

Electrochemistry of Vitamin B₁₂. 2. Redox and Acid–Base Equilibria in the B_{12a}/B_{12r} System

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Abstract: The unusual behavior reported for the reduction of B_{12a} on a mercury electrode renders the derivation of the intrinsic redox properties of the B_{12a}/B_{12r} couple by polarography questionable. Gold and platinum electrodes give more reliable results. However, a preliminary analysis of the system on such an electrode by cyclic voltammetry shows that reversibility is far from being reached even at the lowest usable sweep rates. A spectroelectrochemical method using a platinum electrode with electrolysis durations long enough to allow redox equilibration was therefore employed to determine the standard potential as a function of pH in the range –1 to 11. Joining these results to those previously obtained with the B_{12r}/B_{12s} couple leads to a general picture of the stability ranges of the three oxidation states of aquocobalamin as a function of potential and pH. The characteristic E° and pK_a values thus found are compared with previous determinations. Implications of B_{12r} disproportionation and of the expectable redox behavior of aquocobinamide and cobyrinic acid are discussed.

In the preceding article of this series, devoted to the elucidation of the electrochemical oxidation–reduction mechanism of the B_{12r}/B_{12s} system, several causes of irreversibility were analyzed. Hereby it was possible to select conditions in which the reversibility is achieved in cyclic voltammetry (CV) and thus to determine the standard potentials (E°) and pK_a s featuring the various redox and acid–base equilibria. The E° s thus measured exhibit significant differences with the polarographic half-wave potentials.

In the case of the B_{12a}/B_{12r} system, departure from reversibility in polarography and CV is expected to be even more dramatic as shown by a preliminary CV study on a gold electrode reported hereafter. On the other hand, the polarographic reduction pattern of B_{12a} into B_{12r} is not simple, being composed of two poorly defined waves with the first close to the mercury oxidation background current and the second located in the region –0.3 to –0.6 V vs. SCE.^{3–5} Correspondingly, the cyclic voltammograms exhibit unusual behavior.⁴ Reoxidation of B_{12r} gives rise to a single polarographic wave, the $E_{1/2}$ of which is close to 0 V, its exact value depending on the composition of the medium.^{3,4} The same type of behavior has recently been observed in a spectroelectrochemical investigation on mercury corresponding to much longer electrolysis durations than in polarography.⁶ Our results, obtained in comparable time ranges but on a platinum electrode, show neither two cathodic steps nor any hysteresis upon reoxidation. It may then be hypothesized that, due to the proximity of its oxidation potential, mercury interferes in the electrochemical reduction–oxidation process of the B_{12a}/B_{12r} system. This unusual behavior has also been thought to be related to the interference of the base-off/base-on reaction at the Co^{III} level.⁶ The splitting in two waves of the polarographic reduction of B_{12a} into B_{12r} is, however, not always observed,⁷ and the second wave has been attributed to one impurity.⁷ It follows that reliable conclusions regarding the intrinsic redox properties of the B_{12a}/B_{12r} couple cannot be drawn at present from polarography or other methods employing mercury as electrode material. Moreover, previous electrochemical studies of this system have been carried out around pH 7 and for a very restricted number of other pH values.

It is the purpose of the present paper to determine the standard potentials characterizing the B_{12a}/B_{12r} redox couple as a function of pH in the range –1 to 11. Joining these results to those already obtained for the B_{12r}/B_{12s} couple,^{2,8} a general picture of the range of stability of the three oxidation states as a function of potential and pH will then be given.

Preliminary Cyclic Voltammetric Study. Procedure for Redox Equilibrium Determinations

The cyclic voltammetry of B_{12a} on a gold disk electrode is shown in Figure 1 at four pHs. The separation between the cathodic and anodic peaks indicates that the system is far from being reversible even at the lowest sweep rate (0.02 V s^{–1}, Figure 1a). Raising the sweep rate results in an increase of the peak separation (Figures 1a,b,c). Due presumably to the interference of the acid dissociation of the water molecule beared by the cobalt as a sixth ligand,¹¹ irreversibility increases as shown by comparison of Figures 1b, 1e, and 1f which represent the voltammogram obtained at pH 8.2, 9.7, and 11.7 for the same sweep rate. The results obtained on a platinum disk electrode were essentially the same.

It is therefore desirable, as far as redox equilibrium determinations are concerned, to use a much slower method than CV (or even low-rotating-speed disk electrode voltammetry) and such as the mass transport to the electrode would be slow enough to allow equilibrium to be achieved. We employed for this purpose a thin-layer spectroelectrochemical technique using the cell shown in Figure 2 with a platinum grid working electrode. Starting from a B_{12a} solution, a series of electrolyses was carried out, settling the working electrode potential at more and more negative values up to complete conversion into B_{12r}. The corresponding changes of the B_{12a}/B_{12r} concentration ratio in the solution were monitored by absorption spectroscopy, the light beams crossing the middle of the thin portion of the cell 1 cm below the bottom of the upper portion (Figure 2). In these conditions it was found that a stationary spectrum is obtained at each potential within an electrolysis time ranging from 15 min to 1 h depending upon the pH and the value of the electrolysis potential.

The thickness of the lower part of the cell being about 0.05 cm and its surface area being almost equal to that of the platinum grid, it is clear that exhaustive electrolysis up to the equilibrium B_{12a}/B_{12r} ratio corresponding to the particular value of the electrode potential is not kinetically limited by lateral diffusion but rather by the electrode reaction itself. On the other hand, vertical diffusion of B_{12a} from the upper part of the cell down to the region of the light beam can be neglected. Indeed, the corresponding diffusion layer $(\pi Dt)^{1/2}$ would be 1 cm which corresponds to about 20 h (taking $D \approx 4 \times 10^{-6}$ cm² s^{–1}).² Natural convection is therefore likely to play the major role in the eventual mixing of the solutions contained in the upper and lower parts of the cell. The very fact that a stationary spectrum is obtained within less than 1 h of

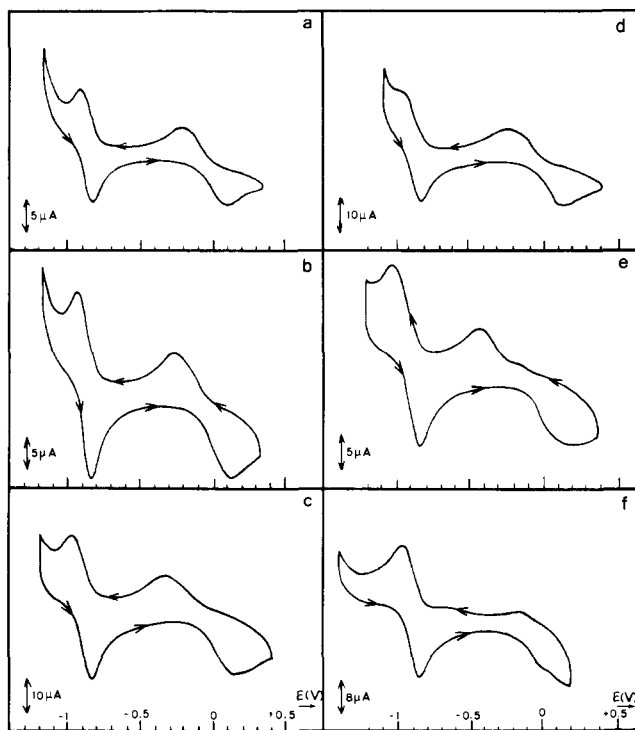


Figure 1. Cyclic voltammetry of B_{12a} on a gold disk electrode; starting potential on the left; B_{12a} concn: a, b, c, e, f: 4 mM, d: 5 mM; pH: a, b, c: 8.2, d: 6.9, e: 9.7, f: 11.7; sweep rate: b, d, e, f: 0.05 V s^{-1} , a: 0.02 V s^{-1} , c: 0.1 V s^{-1} .

electrolysis shows that this factor does not interfere too seriously. It seems, however, to be the cause of the limitation of the method at very high pH values where the electrochemical reaction becomes very slow. At pH 12, for instance, a clearly stationary spectrum could not be obtained, whereas this could be achieved in the pH range -1 to 11 .

Due to the poor electrochemical geometry of the cell, as long as the current flows the potential difference between the solution and the working electrode most probably varies from one point to the other and is not equal to the potential difference imposed by the potentiostat between the working electrode and the reference electrode. However, when a stationary spectrum is obtained, the current is very small and so is the ohmic drop between the working electrode and the equipotential surface passing through the tip of the reference electrode. In these conditions, the imposed potential difference becomes practically equal to that between the working electrode and the solution.

Figures 3-5 show the procedure employed for determining the standard potential E° at a given pH. The results represented in the figures correspond to pH 5. The variations of the stationary state spectrum with the electrode potential up to complete conversion of B_{12a} into B_{12r} are shown in Figure 3a. Five perfectly defined isosbestic points are obtained in the whole potential range, being a first piece of evidence that redox equilibrium is actually achieved. Spectroelectrochemical waves can be derived from this series of spectra featuring a quantity proportional to the concentration of either B_{12a} or B_{12r} as a function of the electrode potential. It is indeed readily shown that at a given wavelength:

$$C_O = (A - A_{R^\circ}) / (\epsilon_O - \epsilon_R)$$

$$C_R = (A - A_{O^\circ}) / (\epsilon_R - \epsilon_O)$$

where C_O and C_R are the B_{12a} and B_{12r} concentrations, ϵ_O and ϵ_R their molar extinction coefficients, A the observed absorbance, A_{O° and A_{R° the absorbances at the same wavelength

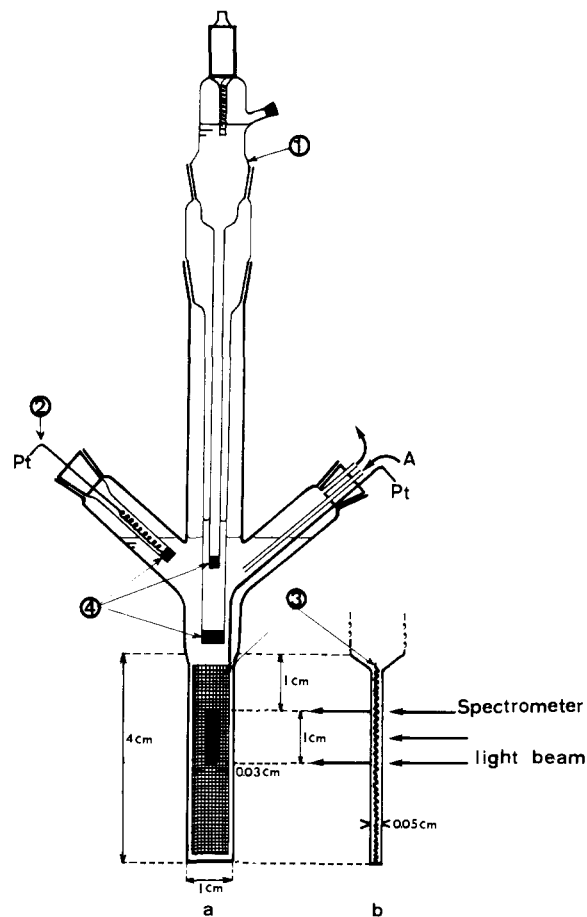


Figure 2. Spectroelectrochemical cell for determination of the redox equilibria: (a) front view, (b) side view; (1) saturated calomel electrode, (2) auxiliary electrode (Pt), (3) platinum grid working electrode, (4) fritted disks.

of pure solutions of B_{12a} and B_{12r} at the same concentration.

Using the γ bands, one obtains the waves represented in Figure 4a. A further test of reversibility is provided by the logarithmic analysis of the waves: $\log [(I_1 - I)/I]$ vs. E (I_1 is the plateau value of I). As seen in Figure 5a these plots are approximately linear and their slope is close to the expected value of 60 mV per logarithm unit. E° is then the value of the potential where the straight line intersects the horizontal axis. Additional evidence that equilibrium is actually reached is provided by repeating the same operations upon reoxidation of the B_{12r} obtained at the end of the reduction experiment and comparing the E° values. The spectra, the spectroelectrochemical waves, and logarithmic analysis for reoxidation are shown in Figures 3b, 4b, and 5b. The E° value thus obtained, -0.048 V , is in fair agreement with the value determined upon reduction, -0.037 V .

Results

Three different spectroelectrochemical behaviors are observed according to the pH. Between pH 2.9 and 7.8 the spectra are those represented in Figure 3, exhibiting five isosbestic points. Between -1 and 2.9 (Figure 6) seven differently located isosbestic points are noted. The spectra obtained between 7.8 and 11 are shown in Figure 7 with again five isosbestic points but at different wavelengths than the previous ones. These changes in number and location of the isosbestic points reflect the existence of two forms of B_{12a} with a $pK_a = 7.8$ and two forms of B_{12r} with a $pK_a = 2.9$. The first form of B_{12a} is the base-on aquocobalamin, whereas the second is the base-on

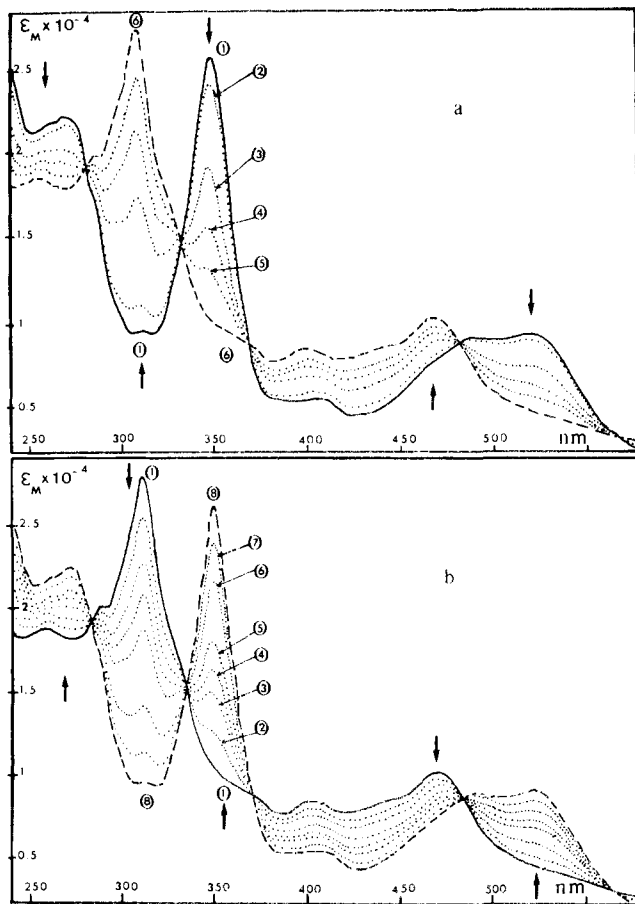


Figure 3. Spectroelectrochemistry of the B_{12a}/B_{12r} couple at pH 5: variation of the spectrum with the electrode potential; B_{12} concn ≈ 0.7 mM; (a) reduction of B_{12a} , electrode potential (V vs. SCE): (1) 0.25, (2) 0, (3) -0.040 , (4) -0.070 , (5) -0.100 , (6) -0.20 ; (b) reoxidation of B_{12r} , electrode potential (V vs. SCE): (1) -0.20 , (2) -0.10 , (3) -0.070 , (4) -0.05 , (5) 0.030 , (6) 0, (7) 0.30 , (8) 0.10 .

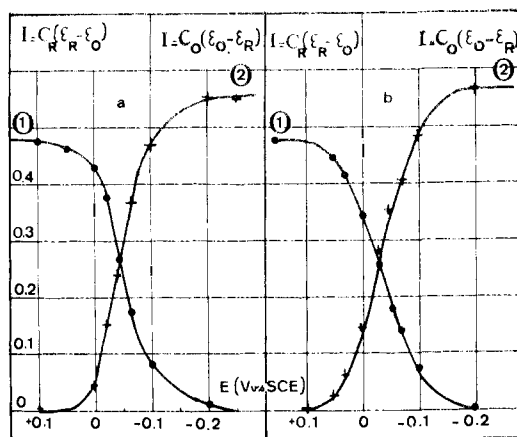


Figure 4. Spectroelectrochemistry of the B_{12a}/B_{12r} couple at pH 5: spectroelectrochemical waves; B_{12} initial concn, 0.7 mM; (a) reduction of B_{12a} : (1) from the intensity of B_{12a} γ band (350 nm), (2) from the intensity of B_{12r} γ band (310 nm); (b) reoxidation of B_{12r} : (1) from the intensity of B_{12a} γ band (350 nm), (2) from the intensity of B_{12r} γ band (310 nm).

hydroxocobalamin. Accordingly, a bathochromic shift of the main bands is observed when crossing pH 7.8 ($272 \rightarrow 278$, $350 \rightarrow 357$, $490 \rightarrow 510$, $522 \rightarrow 535$ nm) which features the replacement of H_2O as a sixth ligand by the more basic OH^- . The spectra of both species are essentially the same as those previously reported.¹²⁻¹⁵ The change in the spectrum of B_{12r} at pH 2.9 features the existence of the protonated base-off form

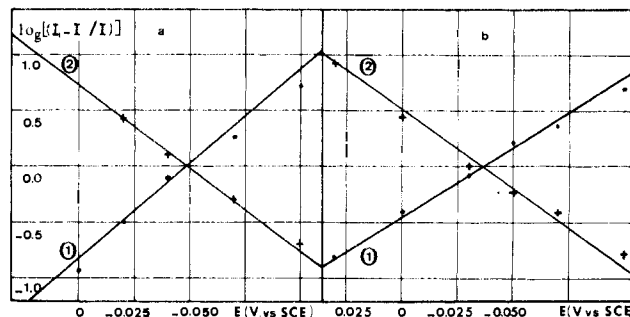


Figure 5. Spectroelectrochemistry of the B_{12a}/B_{12r} couple at pH 5. Logarithmic analysis of the spectroelectrochemical waves of Figure 4. Same notations as in Figure 4.

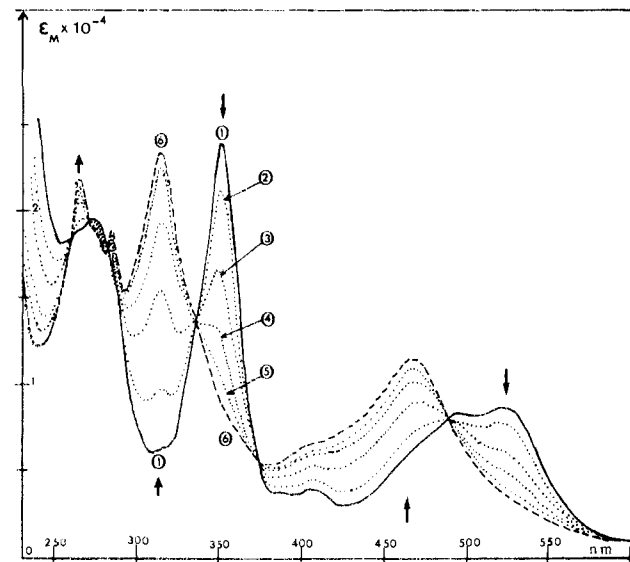


Figure 6. Spectroelectrochemistry of the B_{12a}/B_{12r} couple at pH 0: reduction of B_{12a} , electrode potential (V vs. SCE): (1) 0.30 , (2) 0.20 , (3) 0.15 , (4) 0.10 , (5) 0.050 , (6) -0.10 .

below this pH and of the base-on form above. At pHs larger than 2.9 it has been shown that the ratio of the base-on form over the unprotonated base-off form is 60,² so that the observed spectrum is indeed that of the base-on form with negligible interference of the base-off form. The spectrum observed above pH 2.9 agrees with previously reported descriptions.¹⁶ Below pH 2.9 the band at 284 nm characterizes the protonated 5,6-dimethylbenzimidazole (Bzm) uncoordinated to the cobalt.¹⁷ The main bands previously reported¹⁶ are seen in Figure 6. In addition, a well-resolved band at 265 nm which was not present in the base-on form spectrum now appears. On the contrary, the shoulder at 440 nm (Figures 3 and 7) is no longer present.

The E^0 s obtained at various pHs according to the procedure described above are given in Table I together with the slopes of the logarithmic analysis of the spectroelectrochemical waves. The results were obtained upon reduction of B_{12a} with the exception of pH 8 where they correspond to a reoxidation experiment. Tests of reversibility by reoxidation were carried out at pH 3, 5, 7, and 10 and gave satisfactory results. The accuracy of E^0 determination can be estimated as being on the order of ± 5 mV.

The E^0 -pH diagram is shown in Figure 8. We have reported in the same diagram the previous results regarding the B_{12r}/B_{12s} couple,^{2,8} so as to define the ranges of thermodynamic stability of the various forms in the three oxidation states. On the other hand, it has been verified in a standard electrolysis cell with a platinum cathode that the number of

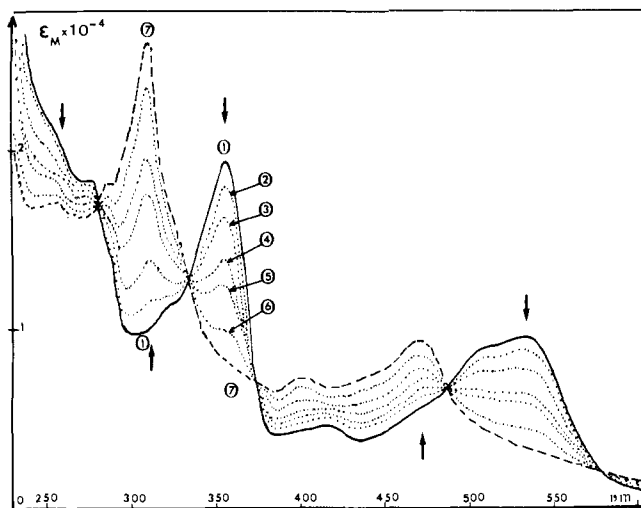


Figure 7. Spectroelectrochemistry of the B_{12a}/B_{12r} couple at pH 10.7: reduction of B_{12a} , electrode potential (V vs. SCE): (1) -0.10, (2) -0.19, (3) -0.22, (4) -0.25, (5) -0.28, (6) -0.35, (7) -0.40.

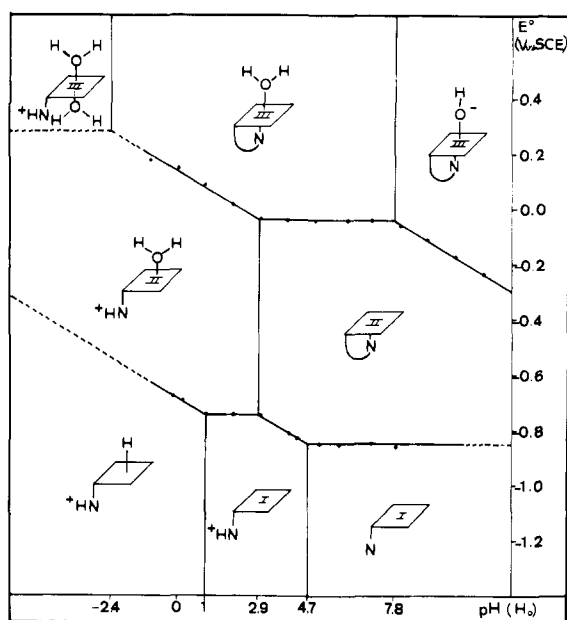


Figure 8. E° -pH diagram. Ranges of thermodynamic stabilities of the various forms in the three oxidation states.

electrons per molecule required to completely reduce B_{12a} into B_{12r} is one with a good accuracy (10%). The initial concentration of B_{12a} was 2 mM and the electrolysis potential was -0.30 V vs. SCE. The pH for this experiment was 6.

Discussion

It appears from the above results that the redox behavior of the B_{12a}/B_{12r} couple is a simple one provided platinum is used as electrode material. The reduction is not stepwise and no hysteresis appears upon reoxidation as was often the case with mercury.

Between pH 2.9 and 7.8 the standard potential does not depend upon pH featuring the equilibrium between the base-on forms of both B_{12a} and B_{12r} . Its value, -0.040 V vs. SCE, is finally close to the polarographic half-wave potentials previously reported,^{3,7} -0.03 and -0.04, and somewhat more negative than the $E_{1/2}$ obtained upon reoxidation of B_{12r} ⁴ (-0.015 and -0.002 in sulfate and phosphate media, respectively).

Below pH 2.9, E° increases by 60 mV per pH unit when the

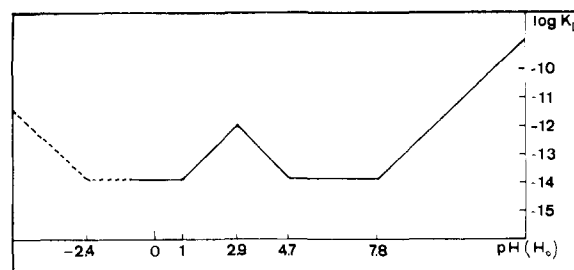


Figure 9. Variation of the disproportionation equilibrium constant; $2 B_{12r} \rightleftharpoons B_{12a} + B_{12s}$, as a function of pH.

Table I. E° vs. pH

pH (H ₀)	From B_{12a} γ band		From B_{12r} γ band	
	E° , V vs. SCE	Slope, mV	E° , V vs. SCE	Slope, mV
	ClO ₄ H			
-1	0.230	65	0.225	60
0	0.150	70	0.155	70
	NO ₃ H			
0	0.150	65	0.155	60
	Britton-Robinson Buffers			
1	0.095	60	0.090	55
2	0.015	60	0.015	60
3	-0.035	65	-0.035	60
4	-0.040	60	-0.040	70
5	-0.043	68	-0.043	68
6.1	-0.045	70	-0.040	70
7	-0.040	55	-0.045	55
8	-0.055	65	-0.065	70
9	-0.110	65	-0.110	65
10	-0.175	70	-0.170	70
11	-0.240	50	-0.245	60

pH decreases. This corresponds to the redox equilibrium being now between base-on B_{12a} and protonated base-off B_{12r} . This leads to a confirmation of the value of 2.9 for the pK_A of Bzm protonation in B_{12r} . The same value was previously derived from the study of the E° -pH plot of the B_{12r}/B_{12s} couple^{2,8} and from acid-base titration of a B_{12r} solution.^{2,8} The 2.9 value appears to be more exact than the previous figure of 2.5 determined by ESR spectroscopy¹⁸ owing to the approximate character of this experiment.

Above pH 7.8, E° decreases by 60 mV per pH unit as the pH is raised. This corresponds to the aquo- B_{12a} being deprotonated into hydroxo- B_{12a} , with a $pK_a = 7.8$. This value is in agreement with the most reliable results^{14,19} obtained in previous studies.^{14,19-22}

The data figured in the E° -pH diagram of Figure 8 allow the estimation of the disproportionation equilibrium constant K_D of B_{12r} into B_{12a} and B_{12s} as a function of pH. Such an estimation has already been made for pH 7.43,⁴ leading to $K_D = 0.8 \times 10^{-15}$. Using the E° values found here, a K_D value of 1.4×10^{-14} , i.e., 18 times larger, is obtained. This does not affect the qualitative conclusion that methylation of B_{12r} cannot, from a kinetic point of view, proceed through prior disproportionation.⁴ The above value of K_D is valid in the pH range 4.7 to 7.8. The variations of K_D with pH are represented in Figure 9.

It is seen that when the pH increases beyond 7.8, the tendency of B_{12r} to disproportionation increases as noted before on observing that the Co^{III}/Co^{II} and Co^{II}/Co^I CV waves tend to merge.² However, even at pH 12 the disproportionation equilibrium is of the order of 10^{-10} . It follows that, although

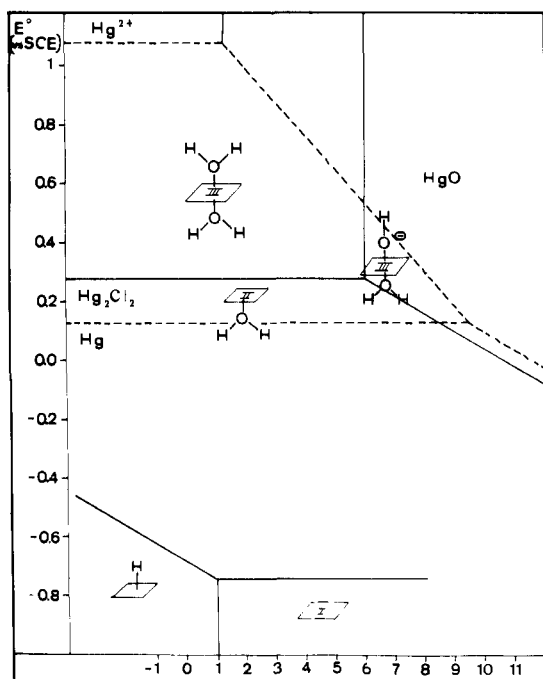


Figure 10. Predicted redox properties of aquocobinamide and cobyrinic acid as a function of pH (solid lines); zones of stabilities of the various oxidation state of mercury for 0.02 M Cl^- (dashed lines).

primarily based on the thermodynamic tendency toward disproportionation, the merging of the waves is enhanced by kinetic factors, mainly by the reduction of B_{12a} becoming slower and slower as the system enters the range of stability of the hydroxo form.

The $\text{p}K_a$ featuring the protonation of Bzm in B_{12a} has been previously determined as being -2.4 .²³ Using this value, the zone of stability of the protonated base-off B_{12a} can be drawn as shown in Figure 8. The standard potential of the base-off B_{12a} /base-off B_{12r} couple may then be estimated as 0.280 V vs. SCE. Assuming, which seems reasonable, that the protonated uncoordinated Bzm side chain extends little influence on the redox properties of the cobalt in the corrin ring, it can be estimated that the E° for the $\text{Co}^{\text{III}}/\text{Co}^{\text{II}}$ couple in diaquocobinamide and diaquocobyrinic acid is close to this value. More generally, on the basis of the same assumption and taking -2.4 and 6 as correct values for the $\text{p}K_a$ of Bzm protonation and the $\text{p}K_a$ of the deprotonation of one trans water molecule, respectively, the redox behaviors of diaquocobinamide and diaquocobyrinic acid can be predicted to be as shown in Figure 10. We have drawn in the same diagram the zones of stabilities of the various oxidation states of mercury for a solution containing 0.02 M chloride ion.²⁴ It is seen that up to pH 8.5 mercury is likely to reduce Co^{III} -diaquocobinamide or cobyrinic acid into the Co^{II} compounds with formation of Hg_2Cl_2 . Beyond this pH the E° s are close. Bearing in mind that the above estimations are approximate (e.g., the temperature of our experiments was 22°C whereas the data for mercury correspond to 25°C), it is well possible that reduction of the Co^{III} species occurs also in this region, at least partially. On this basis it appears likely that the previously reported polarographic wave

at about -0.74 V vs. SCE of Co^{III} -diaquocobinamide is actually the wave of the Co^{II} species resulting from prereduction by mercury. The reduction of the Co^{III} -cobinamide is indeed expected to be located at -0.74 V in absence of overpotential effects (Figure 10). This would be in agreement with the reduction of Co^{III} -diaquocobinamide being easier than that of B_{12a} which seems much more reasonable than the opposite since, in the first case the electron-donating character of the ligand, water, is clearly less than with Bzm. It follows that speculations about the role of the base-off B_{12a} based on the above mentioned polarographic results⁶ are questionable.

Experimental Section

Chemicals. Vitamin B_{12a} had the same origin as previously described.² Britton-Robinson buffers and perchloric acid solutions below pH 2 were used as described earlier.²

Cyclic Voltammetry and Controlled Potential Coulometry. The instrumentation, cell, and electrodes were the same as in ref 2 except for the working electrode in CV which was a gold disk of about 3 mm diameter.

Spectroelectrochemistry. The cell was as represented in Figure 2 with a platinum grid (30 lines/cm) working electrode and a platinum wire counterelectrode. It was fitted in a Cary 15 spectrophotometer.

The temperature of the experiments was 22°C . The H_0 Hammett function is used instead of pH below pH 0.²⁵

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