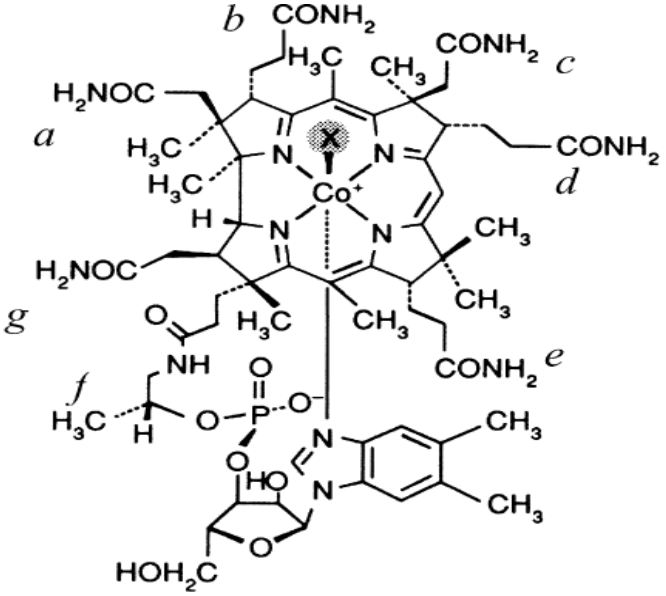
Table 2. Photolysis of Different Formulationsof Cyanocobalamin (I-IV) at pH 4.0-7.0

Formulation	pH	Time (min)	B_{12} (M x 105)	B_{12b} (M x 105)	Total (M x 105)
I	**4.0**	0	2.50	0.55	3.05
		15	2.45	0.59	3.04
		30	2.40	0.65	3.05
		45	2.30	0.75	3.05
		60	2.27	0.78	3.05
	4.5	0	2.55	0.47	3.02
		15	2.50	0.55	3.05
		30	2.46	0.59	3.05
		45	2.45	0.60	3.05
		60	2.35	0.67	3.02
	5.0	0	2.61	0.44	3.05
		15	2.56	0.48	3.04
		30	2.51	0.53	3.04
		45	2.49	0.54	3.03
		60	2.44	0.57	3.01
	6.0	0	2.55	0.50	3.05
		15	2.51	0.53	3.04
		30	2.49	0.54	3.03
		45	2.47	0.58	3.05
		60	2.44	0.61	3.05
	7.0	0	2.51	0.58	3.09
		15	2.48	0.54	3.02
		30	2.47	0.55	3.02
		45	2.44	0.65	3.09
		60	2.42	0.61	3.03
II	**4.0**	0	1.90	1.05	2.95
		15	1.63	1.17	2.80

		30	1.53	1.31	2.84
		45	1.47	1.38	2.85
		60	1.37	1.52	2.89
	4.5	0	2.10	0.85	2.95
		15	2.00	0.94	2.94
		30	1.89	1.06	2.95
		45	1.78	1.16	2.95
		60	1.67	1.28	2.95
	5.0	0	2.25	0.70	2.95
		15	2.17	0.73	2.90
		30	2.09	0.84	2.93
		45	2.00	0.95	2.95
		60	1.90	1.04	2.94
	6.0	0	2.25	0.70	2.95
		15	2.22	0.68	2.90
		30	2.19	0.72	2.91
		45	2.17	0.77	2.94
		60	2.14	0.79	2.93
	7.0	0	2.25	0.70	2.95
		15	2.24	0.70	2.94
		30	2.21	0.72	2.93
		45	2.20	0.75	2.95
		60	2.18	0.76	2.94
III	**4.0**	0	1.88	0.02	1.90
		15	1.78	0.13	1.91
		30	1.70	0.20	1.90
		45	1.68	0.26	1.94
		60	1.52	0.40	1.92

	4.5	0	1.84	0.06	1.90
		15	1.74	0.17	1.91
		30	1.71	0.19	1.90
		45	1.64	0.29	1.93
		60	1.58	0.33	1.91
	5.0	0	1.89	0.01	1.90
		15	1.85	0.03	1.88
		30	1.81	0.06	1.87
		45	1.79	0.09	1.88
		60	1.71	0.10	1.81
	6.0	0	1.86	0.04	1.90
		15	1.84	0.07	1.91
		30	1.82	0.10	1.92
		45	1.80	0.13	1.93
		60	1.79	0.14	1.93
	7.0	0	1.88	0.02	1.90
		15	1.86	0.05	1.91
		30	1.84	0.06	1.90
		45	1.85	0.06	1.91
		60	1.82	0.08	1.90
IV	**4.0**	0	1.82	0.10	1.92
		15	1.78	0.13	1.91
		30	1.75	0.15	1.90
		45	1.73	0.19	1.92
		60	1.65	0.20	1.85
	4.5	0	1.78	0.12	1.90
		15	1.74	0.17	1.91
		30	1.73	0.18	1.91
		45	1.70	0.22	1.92

	5.0	0	1.85	0.05	1.90
		15	1.82	0.09	1.91
		30	1.81	0.09	1.90
		45	1.79	0.13	1.92
		60	1.77	0.16	1.93
	6.0	0	1.86	0.06	1.92
		15	1.83	0.08	1.91
		30	1.82	0.10	1.92
		45	1.80	0.12	1.92
		60	1.79	0.14	1.93
	7.0	0	1.85	0.05	1.90
		15	1.83	0.07	1.90
		30	1.82	0.08	1.90
		45	1.81	0.11	1.92
		60	1.80	0.12	1.92



X = OH → Hydroxo-Cbl
X = CN → Cyano-Cbl (vitamin B_{12})
X = CH_3 → Methyl-Cbl
X = 5'-deoxyadenosyl → Ado-Cbl (coenzyme B_{12})

Fig. 1: Structure of Vitamin B_{12} and biologically relevant cobalamin

3. RESULTS AND DISCUSSION

3.1. CYANOCOBALAMIN

3.1.1. Identification of Degradation Products of Cyanocobalamin

It has been reported that the photochemical degradation of B_{12}leads to the formation of B_{12b} in acidic solution (Connors *et al.,* 1986). It was necessary to confirm the presence of hydroxocobalamin as a degradation product in the photolysed solution of cyanocobalamin before an assay of the two compounds can be carried out in degraded solutions. TLC was perfromed for the degraded solution with the solvent system S_1 and S_2.It was observed that, at pH 4.0 - 7.0,B_{12} is converted into B_{12b}in degraded solution as detected by thin layer chromatography using both solvent systems. Hydroxocobalamin was identified by comparing its color and R_f value with that of an authentic compound. No other product was detected in the degraded solutions of cyanocobalamin. It has been reported that hydroxocobalamin undergoes irreversible oxidation degradation to unknown products (Connors *et al*., 1986). The presence of hydroxocobalamin was confirmed in the photolysed solution of all the single ingredient and multi-ingredient B_{12} preparations. However, the intensity of the spot varied depending upon the degradation rate in different solutions. The major degradation product of cyanocobalamin on visible irradiation is hydroxocobalamin which is formed by replacement of –CN group by –OH group at the cobalt atom (Ahmad *et al*., 1992). The intensity of the spots indicates that the formation of hydroxocobalamin decreases with pH and therefore cyanocobalamin appears to be more stable in neutral solutions. The presence of hydroxocobalamin in photolysed solution of cyanocobalamin was also confirmed by loss of absorbance at 550 nm ($ë_{max}$ of B_{12}) and simultaneous increase in absorbance at 525 nm ($ë_{max}$ of B_{12b}).

3.1.2. Assay of Cyanocobalamin and Hydroxocobalamin in Degraded Solutions

Cyanocobalamin and hydroxocobalamin have overlapping spectra and the British Pharmacopoeia (2022) assay method for cyanocobalamin is grounded on the absorbance measurement at 361 nm. Therefore, this method would not work for the assay of cyanocobalamin accurately in the presence of hydroxocobalamin in degraded solutions. In this situation, it was, therefore, considered necessary to apply a method which can simultaneously determine the concentration of both compounds in the degraded solutions accurately. This is necessary to carry out the kinetic analysis of the degraded solutions. The applied method was reported in literature (Ahmed *et al*., 2017*)* (Fig. 2) for the analysis of above compounds in degraded samples.

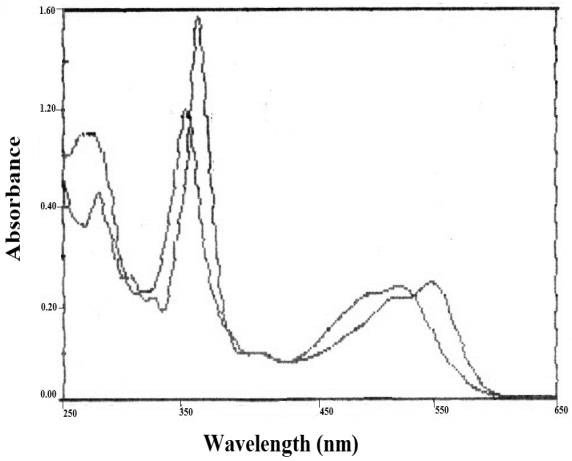


Fig.2: Absorption spectra of cyanocobalamin B12 and hydroxocobalamin B12b and hydroxocobalamin (B12a) at pH 4.0 (Ahmed *et al*., 2017).

It has been found that the method works well in single ingredient B_{12} preparations. Since vitamin B_1 and B_6 as component of multi-ingredient preparations absorb only in the UV region and cause no interference in the region of analytical wavelength (550 and 525 nm) employed for the assay of $B_{12}$and B_{12b} in degraded solutions. This method could also be helpfulfor determination of B_{12} in degraded samples containing other vitamins with no absorption in 500 – 600 nm region. The different parameters used in the development of the method including the choice of wavelength, pH, and applicability of Beer's law and precision have been reported by Ahmed *et al*.(1992).

The analytical results of cyanocobalamin and hydroxocobalamin in single ingredient and multi-ingredient vitamin B_{12} preparations used in current study are stated in (Table 2). The analytical data of the photolytic reactions for all of these preparations were carried out by adjusting the pH at 4.0– 7.0 (0.02M Phosphate buffer). The data shows a gradually decreasing concentration of cyanocobalamin with time and concomitant increase in the concentration of hydroxocobalamin (Table 2). The comparative analysis of different formulations also shows a gradual decrease in concentration of cyanocobalamin with time at between pH 4.0–7.0 (Fig. 3). However, with a rise in the photodegradation of cyanocobalamin, the total molar balance of cyanocobalamin and hydroxocobalamin is decreased due to the formation of the oxidation products. The method for calculating the concentration of oxidation products have been reported by Ahmed *et al*., 2017, obtained by a change in the initial molar concentration of cyanocobalamin and the molar balance of (cyanocobalamin + hydroxocobalamin) at various time intervals through the photo-degradation process. The overall accuracy of the method has been found to be with-in ±3% and is in agreement with that reported by Ahmed *et al.* (2017). The method is rapid, specific and suitable for conveniently analyzing the mixture of the two vitamins in degraded solutions.

3.1.3. Kinetics of Photolysis of Cyanocobalamin in Commercial Preparations

The assay data obtained on photolysed solutions of different single ingredient and multi-ingredient vitamin B_{12} preparations at various pH values were subjected to kinetic treatment. It was observed that under the present conditions cyanocobalamin (Formulation I, II, III, IV) on photolysis follows the first-order kinetics. The rate constant for the degradation of cyanocobalamin in various preparations were calculated from the slopes of the straight line of first order plots. The values of the rate constant indicate a gradual decrease with an increase in pH(4.0-7.0) forbothsingleingredientandmulti-ingredient preparations and are in the rangeof $0.44 – 5.05 \times 10^{-3}$ min^{-1} given in Table 3. The higher rate at pH 4.0 may be explained on the basis of the protonated form of cyanocobalamin (pK_a 3.3 for the base atom B_{12}, Hill *et al*., 1964) which seems to be more vulnerable to photolysis. The rates are about five times higher at pH 4.0 as compared to pH 7.0. This indicates that cyanocobalamin exhibits greater stability in the neutral medium and the pharmaceutical formulation of the vitamin should be maintained between pH 5.0 - 7.0. The same region of pH is reported for cyanocobalamin in the British Pharmacopoeia (2022).

The influence of pH on the degradation rate of cyanocobalamin has been observed by the rate-pH profiles for these preparations were

prepared and are shown in Fig.3. A relatively lower influence of pH on the rate of photodegradation of Formulation IV compared to Formulation Iinjections indicated that the former preparation has a better photostability and may have some stabilizing agent to control the rate of degradation. In the multicomponent preparation Formulation II containing B_1 and B_6 shows relatively more degradation compared to the Formulation III injections containing B_1 and B_6. This could result from relatively lower interaction of the vitamins in Formulation IIIinjections because the degradation may be accelerated by the interaction of these vitamins at a particular pH. The pattern of the pH-rate profile for all preparations is similar. However, there aresome changes in the magnitude of the rate constants at pH 4.0 and 7.0 due to differences in the photostability of these vitamins (Fig. 4). It can be concluded from the kinetic data that rate of degradation of cyanocobalamin is greater in multi-ingredient preparations than the single ingredient preparations and this can only be clarified on the basis of the interaction of these vitamins on exposure to light as indicated by the % loss of cyanocobalamin in different formulations (Fig. 5).

Table 3. Apparent First–Order Rate Constants (k_{obs}) for the Photolysis of Different Formulations of Cyanocobalamin (I-IV) at pH 4.0–7.0

pH	k_{obs} x 10^3 min^{-1}			
	I	II	III	IV
4.0	1.71	5.05	3.22	1.50
4.5	1.20	3.83	2.43	0.93
5.0	1.08	2.80	1.55	0.71
6.0	0.70	0.82	0.66	0.62
7.0	0.61	0.54	0.47	0.44

3.1.4. Mode of Degradation of Cyanocobalamin

The photochemical degradation of cyanocobalamin to hydroxocobalamin has been mentioned by Ahmed *et al.* (1992; 2017). It takes place in acidic environment by the exchange of the –CN group attached to cobalt with that of –OH group and is represented as follows:

[Co-CN] ——— [Co-OH] +CN

CyanocobalaminHydroxocobalamin

[Co-OH] ——————— [Co–OH_2]

Hydroxocobalamin Aquocobalamin

During the photodegradation, the -CN is removed by -OH group devoid of any alteration in the cobalt atom valency. The absorption of light initiatedthe reaction by resulting in the shifting of ð ’! ð* electrons in the corrinnucleus (Pratt, 1972). The value of quantum yield for the photodegradation of cyanocobalamin is reported to be around 10^{-4} (Vogler *et al*., 1976). It is impor

tant to know that the photo-induced degradation of cyanocobalamin is a pH dependent reaction and the stability of the vitamin may be improved by adjusting the pH around the neutral range. Once hydroxocobalamin is formed it may undergo further degradation to irreversible oxidation compounds leading to the loss of potency of cyanocobalamin (Connors *et al*., 1986). Therefore, it is necessary to control the primary step in the degradation of cyanocobalamin to prolong its shelf-life on exposure to light. This can be achieved by the use of suitable stabilizing agents to control the vitamin degradation and adopting an optimum pH for photostability.

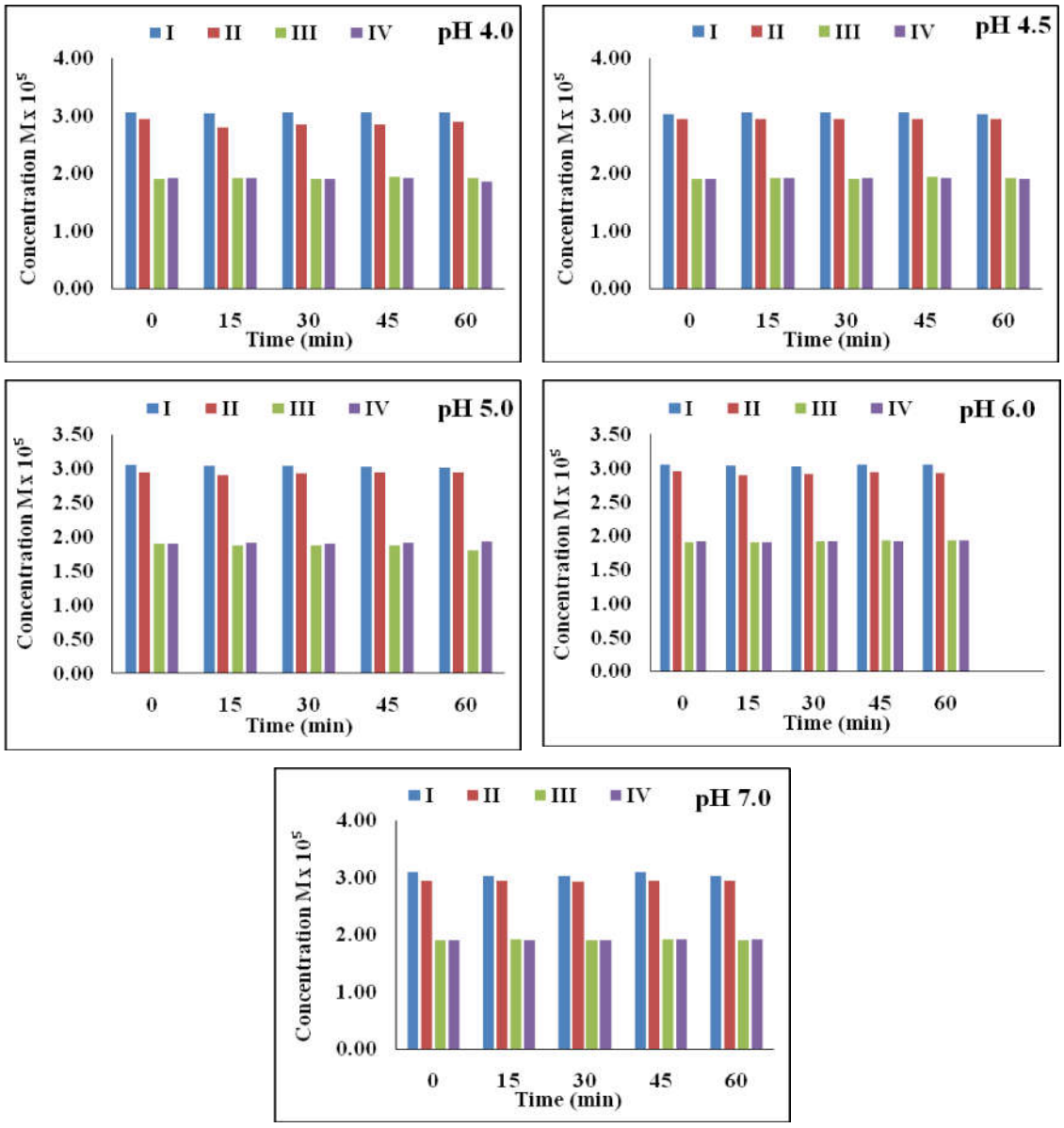


Fig.3: Plots of concentration of cyanocobalamin versus time for the photolysis of cyanocobalamin at pH 4.0–7.0 in different formulations (I-IV)

4. CONCLUSION

Hydroxocobalamin has been detected as the main photo product of cyanocobalamin in acidic medium using thin layer chromatography and spectrophotometry.The analysis of B_{12} and B_{12b} in the photodegraded solutions was performed by a two-component spectro photometric method with a precision of ±3%. The method can rapidly and accurately determine the concentration of the two vitamins in the degraded solutions.Cyanocobalamin undergoes degradation by apparent first-order kinetics. The value of first rate constants range from $0.44 - 5.05 \times 10^{-3}$ min^{-1}(pH 4.0 –7.0). The rates are higher for degradation of cyanocobalamin in the presence of other vitamins due to mutual interactions.The rate-pH profiles have been prepared which shows the rate of photolysis is influenced by the pH. The rate constants have been found to increase with the decrease in pH on account of the protonation of the molecule at lower pH values. Cyanocobalamin is most stable in the neutral pH range and this should be used to maintain the pH of the injectable preparations.

5. REFERENCES

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