Kinetics and Thermodynamics of Iodide Binding to Aquacobalamin in Methanol–Water Solvent Mixtures

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The rates and equilibrium constants for the reaction of aquacobalamin with iodide were measured as a function of solvent composition using methanol–water solutions. The solvent effects are consistent with a reaction that proceeds *via* the formation of an outer-sphere complex which is more stable for the cobalamin–methanol than for the cobalamin–water complex.

During the last 25 years the chemistry of aquacobalamin and related model compounds has been studied extensively.[1–12] In particular the reactions of aquacobalamin with neutral and anionic ligands such as thiosulfate, iodide, thiourea, *etc.*, have been described both from a kinetic and a thermodynamic standpoint. In a series of definitive studies Pratt and co-workers have reported that equilibrium constants for these reactions varied from 1.3 dm^3 mol^{-1} for Br$^-$ (ref. 11) to 2.20 × 10^7 dm^3 mol^{-1} for SO_3^{2-},[12] while Thusius[5] studied the kinetics of such substitutions and showed that the rates varied from 2000 to 170 dm^3 mol^{-1} s^{-1}. Such studies were consistent with a dissociative mechanism involving either S_N1 type intermediates or outer-sphere complexes; however, a subsequent study by Hasinoff,[10] in which activation volumes were measured by high-pressure temperature-jump techniques, concluded that the outer-sphere complex mechanism was operative. On the other hand McGreen and co-workers[13] showed the existence of intermediate complexes during the substitution reactions of a vitamin B_{12} model complex with some aromatic substituents.

In general these studies have been conducted in aqueous solutions since this was thought best to mimic the biological environment in which cobalamin would be active. It is now understood that the microenvironments for important physiological processes involve partially hydrophobic character. More recently, therefore, Balt and co-workers[14,15] have studied the reactions of aquacobalamin with several ligands as a function of solvent composition, *i.e.* in water–dioxane and –acetonitrile mixtures. These studies concluded that in general the axial substitutions of aquacobalamin have a dissociative character for reactions in such mixed solvents and that both the thermodynamic and kinetic parameters are significantly altered by changing the microenvironment about the cobalt centre.

With respect to such studies we now report a related study of the reaction between iodide and aquacobalamin as a function of varying water–methanol solvent composition. Variations in the thermodynamic and kinetic parameters can be correlated to the detailed mechanistic understanding provided by the previous studies.

Experimental

Materials.—Crystalline vitamin B_{12a} in the form of hydroxocobalamin hydrochloride from Fluka was used as purchased. Iodide solutions were prepared using analytical grade sodium iodide (MCB reagents) and anhydrous sodium perchlorate (GFS reagents) without further purification. Methanol (Baker Analyzed Reagents) was employed as received after saturation with N_2. Aqueous or methanolic solutions of aquacobalamin hydrochloride and iodide were prepared in doubly distilled and N_2-saturated water or N_2-saturated methanol. They were stored in the dark at 4 °C.

Because of iodide oxidation in the presence of aquacobalamin,[6] it was necessary to prepare daily fresh solutions of sodium iodide under N_2. The concentrations of stock solutions of aquacobalamin were determined spectrophotometrically by means of its absorption at 350 nm (ε = 2.62 × 10^4 dm^3 mol^{-1} cm^{-1}) as determined by Pratt and Thorp.[11]

All reactions were carried out at 25 ± 0.1 °C with an ionic strength of 0.15 mol dm^{-3}, adjusted by using $NaClO_4$ as inert electrolyte. This salt had no effect upon the spectrum of aquacobalamin solutions either aqueous or methanolic up to concentrations of 4.0 mol dm^{-3}.

Spectroscopic Determination of Equilibrium Constants.—The absorbances of solutions were measured using a Hewlett-Packard diode-array spectrophotometer, or a Perkin-Elmer λ4 workstation, thermostatted at 25 °C.

The procedure for each determination involved mixing equal volumes of a solution of aquacobalamin (2.0 × 10^{-5} mol dm^{-3}) and solutions of sodium iodide of different concentrations (0.5 × 10^{-3}–1.2 × 10^{-1} mol dm^{-3}), all solutions having the same ionic strength (0.15 mol dm^{-3}). In all cases the iodide concentration was at least 25 times higher than that of the aquacobalamin. All solutions were thermostatted at 25 °C for at least 30 min prior to spectral analysis. To obtain the value of the initial absorbance, a solution of aquacobalamin was mixed with a solution of $NaClO_4$ at the same ionic strength but in the absence of sodium iodide.

Kinetic Studies.—The formation reaction was followed in the visible region from 300 to 550 nm by means of a Dionex stopped-flow spectrophotometer with a cell of 2 cm pathlength, and a dead time of 3 ms. Solutions of sodium iodide at ionic strength 0.15 mol dm^{-3} were mixed with aquacobalamin solutions at the same pH and ionic strength. In order to assure thermal stability, all solutions were left inside the stopped-flow bath for 30 min prior to each reaction.

The pseudo-first-order rate constants were calculated using the acquisition program from OLIS Inc. Jefferson GA. 30549 which determines the value of the observed constant by means of a non-linear fitting of the observed data (normally absorbance *vs.* time). The rate constants obtained are the averages of at least 15–20 experiments for each concentration. Changes in absorbance were observed at 320, 350, 375, 380, 457 (isosbestic point), 500, 560 and 570 nm. The average deviation from the mean of k_{obs} for each λ was less than 5%.

Results

Equilibrium Studies.—The reaction studied can be considered as the replacement of water from the aquacobalamin molecule

Table 1 Equilibrium constants for the reaction of cobalamin and iodide in aqueous methanol solutions at 25 °C and ionic strength 0.15 mol dm^{-3} NaClO$_4$

[MeOH]				
% v/v	mol dm^{-3}	[H$_2$O]/mol dm^{-3}	K_{eq}^{k}	K_{eq}^{e}
0.0	0.0	55.4	21.0	26.0
20.0	4.9	44.3	62.0	60.0
40.0	9.8	33.2	157.0	153.0
50.0	12.3	27.7	207.0	258.0
60.0	14.7	22.2	335.0	300.0
70.0	17.2	16.6	800.0	888.0
80.0	19.7	11.1	1402.0	1305.0
90.0	22.1	5.5	4300.0	4227.0
100.0	24.6	0.0	—	9042.0

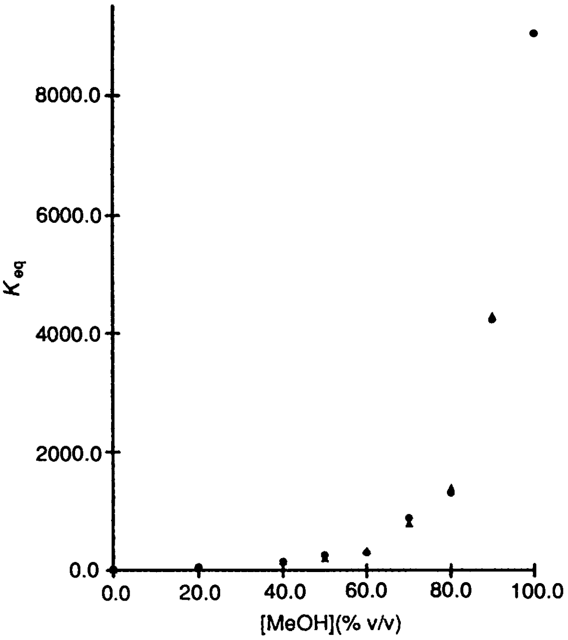

Fig. 1 Equilibrium constants, K_{eq}^{e} (●) and K_{eq}^{k} (▲), for reaction (1) at 25 °C and I = 0.15 mol dm^{-3} NaClO$_4$

(represented as Co–OH$_2$) by iodide and the reverse reaction to give the equilibrium (1).[5,10] Spectrophotometric measurements

$$\mathrm{Co{-}OH_2^+ + NaI \underset{k_r}{\overset{k_f}{\rightleftharpoons}} Co{-}I + H_2O + Na^+} \qquad (1)$$

of solutions in which reaction (1) has reached equilibrium were employed to calculate the numerical value of the equilibrium constant. This procedure involves the use of the equation (2)[16]

$$(A^{\lambda}_{\infty} - A^{\lambda}_{0}) = \frac{(\varepsilon^{\lambda}_{\mathrm{Co{-}I}} - \varepsilon^{\lambda}_{\mathrm{Co{-}OH_2}})K_{eq}[\mathrm{Co{-}OH_2}][\mathrm{NaI}]}{1 + K_{eq}[\mathrm{NaI}]} \qquad (2)$$

where A^{λ}_{∞} = absorbance of the system at equilibrium for the specified [I$^-$], A^{λ}_{0} = absorbance of a solution of aquacobalamin with no iodide at the same total aquacobalamin concentration, $\varepsilon^{\lambda}_{\mathrm{Co{-}OH_2}}$ and $\varepsilon^{\lambda}_{\mathrm{Co{-}I}}$ the molar absorption coefficients for Co–OH$_2$ and Co–I respectively and [Co–OH$_2$] is the total concentration of aquacobalamin.

The absorbance of solutions at equilibrium was measured at 350, 380, 500, 520, 560 and 570 nm over a range of iodide concentration (0–1.2 mmol dm^3) and an aquacobalamin concentration of 2 × 10^{-5} mol dm^{-3}. A non-linear least-squares program was used to obtain the values of the equilibrium constant, for each of the solutions with different water–methanol compositions ranging from 0 to 100% (v/v) of methanol. The values for K_{eq} obtained with this method have an average deviation from the mean of 7% for between 5 and 10 individual experiments. The values are shown in Table 1 and plotted as a function of the solution composition in Fig. 1.

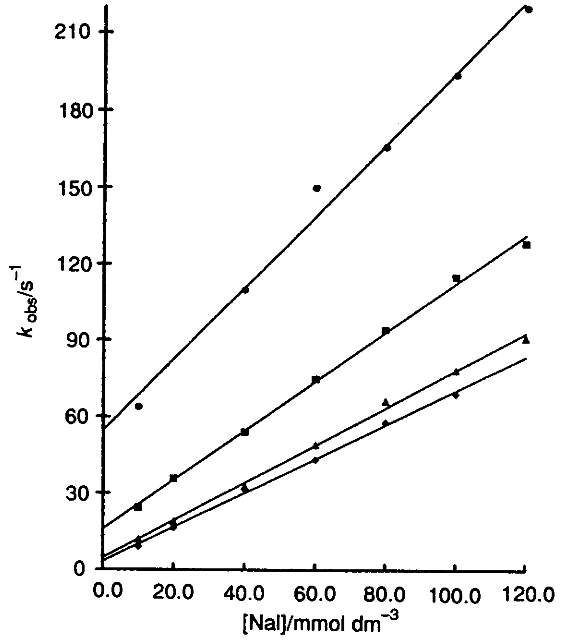

Fig. 2 Observed behaviour of the first-order rate constant k_{obs} vs. [I$^-$] in aqueous methanol solutions at 25 °C and I = 0.15 mol dm^{-3} NaClO$_4$. Methanol concentrations of 0 (●), 20 (■), 40 (▲) and 50% (◆)

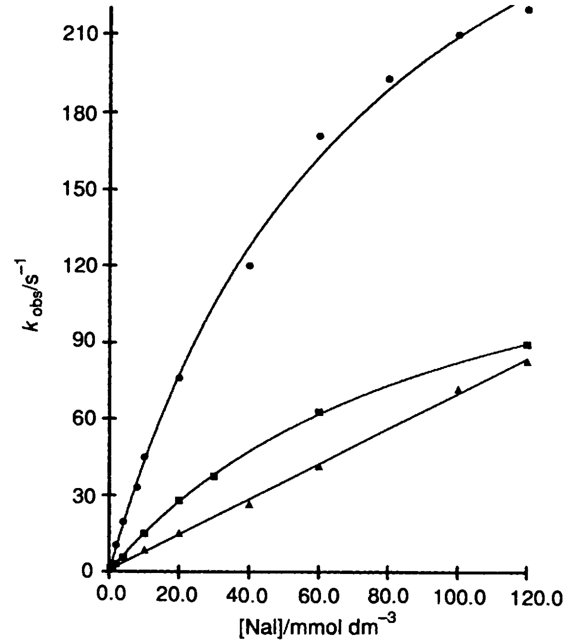

Fig. 3 Observed behaviour of the first-order rate constant k_{obs} vs. [I$^-$] in aqueous methanol solutions at 25 °C and I = 0.15 mol dm^{-3} NaClO$_4$. Methanol concentrations of 70 (▲), 90 (■) and 100% (●)

A spectroscopic study of the solutions in 100% methanol indicated that a substitution reaction between water and methanol on the cobalamin moiety occurs. The equilibrium constant for this process (3) was evaluated through the procedure described

$$\mathrm{Co{-}HOMe + H_2O \rightleftharpoons Co{-}OH_2 + MeOH} \qquad (3)$$

Table 2 Observed behaviour of the rate constant k_{obs} (s^{-1}) *vs.* $[I^-]$/mmol dm^{-3} in aqueous methanol solutions at 25 °C and $I = 0.15$ mol dm^{-3} ($NaClO_4$)

	[MeOH] (% v/v)*								
$[I^-]$	0	20	40	50	60	70	80	90	100
0.0									
0.5								1.5 (0.1)	
1.0							1.4 (0.3)	1.7 (0.1)	3.1 (0.2)
2.0							2.3 (0.1)	3.0 (0.2)	10.4 (0.3)
4.0							3.8 (0.5)	5.6 (0.3)	19.5 (0.1)
8.0									33.0 (2.0)
10.0	63.9 (5.3)	24.2 (1.7)	12.0 (0.9)	9.1 (0.8)	7.9 (1.5)	8.6 (0.8)	8.1 (1.1)	14.9 (0.1)	44.9 (2.2)
20.0		35.8 (1.9)	19.0 (0.8)	16.5 (0.7)	15.2 (2.0)	15.2 (1.7)	16.4 (1.8)	28.0 (1.2)	76.0 (3.0)
30.0								37.5 (2.0)	
40.0	109.9 (7.2)	54.1 (3.3)	32.9 (1.1)	31.5 (1.5)	29.6 (3.0)	26.7 (1.2)	28.7 (2.3)		120.0 (5.7)
60.0	150.0 (8.3)	74.8 (2.9)	49.1 (1.7)	43.3 (3.0)	44.1 (1.4)	41.6 (2.2)	44.6 (3.0)	62.7 (3.0)	171.0 (6.2)
80.0	166.0 (9.0)	94.2 (4.3)	66.4 (3.2)	57.9 (4.0)	56.3 (1.0)				193.0 (5.4)
100.0	194.0 (10.0)	115.1 (8.9)	78.4 (7.7)	69.1 (4.0)	68.7 (2.0)	72.0 (4.0)	76.0 (3.0)		210.0 (4.2)
120.0	220.0 (15.0)	128.5 (7.3)	91.1 (6.5)		81.9 (4.0)	83.0 (3.7)	85.7 (4.2)	89.6 (4.3)	220.0 (8.0)

* Values of 3σ are given in parentheses.

above. The value for $K = [\text{Co–OH}_2][\text{MeOH}]/[\text{Co–HOMe}][H_2O]$, $= 3.69 \pm 0.1$, was obtained under conditions in which $[H_2O] \ll [\text{MeOH}]$. The experimental data, together with the calculated curve, is presented in Table 4 and Fig. 7, λ_{max}(Co–HOMe) = 351 nm ($\varepsilon = 2.06 \times 10^4$ dm^3 mol^{-1} cm^{-1}).

The possibility of the presence of Co–OH due to the change in relative permittivity upon solvent change may be ruled out since the spectrum of Co–OH is distinctly different from that of Co–OH_2, *i.e.* $\lambda_{max} = 356$ ($\varepsilon = 1.92 \times 10^4$ dm^3 mol^{-1} cm^{-1}.[17] Furthermore we monitored the spectra of our system as a function of added H^+ and observed no changes.

Kinetic Studies.—As noted in the Introduction, Hasinoff[10] has shown that the substitution of aquacobalamin by iodide proceeds *via* an outer-sphere mechanism. This process, as illustrated in reactions (4) and (5), is well established and

$$\text{Co–solv}^+ + I^- \overset{K_{os}}{\rightleftharpoons} \{(\text{Co–solv})\text{–}(I)\} \tag{4}$$

$$\{(\text{Co–solv})\text{–}(I)\} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{Co–I} + \text{solv} \tag{5}$$

described by Eigen and Wilkins.[18,19] Here solv = solvent and K_{os} = equilibrium constant for the formation of the outer-sphere complex. Such a mechanism has an overall rate law which depends upon the extent of reversibility of the final solvent expulsion from the outer-sphere complex. In the event that this process is irreversible, *i.e.* k_{-2} is effectively zero, the rate equation (6) is applicable. This expression will result in a

$$k_{obs} = \frac{k_2 K_{os}[I^-]}{1 + K_{os}[I^-]} \tag{6}$$

hyperbolic plot of k_{obs} *vs.* $[I^-]$, with no intercept at $[I^-] = 0$.[16] If the solvent expulsion is reversible, then equation (7) is

$$k_{obs} = \frac{k_1[I^-] + (k_{-1}k_2/k_{-2})}{(k_{-1}/k_2) + [I^-]} \tag{7}$$

applicable.[20] Plots of k_{obs} *vs.* $[I^-]$ for this expression should also be parabolic, with an intercept at $[I^-] = 0$ from which the value of k_{-2} can be evaluated.

The observed behaviour of the rate constant k_{obs} *vs.* $[I^-]$ for the present system is presented in Table 2 and Figs. 2 and 3. It is clear that there are two distinct types of behaviour. At low methanol concentrations, which we term water-dominated systems, linear behaviour is noted with intercepts at $[I^-] = 0$, whereas in the methanol-dominated systems, *i.e.* greater than 80% MeOH, parabolic behaviour with no intercept is observed. When the observed data are fitted by equation (6) or (7) the solid lines shown in Figs. 2 and 3 are obtained, showing a good correlation between experimental and theoretical points.

Discussion

Thermodynamic Behaviour.—The reaction between the iodide ligand and aquacobalamin is significantly affected by the progressive substitution of water by methanol in the reaction medium. The formation constant varies from 26 in water to 9.042×10^3 dm^3 mol^{-1} in methanol. Such a change is in accord with the work of Balt *et al.*[21] on substitution of water by SCN^- as a function of changing solvent from water to acetonitrile, *i.e.* values of 1.9×10^3 and 1.4×10^4 dm^3 mol^{-1} respectively. The variations for the I^- system are larger than for SCN^-, and both are significantly greater than those observed for neutral-ligand displacement reactions. For example, in the system aquacobalamin–urea in water–dioxane solvent mixtures, K_{eq} varied from 13 to 17 dm^3 mol^{-1}.[15] Such results are in accord with expectation for reactions between charged or uncharged species as a function of solvent polarity.[22] This result is further illustrated by plotting ln *K* *vs.* 1/ε, the inverse of the solvent relative permittivity, Fig. 5. A more detailed understanding of this behaviour is obtained from the study of the kinetic aspects of our investigation and is discussed below.

The observation that reaction (3) exists with $K = 3.69$ is significant. Furthermore we note that this value does not change as a function of solvent composition, in direct contrast to the ionic reaction involving iodide co-ordination.

Kinetic Behaviour.—It is clear from the data recorded in Table 2 and Figs. 1 and 2 that the expected kinetic behaviour for an outer-sphere complex mechanism is not exhibited for water-dominant solvent systems, but it is clearly observed for the methanol-dominant systems. The explanation stems from the fact that the reaction (3) of aquacobalamin and methanol has an equilibrium constant of 3.69 ± 0.1, forming significant amounts of Co–HOMe as noted in Table 4. Thus, the reaction we have studied involves two discrete intermediates, the aqueous or methanol-substituted cobalamins. The outer-sphere complex mechanism, as noted earlier, involves two rate equations depending upon the degree of reversibility of the final solvent expulsion. It is apparent that in the case of the methanol-dominant solvent system the rate equation (6) applies, and indeed the 'expected' parabolic behaviour is observed, Fig. 3.

Table 3 Observed second-order rate constants ($dm^3\ mol^{-1}\ s^{-1}$) k_f and k_r for the reaction of cobalamin and iodide in aqueous methanol solutions at 25 °C and I = 0.15 mol dm^{-3} ($NaClO_4$)

[MeOH]				
% v/v	mol dm^{-3}	$[H_2O]$/mol dm^{-3}	k_f	k_r
0.0	0.0	55.4	1404.8	54.6
20.0	4.9	44.3	963.9	16.1
40.0	9.8	33.2	733.0	4.8
50.0	12.3	27.7	690.1	2.7
60.0	14.7	22.2	669.9	2.2
70.0	17.2	16.6	692.8	0.8
80.0	19.7	11.1	782.7	0.6
90.0	22.1	5.5	1395.0	0.3
100.0	24.6	0.0	4340.0	0.5

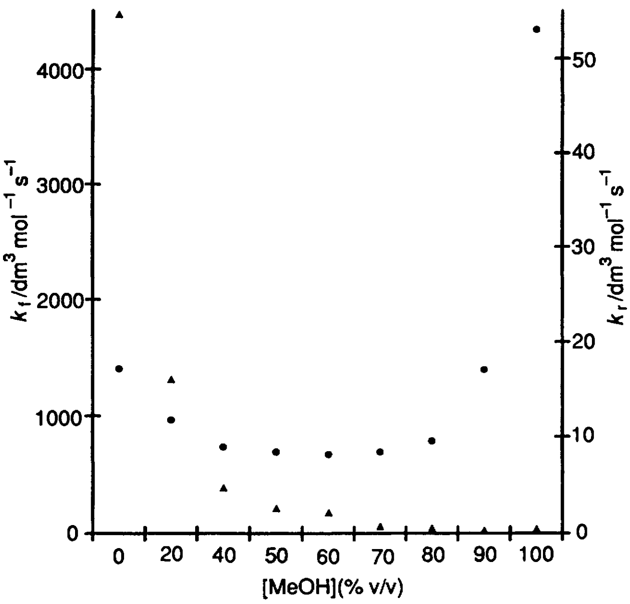

Fig. 4 Behaviour of the second-order rate constants k_f (▲) and k_r (●) *vs.* methanol (% v/v)

In the case of the water-dominant media, whereas such parabolic behaviour is not observed, a positive intercept is noted at $[I^-] = 0$, clearly indicating a reversible solvent expulsion from the outer-sphere complex. We do not observe the parabolic behaviour because k_2 is so high that the iodide concentrations needed for limiting k_{obs} values have not been attained in our study. The smaller rate of methanol loss compared to that of water, approximately two orders of magnitude, has been previously established from studies on exchange of methanol or water in metal complexes.[23] It is also possible that our results mirror a dramatic change in K_{os} around a solvent composition of 90% MeOH.*

Notwithstanding the fact that the outer-sphere complex mechanism is operative in our system, it is instructive to analyse our data in accord with the simple scheme outlined in equation (1). For such a process, plots of k_{obs} *vs.* $[I^-]$ should be linear with slope k_f and intercept k_r. In the water-dominant systems linear plots are obtained, as they are in the low $[I^-]$ regions of the experiments performed in methanol-rich solvents. These data are reported in Table 3, and may be used to determine the kinetic formation constants, K^k_{eq}. The resulting values are recorded in Table 1 along with those obtained from the spectroscopic experiments, K^e_{eq}. The two sets of data are remarkably similar, justifying such an analysis. The value for K^k_{eq} in 100% methanol could not be obtained from the kinetic data since, as noted above, the reaction in this solvent obeys the rate equation (6), *i.e.* the expulsion of MeOH is irreversible, thus no sensible value of k_r from equation (1) could be obtained.

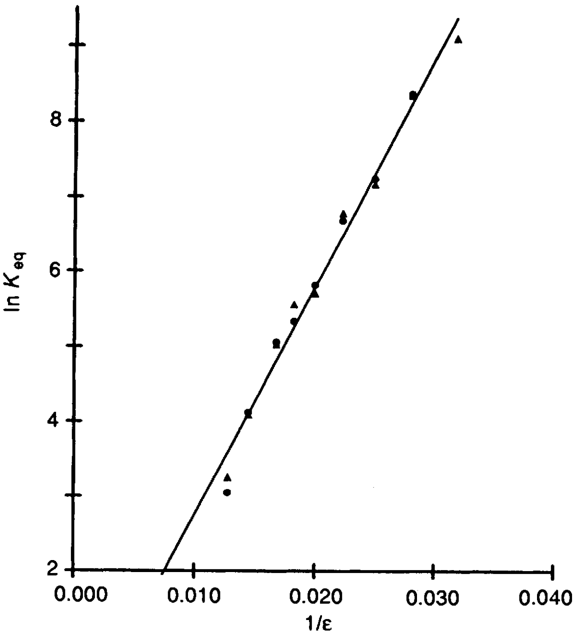

Fig. 5 Plot of ln K_{eq} *vs.* 1/ε of solvent for kinetic (●) and spectroscopic (▲) measurements

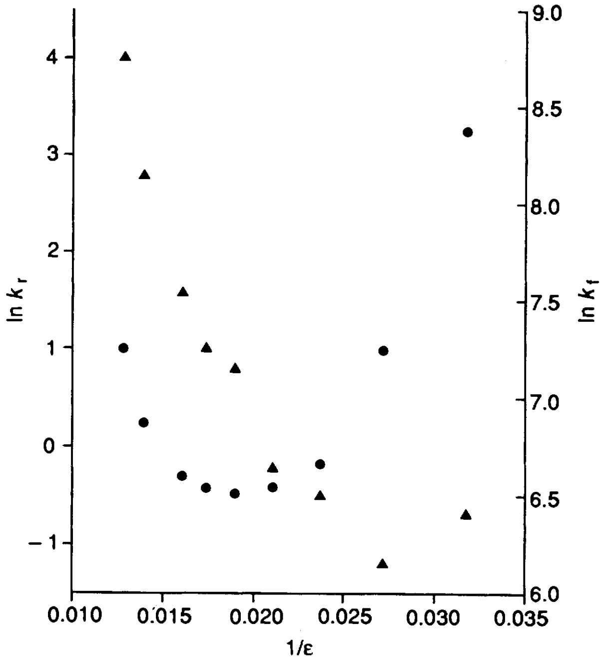

Fig. 6 Plot of ln k_f (▲) and ln k_r (●) *vs.* 1/ε of solvent

The major change in the values of K_{eq} as a function of solvent composition can now be understood in terms of the various rate data. The values taken from Table 3 are plotted as a function of solvent composition in Fig. 4. For a reaction between two oppositely charged ions a linear relationship between ln k_f and 1/ε, is expected.[22] In the present case this relationship is only observed up to 40% methanol, or, from our interpretation of the Balt data, 70% acetonitrile.[21] After this limit our system changes significantly. The slope dramatically changes sign and this cannot be explained in terms of the normal variation of rate with the relative permittivity. These data are presented in Fig. 6. Such a clear distinction cannot be made from the Balt data since that investigation involved a more restricted solvent composition range. On the other hand, k_r decreases with increasing methanol concentration. This behaviour agrees with

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* The authors thank a referee for pointing out this possibility.

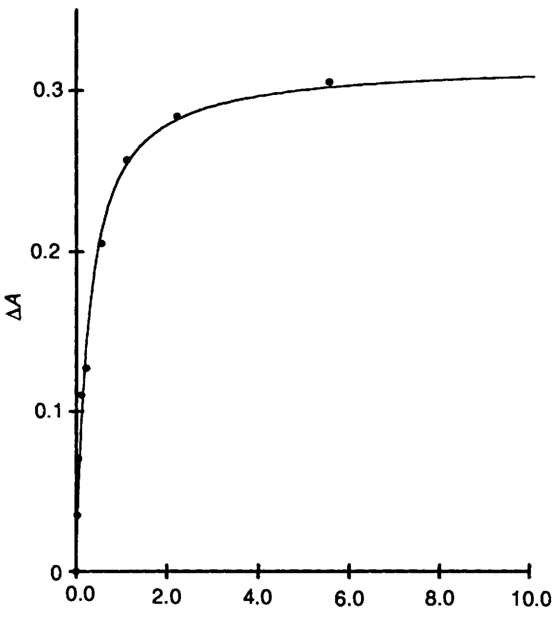

Fig. 7 Plot of ΔA observed (●) and calculated (—) [with $K = 3.69$, using an equivalent equation to (2)] *vs.* $[H_2O]$ for reaction (3)

Table 4 Equilibrium concentrations of Co–HOMe in water–methanol solutions at 25 °C and $I = 0.15$ mol dm^{-3} ($NaClO_4$)

[MeOH]			
% v/v	mol dm^{-3}	$[H_2O]$/mol dm^{-3}	Co–OH_2 (%)
0.0	0.0	55.4	100.00
10.0	2.5	49.9	99.95
20.0	4.9	44.3	99.88
30.0	7.4	38.8	99.79
40.0	9.8	33.2	99.68
50.0	12.3	27.7	99.52
60.0	14.7	22.2	99.28
70.0	17.2	16.6	98.88
80.0	19.7	11.1	98.10
90.0	22.1	5.5	95.82
100.0	24.6	0.0	0.00

the Balt data.[21] As a result the value of the equilibrium constant increases greatly as the mole fraction of methanol in the mixture increases. In other words the overall substitution reaction becomes more irreversible as the solvent polarity is decreased.

In summary, the present work demonstrates unambiguously in methanol-dominated solvent systems the levelling effect of the incoming ligand concentration upon the overall rate of substitution at cobalt expected from the outer-sphere complex mechanism. The results also illustrate the significant increase in stability of the iodocobalamin complex upon progressive substitution of methanol for water.

Acknowledgements

Support of this research by the National Institutes of Health *via* grants RR-08012 and GM-08048 is gratefully acknowledged. R. M.-E. thanks the Facultad de Quimica of the Universidad Nacional Autonoma de Mexico for a leave of absence.

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Received 24*th September* 1991; *Paper* 1/04907K