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Kinetic and thermodynamic studies of the ligand substitution reactions of the cobalamins

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CHAPTER 3

pK_a DETERMINATION OF AQUACOBALAMIN, AQUA-10-CHLORO-COBALAMIN, AQUA-10-NITROSOCOBALAMIN, AND AQUA-COBALAMIN IN 70% ETHANOL

3.1 Introduction

Marques *et al*.[1] were the first to report the values for the enthalpy and entropy of the proton dissociation of the water ligand of aquacobalamin. These values are ΔH = 28.6 ± 0.3 kJ mol^{-1} and ΔS = –59 ± 1 J K^{-1} mol^{-1}. Hence, the acid dissociation constant (pK_a) is calculated from these activation parameters, at 25 ºC, to be 8.1 at I = 1.0 M with a KCl background. It is essential to know the values of this ionisation of water to enable adjustments to be made to all the kinetic and formation constant studies in order to obtain pH-independent values. This is particularly important when the axial water group loses its proton and hydroxocobalamin is formed, since this complex is inert to substitution.

Marques *et al*.[1] also determined the pK_a for aquacobalamin bound to haptocorrin and found that the pK_a was raised from 8.10 to 8.29 at 25 ºC. They concluded that this small difference meant that the micro-environment of the coordinated water was not significantly different from that of the free aquacobalamin in bulk water.

In this work, the pK_a of aquacobalamin was redetermined so that all conditions are the same ensuring that the results are comparable with those of aqua-10-chlorocobalamin and aqua-10-nitrosocobalamin. A solvent study was undertaken with the reaction of aquacobalamin and pyridine in 70% ethanol and hence this pK_a was also determined.

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3.2 Results and Discussion

3.2.1 pK_a Determination of Aquacobalamin

The acid dissociation constant for the coordinated water ligand of aquacobalamin was determined spectrophotometrically at four temperatures ranging between 5.0 and 35.0 ºC. The experimental data were fitted to an ionisation isotherm (Equation 3.1), which is relevant when only one acid/base equilibrium is present.

$$A_T = A_0\left[\frac{1}{1+\frac{K_a}{[H^+]}}\right] + A_1\left[\frac{1}{1+\frac{[H^+]}{K_a}}\right] \qquad (3.1)$$

In Equation 3.1, A_T is the absorbance at the monitoring wavelength, K_a is the ionisation constant, A_0 the initial absorbance and A_1 the final absorbance. Examples of this fit for the ionisation of the water ligand of aquacobalamin at all four of the temperatures studied are shown in Figure 3.1. Ionisation constants were obtained for each of the four temperatures and used in a plot of ln K against $1/T$ (Figure 3.2) in order to obtain the values of ΔH and ΔS. These were determined as $\Delta H = 36.0 \pm 1.9$ kJ mol^{-1} and $\Delta S = -34 \pm 6$ J K^{-1} mol^{-1} resulting in a pK_a value of 8.09 ± 0.02 at 25 ºC, (I = 0.5 M, $NaNO_3$). The discrepancy between these values and those previously reported[1] ($\Delta H = 28.6 \pm 0.3$ kJ mol^{-1} and $\Delta S = -59 \pm 1$ J K^{-1} mol^{-1}) may be due to aquacobalamin coordinating some of the Cl^- from the KCl ionic strength adjustor. This possibility will be discussed further in Chapter 4.

Rubinson *et al*.[2] determined the acid dissociation constant for aquacobalamin by spectroelectrochemical experiments to be 7.8. Once again there may be some coordination of Cl^- to aquacobalamin since this value was determined in 0.5 M KCl. However, the authors did state that the electrolyte had no effect on the electrochemical potentials. Only slight differences arise in rate constants and

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equilibrium values if 7.8 is used instead of the 8.09 determined here, and so for comparative purposes, the value of 8.09 will be used throughout this thesis.

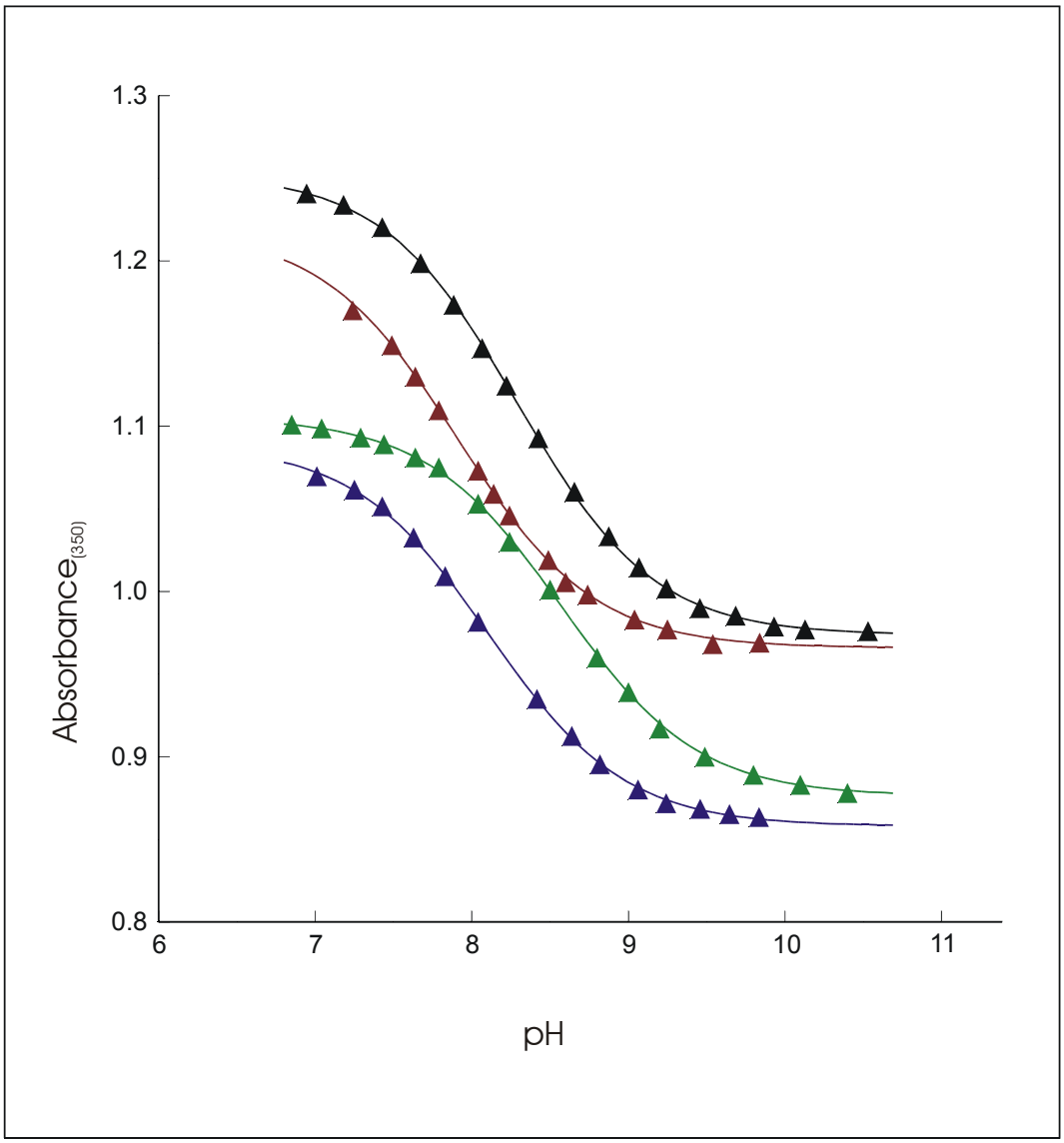

Figure 3.1 Spectrophotometric titration of an aquacobalamin solution in a 2.00 cm pathlength cell at the temperatures 5.0 (green), 15.0 (black), 25.0 (blue) and 35.0 ºC (red), (I = 0.5 M, $NaNO_3$). The solid lines are non-linear least-squares fits of Equation 3.1 to the experimental data.

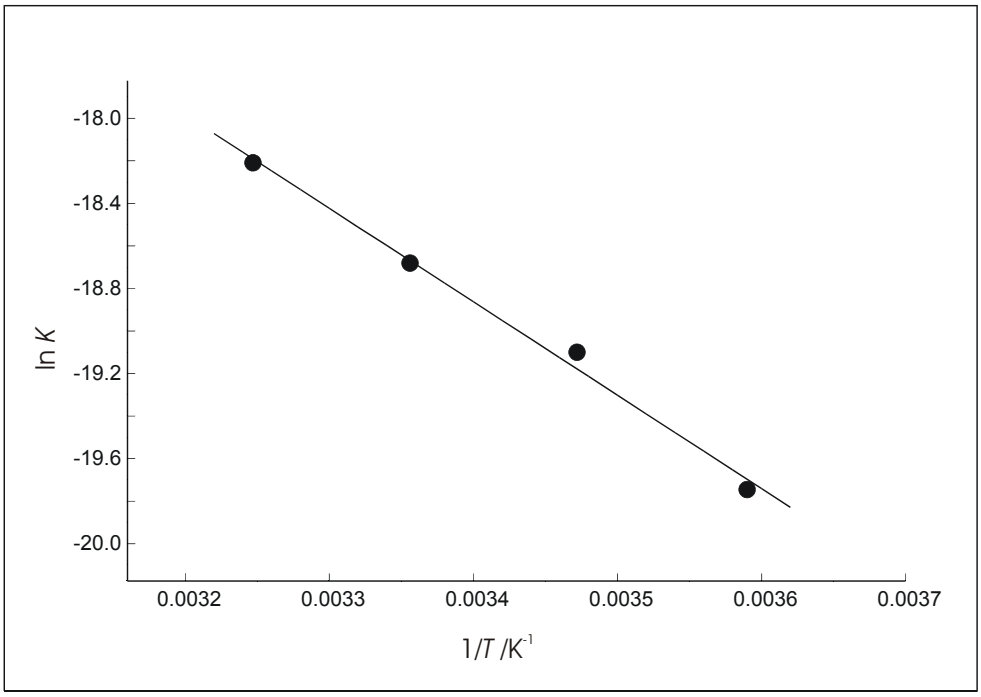

Figure 3.2 Temperature dependence of K_a for the self-ionisation of the water ligand in aquacobalamin for the measurements performed at 350 nm.

3.2.2 pK_a Determination of Aqua-10-chlorocobalamin

The data obtained for aqua-10-chlorocobalamin were treated in the same manner as for aquacobalamin. An example of the spectrophotometric titration at 25.0 ºC can be seen in Figure 3.3 and the temperature dependence of the equilibrium constant is shown in Figure 3.4.

From Figure 3.3, ΔH and ΔS were calculated as 32.9 ± 1.5 kJ mol^{-1} and –36 ± 6 J K^{-1} mol^{-1}, respectively and the pK_a was determined, from these parameters, to be 7.65 ± 0.05 at 25.0 ºC (I = 0.5M, $NaNO_3$).

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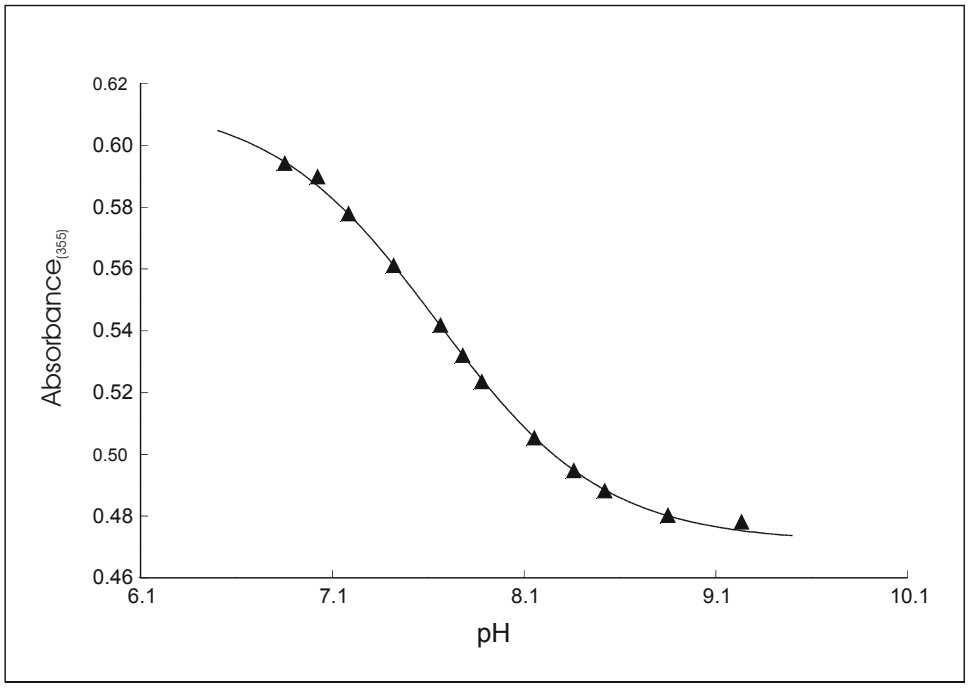

Figure 3.3 Spectrophotometric titration of an aqua-10-chlorocobalamin solution in a 2.00 cm pathlength cell at 25.0 ºC, (I = 0.5 M, $NaNO_3$). The solid line is a non-linear least squares fit to the experimental data.

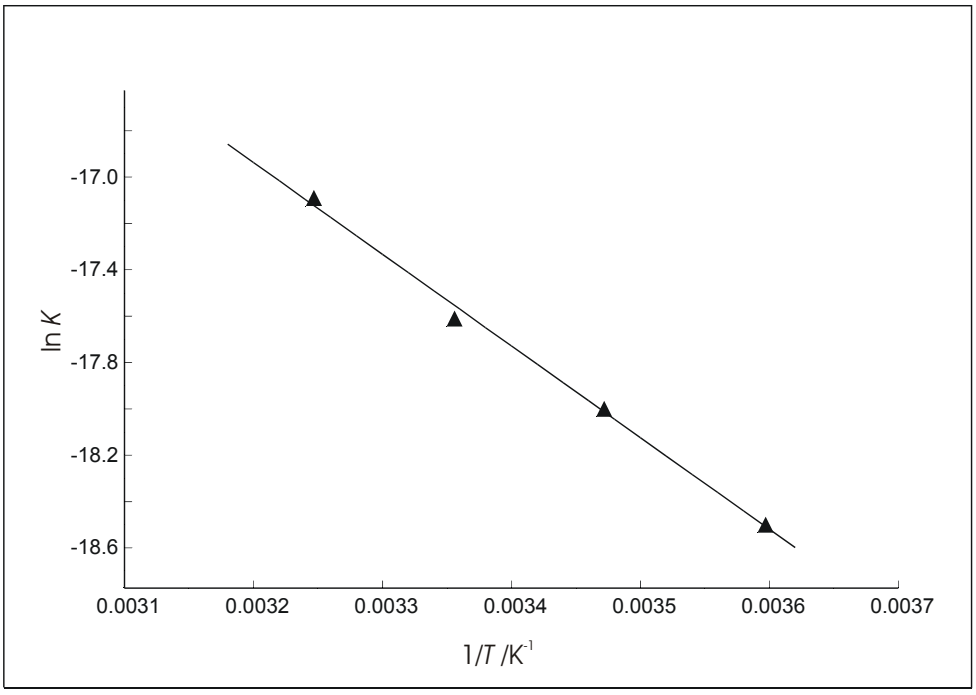

Figure 3.4 Plot of ln K_a against $1/T$ for the values obtained at 355 nm in order to obtain the activation parameters.

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3.2.3 pK_a Determination of Aqua-10-nitrosocobalamin

The spectrophotometric titration for aqua-10-nitrosocobalamin was more problematic than that of aquacobalamin and aqua-10-chlorocobalamin. The pK_a of aqua-10-nitrosocobalamin turned out to be much higher than those of aquacobalamin and aqua-10-chlorocobalamin and the experiment had to be performed at a higher pH. Therefore, the problems experienced were the result of the limitations of the glass electrode at higher pH values. As a result the fits were not as good as those obtained for aquacobalamin and aqua-10-chlorocobalamin and a titration was performed at an additional temperature.

Figure 3.5 shows the data obtained from the titration performed at 30.0 ºC. The temperature dependence of the equilibrium constant can be seen in Figure 3.6.

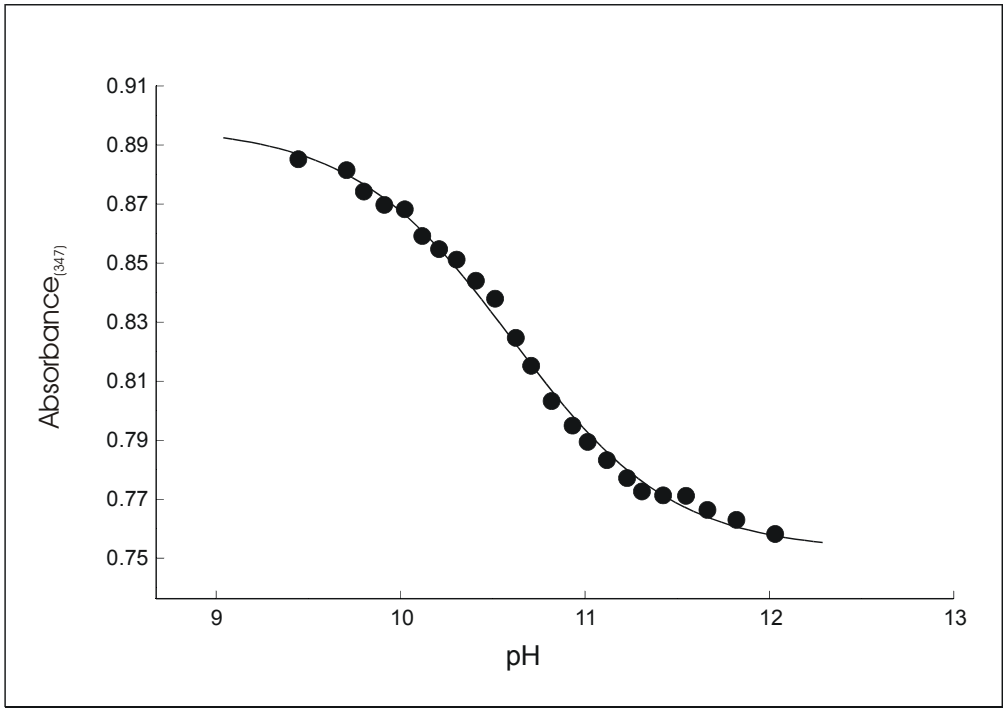

Figure 3.5 Spectrophotometric titration of an aqua-10-nitrosocobalamin solution in a 2.00 cm pathlength cell at 25 ºC, (I = 0.5 M, $NaClO_4$).

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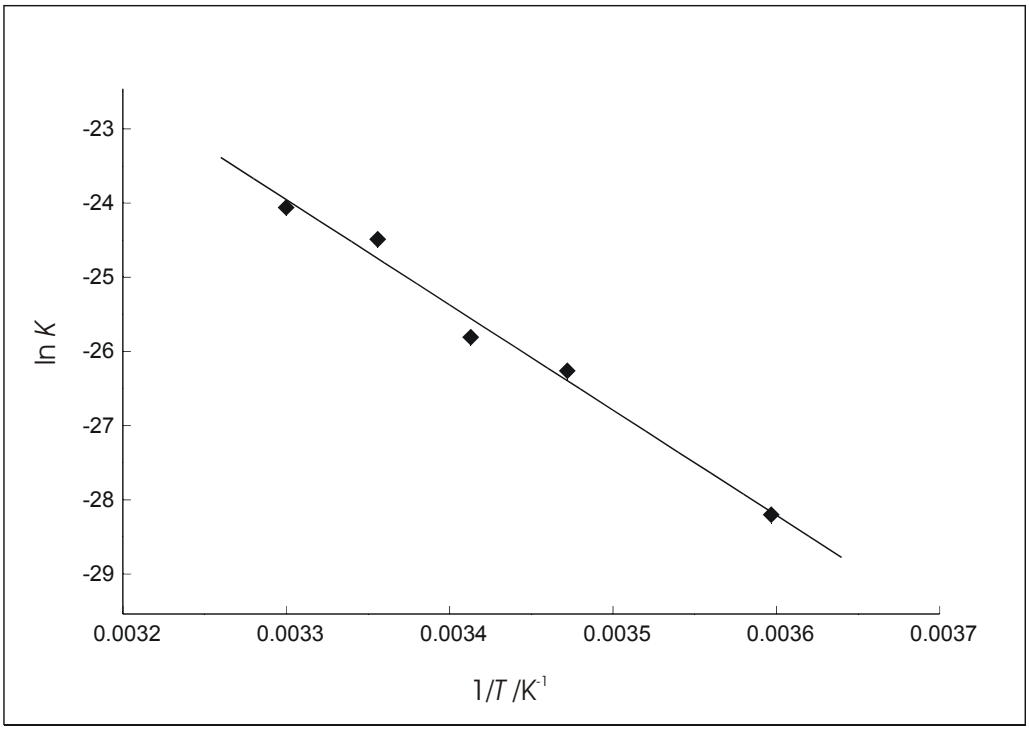

Figure 3.6 Temperature dependence of K_a for the self-ionisation of the water ligand in aqua-10-nitrosocobalamin.

The thermodynamic parameters for aqua-10-nitrosocobalamin are ΔH = 120 ± 11 kJ mol^{-1} and ΔS = 198 ± 38 J K^{-1} mol^{-1}, with the pK_a being calculated from these values as 10.71 ± 0.06 at 25 ºC (I = 0.5M, $NaClO_4$).

3.2.4 pK_a Determination of Aquacobalamin in 70% Ethanol

Solvent-effect studies were performed on the reaction of aquacobalamin with pyridine (see Chapter 5). Since pH adjustments need to be made for these data measurements, it is necessary to determine whether the pK_a of the ionisation of the water ligand of aquacobalamin is very different in 70% ethanol from that in water. The titration was problematic because of the slow response time of the electrode in a 70% ethanol solution. Also at high NaOH concentrations, the ethanol was deprotonated after a few hours, observed by a colour change from clear to yellow. Therefore, one had to work quickly to prevent the degradation of the ethanol, but

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the titration was slowed down because of the slow electrode response. As a result the titration was only performed at one temperature and activation parameters could not be determined.

The titration curve performed at 25 ºC and 522 nm is shown in Figure 3.7. The pK_a obtained from this curve is 8.26 ± 0.03. This value is not significantly different from that of aquacobalamin in water (8.09) and this implies that the micro-environment of the coordinated water is similar in both solvents.

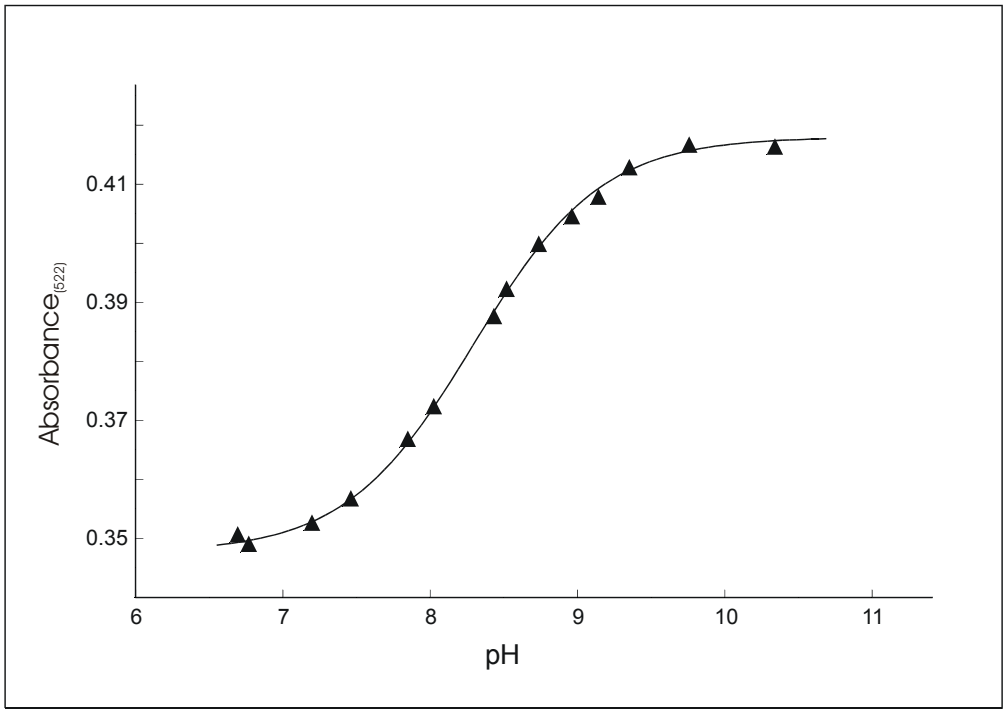

Figure 3.7 Spectrophotometric titration of an aquacobalamin solution in 70% ethanol performed in a 2.00 cm pathlength cell at 25 ºC and 522 nm. The solid line is a non-linear least-squares fit to the experimental data.

A summary of the thermodynamic parameters and resulting pK_a values obtained for the dissociation of the coordinated water ligand for aquacobalamin, aqua-10-chlorocobalamin and aqua-10-nitrosocobalamin can be seen in Table 3.1.

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Table 3.1 Thermodynamic parameters from the ionisation of the water ligand in aquacobalamin, aqua-10-chlorocobalamin and aqua-10-nitrosocobalamin.

Compound	**ΔH /kJ mol^{-1}**	**ΔS /J K^{-1} mol^{-1}**	**pK_a(25 °C)**
Aquacobalamin	36.0(1.9)	–34(6)	8.09(0.02)
Aqua-10-chlorocobalamin	32.9(1.5)	–36(6)	7.65(0.05)
Aqua-10-nitrosocobalamin	120(11)	198(38)	10.71(0.06)

3.2.5 Molecular Orbital Calculations

Molecular orbital calculations were performed on aquacobalamin[3], aqua-10-chlorocobalamin[3] and aqua-10-nitrosocobalamin[4] using the ZINDO/1 model. Since diffraction-quality crystals of aqua-10-nitrosocobalamin have been unattainable, molecular orbital calculations were performed on aqua-10-chlorocobalamin, where a NO group replaced the Cl at the C10 position. The partial charges found on the oxygen atom of the coordinated OH^- for aquacobalamin, aqua-10-chlorocobalamin and aqua-10-nitrosocobalamin are –0.503, –0.456 and –0.566, respectively. This indicates that the charge density on the coordinated OH^- group increases as the electron-withdrawing properties of the C10 substituent increases. Thus, as the pK_a value of the complexes increases the charge density on the O atom increases (R^2 = 0.91) and the metal hydroxide bond becomes more ionic. A plot of this correlation is shown in Figure 3.8.

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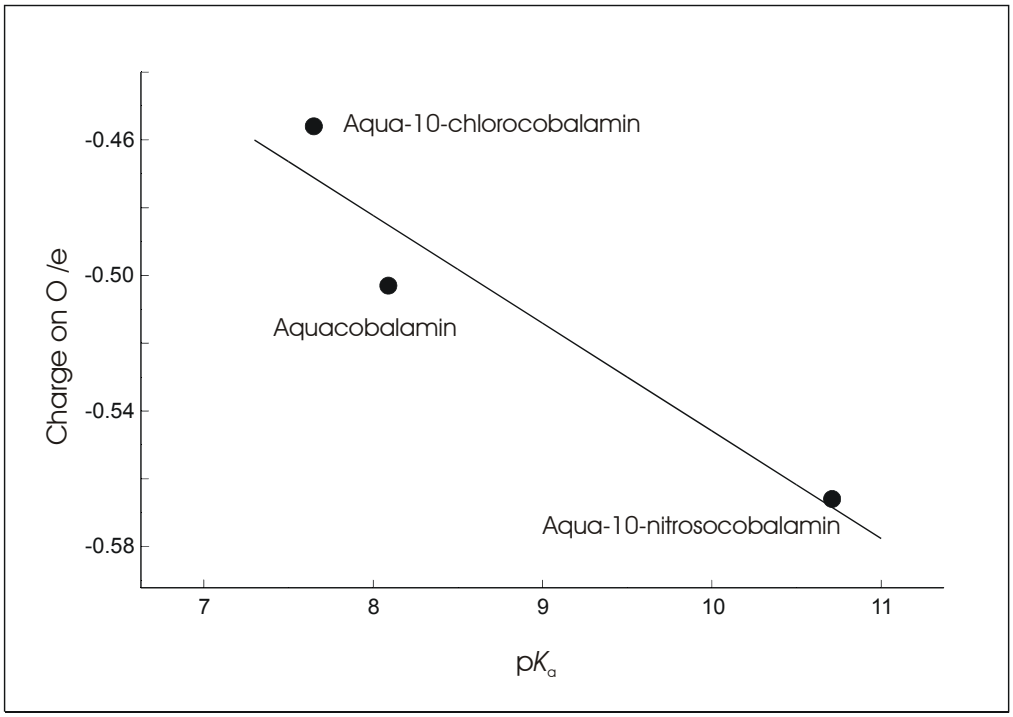

Figure 3.8 A plot of the partial charge on oxygen (determined from the ZINDO/1 model) for aquacobalamin, aqua-10-chlorocobalamin and aqua-10-nitrosocobalamin against the pK_a.

3.2.6 Compensation Effects

Munro and Marques[5] determined the parameters for the ionisation of the water ligand on AcMP8 as ΔH = 48.1 kJ mol^{-1} and ΔS = –23 J K^{-1} mol^{-1}, with a pK_a of 9.59 at 25 ºC. They then compared these values with those from various ferric hemoproteins and found that a plot of ΔH against ΔS was linear, indicating compensation effects between these two parameters. This compensation is seen when water is used as the solvent.[6] Compensation effects are also seen here; aqua-10-chlorocobalamin and aquacobalamin have very similar activation parameters, but the effects are very clear with aqua-10-nitrosocobalamin where a large favourable enthalpic effect is compensated by a large adverse entropic effect. The compensation effects seen with the cobalamins will be discussed further in Chapter 4.

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3.3 Conclusion

The pK_a values were determined for aquacobalamin, aqua-10-chlorocobalamin and aqua-10-nitrosocobalamin, as well as for aquacobalamin in 70% ethanol, in order to make pH adjustments to all the reactions that have been studied in this thesis. The values were determined to be 8.09, 7.65 and 10.71 for aquacobalamin, aqua-10-chlorocobalamin and aqua-10-nitrosocobalamin, respectively, at 25 ºC. The pK_a increases as the electron-withdrawing ability of the C10 substituent increases and molecular orbital calculations[3,4] show that the charge on the O atom of the coordinated OH^- group also increases with this trend. The Co–OH^- bond thus becomes more ionic as the pK_a increases.

The outcome of the titration of aquacobalamin in 70% ethanol was not very different from that in water with the pK_a values being 8.26 and 8.09, respectively. This implies that the micro-environment of the coordinated water in aquacobalamin is similar in both solvents.

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