Cobalamins and the spectrochemical series†

Susan M. Chemaly*

Received 25th April 2008, Accepted 18th July 2008
First published as an Advance Article on the web 10th September 2008
DOI: 10.1039/b806927a

UV-visible-NIR spectra of a variety of cobalamins were run in water and methanol. A broad absorption band (band A) with extinction coefficients of about an order of magnitude less than those of the αβ bands was found in the red and NIR regions for Cl-cobalamin (Cl-cbl), Br-cbl, I-cbl, $SC(NH_2)_2$-cbl$^+$ and SeCN-cbl. $OCrO_3$-cbl$^-$, which also has a broad absorption band in the NIR was prepared for the first time. After deconvolution, similar broad bands were seen in the visible region for many other cobalamins. The wavelengths for band A placed the cobalamins in an order similar to the spectrochemical series but different from that of the αβ and γ bands (π–π* transitions), which follow the nephelauxetic series. Band A was ascribed to a ligand-to-metal charge transfer (LMCT) transition from a π orbital in the corrin ring to Co(III). This is the first systematic study of LMCT bands in cobalamins.

Introduction

The intense red colour of vitamin B_{12} (cyanocobalamin, Fig. 1) played an important part in its isolation and purification in 1948 (by Karl Folkers and co-workers at Merck and almost simultaneously by E. Lester Smith and co-workers at Glaxo).[1] The UV-visible spectrum of vitamin B_{12} was first reported in 1949 (by Ellis and co-workers at BDH)[2] and UV-visible spectroscopy has, ever since, been important in the study of cobalamins. Cobalamins, like all corrinoids, show intense spectra, arising from π–π* transitions of the conjugated corrin ring, in both the visible and near UV regions.[3–7] The absorption bands due to corrin π–π* transitions are designated as αβ, D/E and γ bands (Fig. 2) and shift to higher energies with increasing σ-donor ability of the upper axial ligand in cobalamins[3–7] (Fig. 1). UV-visible spectra have been said to "metaphorically open a window on the machine room of the corrin ligand"[5] and an understanding of the electronic structure of cobalamins is basic to an understanding of their function as co-enzymes.

Absorption bands at longer wavelengths and lower intensities than the αβ bands of cobalamins were first reported by Pratt and Thorp in 1966.[8] These were especially noticeable for I-cbl (~740 nm), SeCN-cbl (~730 nm) and S_2O_3-cbl$^-$ (~680 nm) and were ascribed to spin-forbidden bands.[3] More recently, similar bands were also observed by Perry and Marques.[9] Brunold and co-workers[7] studied H_2O-cbl$^+$ and CN-cbl by UV-visible spectroscopy, circular dichroism (CD), magnetic circular dichroism (MCD) and density functional theory (DFT). They found a weak positive feature in the CD spectrum of H_2O-cbl$^+$ at 17 000 cm^{-1}/588 nm, which occurred at a lower energy than the αβ band but showed no obvious equivalent in the visible spectrum. They assigned this to a LMCT transition, specifically the corrin π → Co $3d_{z^2}$ transition, using DFT.

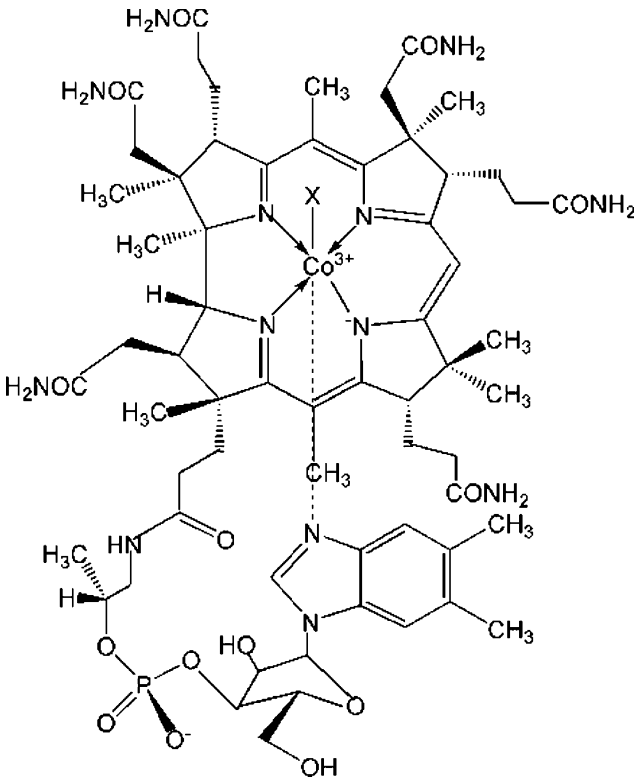

Fig. 1 The structure of cobalamins. Co(III) is coordinated to the four N atoms of the delocalized corrin ring in the equatorial plane and to the N of the 5,6-dimethylbenzimidazole (dbzm) ligand below the plane of the ring. The upper ligand X is CN^- in cyanocobalamin (vitamin B_{12}) and H_2O in aquocobalamin (vitamin B_{12a}). H_2O can be substituted by a wide variety of ligands.

The objectives of this study were to survey the visible and near infrared (NIR) spectra of cobalamins in solution for absorption bands of longer wavelength than the αβ bands (>600 nm) and to determine their origin. In order to do this, solutions of cobalamins were prepared in a spectrophotometer cell and their UV-visible-NIR spectra obtained. Cobalamins are soluble in only a limited

School of Chemistry, Faculty of Science, University of the Witwatersrand, Johannesburg, South Africa. E-mail: susan.chemaly@wits.ac.za

† Electronic supplementary information (ESI) available: Parameters obtained from Gaussian deconvolution of the visible-NIR spectra of cobalamins. See DOI: 10.1039/b806927a

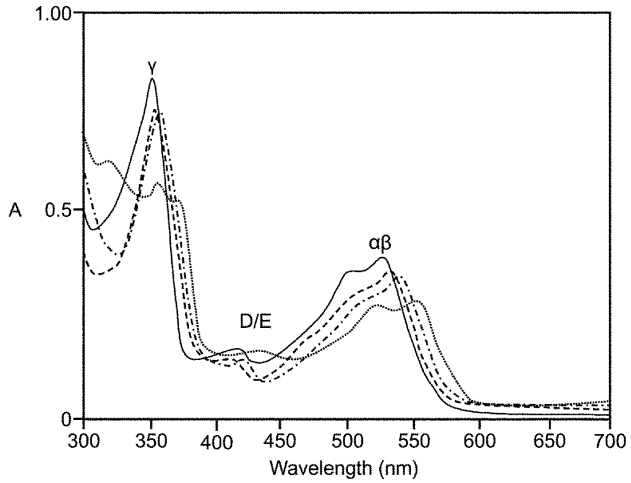

Fig. 2 UV-visible spectra of F-cbl (solid line), Cl-cbl (dashed line), Br-cbl (dot-dash line) and I-cbl (dotted line) (3.8×10^{-5} mol L^{-1}) in methanol, showing the αβ, D/E and γ bands.

range of solvents, including water, lower alcohols, DMSO[10] and acetic acid,[11] and most spectra of cobalamins to date have been run with water as the solvent. In order to determine the effect of solvent, the spectra for each cobalamin were run in at least two solvents when possible, usually water and methanol, but occasionally acetonitrile–water (80 : 20), acetic acid and DMSO.

For cobalamins where there is a possibility of coordination through more than one ligand donor atom, it is necessary to specify the donor atom(s) coordinated to Co(III) in solution. For the ambidentate cobalamins used in this study, the following applies: $(NH_2)_2CS$-cbl$^+$,[12,13] S_2O_3-cbl$^-$,[14] and SO_3-cbl$^-$,[12,13] bond through S in the solid state and their UV-visible spectra in aqueous solution are consistent with bonding through S.[3] SCN-cbl is bonded through both N and S to give the two isomers, NCS-cbl and SCN-cbl, in aqueous solution[14,15] and both isomers are also found in the solid state.[14,16] SeCN is bonded through Se in aqueous solution[14] but both isomers are found in the solid state.[14,16] NO_2-cbl bonds through N in the solid state[14,16] and in solution.[14] The OCN^- ligand can potentially bind through C (fulminate), O (cyanate) or N (isocyanate). It has been proposed, based on its UV-visible spectrum (γ band at 357 nm),[3,17] that OCN-cbl is bonded through N. The X-ray crystal structure of OCN-cbl has not been performed. However, X-ray crystal structures of Co(III) complexes with OCN^- bonded through the C atom, in the fulminato complex $[AsPh_4]_3[Co(CNO)_6]$,[18,19] and the N atom, in the Schiff base complex $[Co\{NH_2C(CH_3)_2C(CH_3)=NH\}_2(NH_3)NCO](ClO_4)_2$,[20] but not the O atom, have been determined. It seems likely that OCN-cbl binds through N, as in the Schiff base complex. $P(OC_2H_5)_2O$-cbl and $P(OC_2H_5)_3$-cbl$^+$ bond through the P atom.[21] CN-cbl bonds through C.[22]

Results and discussion

UV-visible-NIR spectra

The cobalamins prepared and their UV-visible-NIR spectra are given in Table 1. The dbzm base is coordinated to Co(III) in all cases, as shown by the presence of the characteristic poorly resolved band at 288 nm.[5] The UV-visible spectra of Cl-cbl, Br-cbl, I-cbl,[8,9,14] H_2O-cbl$^+$,[23] OH-cbl,[23] *S*CN-cbl/*N*CS-cbl,[8,9,14] $(NH_2)_2CS$-cbl$^+$,[17] SO_3-cbl$^-$,[9,14,17] S_2O_3-cbl$^-$,[8,14] *Se*CN-cbl,[8,9,14] OCN-cbl,[8] NO_2-cbl,[9,14,17] N_3-cbl,[9,17] $P(OC_2H_5)_3$-cbl$^+$, $P(OC_2H_5)_2O$-cbl,[21] CN-cbl[22] and CH_3-cbl[9,14] in water and that of CH_3OH-cbl$^+$ in methanol[24] are similar to those previously reported. F-cbl was unstable in water[8] but its UV-visible spectrum could be obtained in methanol (Fig. 2), as the formation constants of cobalamins are smaller in methanol than in water.[24] The UV-visible spectra of Cl-cbl, Br-cbl, I-cbl (Fig. 2), *S*CN-cbl/*N*CS-cbl, $(NH_2)_2CS$-cbl$^+$, *Se*CN-cbl and OCN-cbl in methanol are similar to those in water, with only slight shifts in wavelength and intensity, the most noticeable of these being the shift in intensity towards the β region of the αβ band in *Se*CN-cbl. The UV-visible spectra of H_2O-cbl$^+$ in methanol–water 80 : 20 (H_2O is replaced by CH_3OH in pure methanol)[24] and acetonitrile–water 80 : 20 (vitamin B_{12a} is insoluble in pure acetonitrile)[25] are similar to those in water. CH_3OH-cbl$^+$ in methanol, CH_3COOH-cbl$^+$ in acetic acid; $(CH_3)_2SO$-cbl$^+$ and $(NH_2)_2CO$-cbl$^+$ in DMSO are assumed to bind through the O atom and the spectra are consistent with this.[3]

Cl-cbl, Br-cbl and I-cbl in both water and methanol show a broad absorption band in the red-NIR region of the spectrum, which is not seen in F-cbl, and is found at shorter wavelengths in methanol than in water (Fig. 3a and b). The red-NIR spectrum of H_2O-cbl$^+$ in water (Fig. 3a) at concentrations of about 2×10^{-4} mol dm^{-3} and higher shows a shoulder, which increases in intensity with increasing concentration, on the red side of the αβ band. CH_3OH-cbl$^+$, CH_3COOH-cbl$^+$, $(CH_3)_2SO$-cbl$^+$ and $(NH_2)_2CO$-cbl$^+$ show a shoulder similar to that for H_2O-cbl$^+$ on the red side of the αβ band. $(NH_2)_2CS$-cbl$^+$ and *Se*CN-cbl in water (Fig. 3c), methanol and DMSO show broad bands in the red-NIR region (similarly to the halogenocobalamins). *S*CN-cbl/*N*CS-cbl, where there is an obvious shoulder in the red-NIR region, and OCN-cbl, where there is no absorption in this region, are also shown in Fig. 3c.

The spectrum of chromatocobalamin, $OCrO_3$-cbl$^-$, is reported for the first time (Fig. 3d). $OCrO_3$-cbl$^-$ (from vitamin B_{12a} in 2.5 mol dm^{-3} $Cr_2O_7^{2-}$) has a broad band in the NIR at 756 nm but only the spectrum above ~600 nm can be seen (except at very low concentrations of $Cr_2O_7^{2-}$) because of strong absorption by $Cr_2O_7^{2-}/CrO_4^{2-}$ in the visible region. Assuming 90% formation of $OCrO_3$-cbl$^-$ at 2.5 mol dm^{-3}, $Cr_2O_7^{2-}$ gives a formation constant of ~2 dm^3 mol^{-1}.

Gaussian deconvolution of spectra

The wavelengths and molar extinction coefficients of the broad band in the red-NIR region obtained by Gaussian deconvolution of the cobalamin spectra, now designated band A, are given in Table 2. The absorbance values, wavenumbers and half-widths of the Gaussian components, together with the standard errors, are given in the ESI, Table S1.† Band A is very broad compared to the bands in the visible and UV region and the extinction coefficients are approximately an order of magnitude smaller than those of the αβ bands. Examples of the Gaussian deconvolution are shown for I-cbl (Fig. 4a), SeCN-cbl (Fig. 4b) and Cl-cbl, Br-cbl and $SC(NH_2)_2$-cbl$^+$ behave similarly. For H_2O-cbl$^+$ (Fig. 4c), CH_3OH-cbl$^+$, CH_3COOH-cbl$^+$, $(CH_3)_2SO$-cbl$^+$, SCN-cbl, S_2O_3-cbl$^-$, NO_2-cbl and N_3-cbl, band A appears as a shoulder ($\Delta A \leq 0.001$) in the red or NIR, rather than a distinct peak, and is resolved by Gaussian deconvolution. For F-cbl (Fig. 4d), SO_3-cbl$^-$, OH-cbl

Table 1 UV-visible-NIR spectra of cobalamins in water, methanol and other solvents. Cobalamins are grouped according to the donor atom: halide, O, S, Se, N, P, C and then arranged in order of increasing wavelength of the γ band

Ligand X[a]	Solvent	γ band λ/nm (ε/dm^3 mol^{-1} cm^{-1})	αβ band λ/nm[b] (ε/dm^3 mol^{-1} cm^{-1})	NIR-red band λ/nm (ε/dm^3 mol^{-1} cm^{-1})
$F^{-c,d}$	Water	352	α 526	—[e]
F^{-d}	Methanol	355 (2.21×10^4)	α 532 (8.65×10^3)	—[e]
Cl^-	Water	356 (2.25×10^4)	α 537 (8.66×10^3)	687 (4.98×10^2)
Cl^-	Methanol	356 (1.93×10^4)	α 540 (8.94×10^3)	sh[f]
Br^-	Water	359 (2.24×10^4)	α 542 (8.66×10^3)	716 (6.45×10^2)
Br^-	Methanol	360 (1.99×10^4)	α 547 (8.93×10^3)	686 (7.19×10^2)
I^-[g]	Water	314 (1.77×10^4)	α 560 (7.46×10^3)	749 (1.18×10^3)
		361 (1.94×10^4)	—	—
		~375 (s) (1.7×10^4)	—	—
I^-	Methanol	322 (1.63×10^4)	α 558 (7.47×10^3)	722 (1.06×10^3)
		358 (1.54×10^4)	—	—
		372 (1.41×10^4)	—	—
$I^{-c,d}$	DMSO	323	α 550	720–760
		360	—	—
H_2O	Water	351 (2.66×10^4)	α 524 (8.77×10^3)	sh
H_2O[h]	Methanol–water 80 : 20	351 (2.52×10^4)	α 523 (8.83×10^3)	sh
H_2O[i]	Acetonitrile–water 80 : 20	352 (2.67×10^4)	α 524 (9.07×10^3)	sh
CH_3OH	Methanol	352 (2.17×10^4)	αβ 525 (8.54×10^3)	sh
$(NH_2)_2CO^{c,d}$	DMSO	355	αβ 523	sh
$(CH_3)_2SO$	DMSO	355	α 539	sh
CH_3COOH	Acetic acid	~322 (s) 359	α 541	sh
OH^-	Water	357 (2.14×10^4)	α 536 (9.98×10^3)	—[e]
NCS^-/SCN^-	Water	~315 (s) (1.1×10^4)	α 538 (8.00×10^3)	sh
		357 (2.17×10^4)	—	—
NCS^-/SCN^-	Methanol	~317 (s) (1.1×10^4)	α 537 (7.95×10^3)	sh
		357 (2.15×10^4)	—	—
$SC(NH_2)_2$	Water	~360[j]	α 548 (7.61×10^3)	675 (7.29×10^2)
$SC(NH_2)_2$	Methanol	356 (1.68×10^4)	α 547 (7.28×10^3)	671 (7.05×10^2)
$SC(NH_2)_2^{c,d}$	DMSO	353	α 545	sh
SO_3^{2-}	Water	312 (1.93×10^4)	α 538	—[e]
		364 (2.00×10^4)	β 517 (9.45×10^3)	—
$S_2O_3^{2-}$[k]	Water	329 (1.67×10^4)	α544(7.88 $\times 10^3$)	sh
		366 (1.68×10^4)	—	—
$SeCN^-$	Water	330 (1.86×10^4)	αβ 544 (8.31×10^3)	693 (1.08×10^3)
		357 (1.85×10^4)	—	—
		371 (1.86×10^4)	—	—
$SeCN^-$	Methanol	330 (1.76×10^4)	αβ 539 (7.41×10^3)	684 (9.39×10^2)
		356 (1.67×10^4)	—	—
		370 (1.65×10^4)	—	—
$SeCN^{-c,d}$	DMSO	326	αβ 537	670–680
		358	—	—
OCN^-	Water	~321 (s) (9.8×10^3)	α 537 (9.45×10^3)	—[e]
		357 (2.31×10^4)	—	—
OCN^-	Methanol	~322 (s) (1.1×10^4)	α 536 (8.79×10^3)	—[e]
		357 (2.09×10^4)	—	—
NO_2^-	Water	355 (2.57×10^4)	α 532 (9.99×10^3)	sh
N_3^-	Water	359 (2.40 x10^4)	α 541 (9.61×10^3)	sh
$P(OC_2H_5)_2O^-$	Water	310, 325, 364	β 541	—[e]
$P(OC_2H_5)_3$	Water	314, 335, 364	β 550	—[e]
CN^-	Water	361	β 549	—[e]
CH_3^-	Water	343	αβ 522	—[e]
$OCrO_3^{2-}$[l]	Water	—	—	756

[a] The ligand atom that is coordinated to cobalamin is italicized. [b] α indicates that the α band is more intense, β indicates that the β band is more intense, αβ indicates that there is no separation between the α and β bands. [c] Reaction not complete. [d] Near-saturated solution. [e] No absorption in the NIR region. [f] sh = shoulder. [g] KI in water showed a peak at 348 nm due to I_3^-. A solution of KI in water at the same concentration as the I-cbl solution was used as blank. See Ref. 8 and Ref. 17, and references therein. [h] A small amount of CH_3OH-cbl$^+$ is present. [i] A small amount of CH_3CN-cbl$^+$ (γ band 358 nm, αβ band 541 nm) is present. [j] The spectrum of $SC(NH_2)_2$-cbl$^+$ is blocked out below 350 nm due to the high absorbance of thiourea. [k] Spectra run at faster speed and lower resolution (2880 nm min^{-1}, 2 nm) due to rapid appearance of SO_3-cbl$^-$. [l] $Cr_2O_7^{2-}$ absorbs strongly in the UV-visible region and the cut-off point is about 600 nm in 2.50 mol L^{-1} solution.

and OCN-cbl, which do not absorb in the red-NIR region, broad bands with low extinction coefficients, in the visible rather than the NIR, appear after Gaussian deconvolution. The wavelengths of the bands obtained by Gaussian deconvolution (when there is only a shoulder in the red-NIR region) are rather sensitive to the modelling of the αβ region and are not precisely determined. For example, deconvolution of triplicate spectra of H_2O-cbl$^+$ in water (run under the same conditions) gave a wavelength of 656 ± 3 nm

Table 2 NIR spectra of cobalamins and bands obtained by Gaussian deconvolution (band A) in water, methanol and other solvents. Cobalamins are grouped according to their donor atom: halide, O, S, Se, N, P, C and then arranged in order of increasing wavelength of their γ band (as in Table 1)

	NIR-red band λ/nm[a] (ε/dm^3 mol^{-1} cm^{-1})		Band A λ/nm (ε/dm^3 mol^{-1} cm^{-1})	
Cobalamin	Water	Methanol	Water	Methanol
F-cbl	—	—	—	542 (446)
Cl-cbl	687 (498)	sh	687 (494)	652 (555)
Br-cbl	716 (645)	686 (719)	724 (513)	684 (647)
I-cbl	749 (1178)	722 (1060)	747 (981)	727 (995)
H_2O-cbl$^+$	sh	sh	656 (252)	642(246)
H_2O-cbl$^+$ in acetonitrile–water 80 : 20	sh	—	649 (268)	—
CH_3*O*H-cbl$^+$	—	sh	—	648 (428)
$(CH_3)_2$*S*O-cbl$^+$ in DMSO	sh	—	652 (498)	—
CH_3CO*O*H-cbl$^+$ in acetic acid	sh	—	656 (526)	—
OH-cbl	—	—	430 (565)	—
*S*CN-cbl/*N*CS-cbl	sh	sh	652 (566)	637 (503)
$(NH_2)_2$C*S*-cbl$^+$	675 (729)	671 (705)	679 (723)	679 (681)
*S*O$_3$-cbl$^-$	—	—	527 (470)	—
S$_2$O$_3$-cbl$^-$[b]	sh	—	642 (615)	—
*Se*CN-cbl	693 (1077)	684 (939)	687 (1037)	681 (938)
OCN-cbl	—	—	638 (273)	633 (321)
*N*O$_2$-cbl	sh	—	621 (680)	—
N$_3$-cbl	sh	—	640 (594)	—
*O*CrO$_3$-cbl	756	—	—	—

[a] From Table 1. [b] Solvent 80 : 20 v/v methanol–water. [c] Spectrum deconvoluted using only 250 points (from faster scan), errors are much greater than for other cobalamins (see ESI, Table S1†).

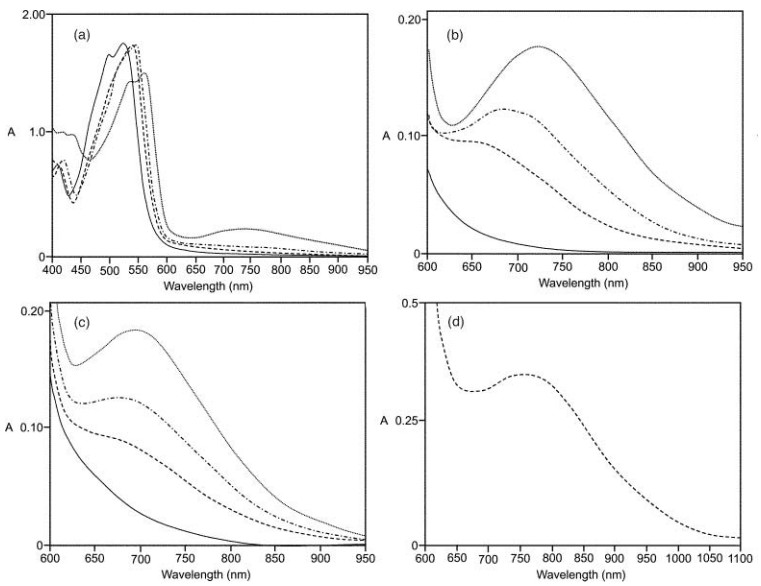

Fig. 3 Visible-NIR spectra of (a) H_2O-cbl$^+$ (solid line), Cl-cbl (dashed line), Br-cbl (dot-dash line) and I-cbl (dotted line) (2.0×10^{-4} mol L^{-1}) in aqueous solution; (b) F-cbl (solid line), Cl-cbl (dashed line), Br-cbl (dot-dash line) and I-cbl (dotted line) (2.0×10^{-4} mol L^{-1}) in methanol; (c) OCN-cbl (solid line), *N*CS/*S*CN-cbl (dashed line), *S*C(NH$_2$)$_2$-cbl (dot-dash line) and *Se*CN-cbl (dotted line) (2.0×10^{-4} mol L^{-1}) in aqueous solution; (d) *O*CrO$_3$-cbl$^-$ in 2.5 mol L^{-1} aqueous $Na_2Cr_2O_7$.

for the Gaussian corresponding to band A but the other Gaussians had a maximum deviation of ± 0.3 nm. For P(OC$_2$H$_5$)$_3$-cbl$^+$, P(OC$_2$H$_5$)$_2$O-cbl, CN-cbl and CH$_3$-cbl, no band corresponding to band A was detected between 400–900 nm.

Wavelength shifts of band A

For the halogenocobalamins, band A shifts to longer wavelengths in the order: F-cbl (methanol only) < Cl-cbl < Br-cbl < I-cbl and the shifts of the αβ and γ bands show the same order. For cobalamins coordinated through O, band A shifts to longer wavelengths in the order: OH-cbl < CH_3OH-cbl$^+$ < H_2O-cbl$^+$ ≈ $(CH_3)_2SO$-cbl$^+$ < CH_3COOH-cbl$^+$; for S: SO_3-cbl$^-$ < S_2O_3-cbl$^-$ < (SCN-cbl) < SC$(NH_2)_2$-cbl$^+$; for N: NO_2-cbl < OCN-cbl < N_3-cbl < (SCN-cbl) (where SCN-cbl is a mixture of S and N bonded isomers). The order for band A of O, S and N donor ligands does not correlate with the shifts of αβ and γ bands.

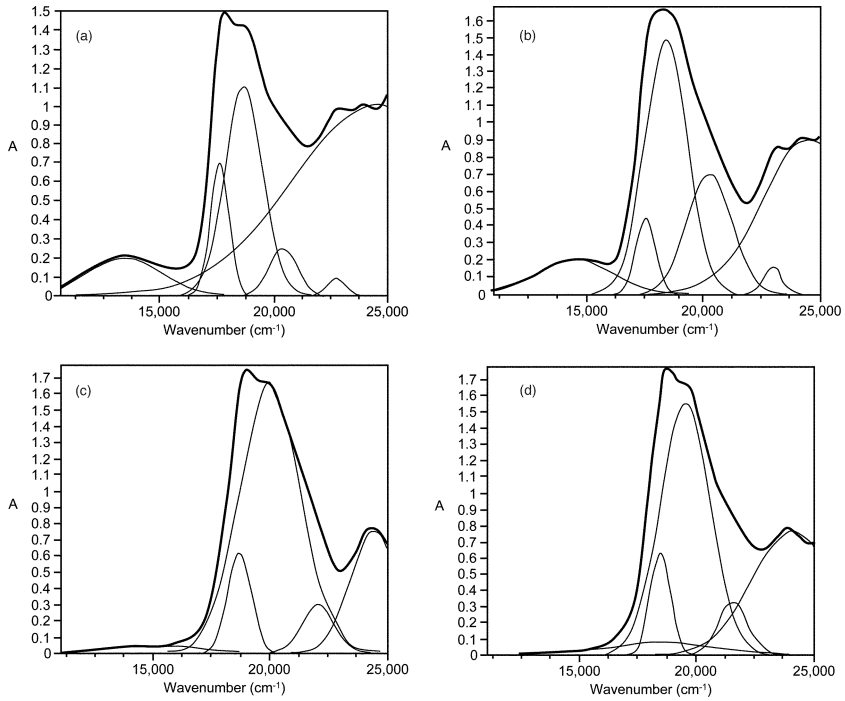

Fig. 4 Deconvoluted visible-NIR spectra (2.0×10^{-4} mol L^{-1}) of (a) I-cbl in water, (b) SeCN-cbl in water, (c) H_2O-cbl$^+$ in water and (d) F-cbl in methanol. The thick black line is the experimental spectrum and the thin black lines are the Gaussian components of the fit to the experimental spectrum.

Band A shifts to shorter wavelengths when the solvent is changed from water (dielectric constant 80)[26] to methanol (dielectric constant 33)[26] for Cl-cbl (35 nm), Br-cbl (40 nm), I-cbl (20 nm), SCN/NCS-cbl (15 nm) and SeCN-cbl (6 nm), where the axial ligand is charged, but there is no shift for $SC(NH_2)_2$-cbl$^+$, where the ligand is neutral. In contrast, the αβ and γ bands show only slight changes in wavelength when the solvent is changed from water to methanol. For H_2O-cbl$^+$, the αβ and γ bands show only slight shifts between water and 80 : 20 acetonitrile–water (dielectric constant 40,[27] considered to be a non-aqueous solvent[25]) or 80 : 20 methanol–water (dielectric constant 42),[28] but band A shifts 7 nm and 14 nm, respectively.

Correlation of wavelength shift of the αβ and γ bands with the nephelauxetic series

It is known[3,29] that the wavelength shifts of the αβ and γ bands (from π–π* transitions in the corrin ring), resulting from changing the donor atom of the axial ligand in cobalamins, correlate well with the nephelauxetic series: (free ion) > F > O > N > Cl = *C*N$^-$ > Br > I > S > Se > As[30] and $P(OC_2H_2)_3CH_3$ > *C*N$^-$,[31] and that the correlation arises from the donation of negative charge from the axial ligand to cobalt through the σ-bond.[3,29] The αβ and γ bands of cobalamins containing axial ligands with more electronegative donor atoms are found at lower wavelengths and those with less electronegative donor atoms are found at higher wavelengths.[3,29] The results obtained in this paper (in terms of donor atoms), F-cbl ≈ O-cbl ≈ N-cbl ≈ Cl-cbl > Br-cbl > I-cbl ≈ S-cbl ≈ Se-cbl ≈P-cbl and Cl-cbl > CN ≈ Br-cbl are consistent with the nephelauxetic series and show that the αβ and γ bands shift to higher wavelengths with increasing polarizability of the ligands.

Correlation of wavelength shift of band A with the spectrochemical series

Arranging the cobalamins in order of decreasing energy (increasing wavelength) of band A gives the following series: *P*$(OC_2H_5)_3$-cbl$^+$, *P*$(OC_2H_5)_2$O-cbl, *C*N-cbl, CH_3-cbl > OH-cbl > *S*O_3-cbl$^-$ > *N*O_2-cbl > OCN-cbl > N_3-cbl > *S*$_2O_3$-cbl$^-$ > CH_3OH-cbl$^+$ > *S*CN/*N*CS-cbl ≈ H_2O-cbl$^+$ ≈ $(CH_3)_2SO$-cbl$^+$ > CH_3COOH-cbl$^+$ > *S*$C(NH_2)_2$-cbl$^+$ > Cl-cbl ≈ *Se*CN-cbl > Br-cbl > I-cbl > *O*CrO_3-cbl$^-$ and F-cbl > H_2O-cbl$^+$. The ligands $P(OC_2H_5)_3$-cbl$^+$, $P(OC_2H_5)_2$O-cbl, CN-cbl and CH_3-cbl are placed at the beginning of the series because they do not show a NIR deconvoluted band above 400 nm and band A, if it exists, has probably been shifted to the γ or UV regions where it would be hidden among many intense bands. This arrangement of cobalamins places polarizable ligands at each end of the series and is obviously not consistent with the nephelauxetic series. It has more in common with the general spectrochemical series (order of ligands according to increase in ligand field strength *Δ*): *C*O = *P*$(OC_2H_2)_3CH_3$ > *C*N$^-$ > *C*NO$^-$ > CH_3^- > *N*O_2^- > *S*O_3^{2-} > *N*CS$^-$ > H_2O > *O*NO$^-$ > C_2H_5OH > CH_3COOH > $(NH_2)_2CO$ = $(CH_3)_2SO$ = OH$^-$ > *N*CO$^-$ > F$^-$ > N_3^- > Cl$^-$ > *S*CN$^-$ > *O*CrO_3^{2-} > Br$^-$ > I$^-$[30–33] and Cl$^-$ > S = Se > Br$^-$.[30] The cobalamin series, like the spectrochemical series, groups the P and C donor atoms together at the top of the series and the halides (I, Br, Cl) and S and Se donor ligands, in order, at the bottom of the series. Both the cobalamin and the spectrochemical series give very similar orders for the ligands H_2O, CH_3OH/C_2H_5OH (assuming that CH_3OH and C_2H_5OH have the same position), $(CH_3)_2SO$ and CH_3COOH in the middle of the series. The position of $(NH_2)_2CO$-cbl, although the wavelength of band A is not accurately determined, is similar to that of $(CH_3)_2SO$ and CH_3COOH in the cobalamin series, as in the spectrochemical

series. *O*CrO_3-cbl$^-$ is at the bottom of the cobalamin series and is at the low end of the spectrochemical series.

When spectrochemical series based only on octahedral Co(III) complexes are considered,

$[Co(NH_3)_5X]^{2+/3+}$, X = *C*N^- > *N*O_2^- > CH_3^- > *N*CS^- > H_2O > F^- > N_3^- > Cl^- > Br^- > I^-,[30,34,35]

$[Co(CN)_5X]^{4-/3-/2-}$, X = *C*N^- > *S*O_3^{2-} > *N*O_2^- > *N*CS^- > *S*CN^- > H_2O > F^- > N_3^- > Cl^- > Br^- > I^-,[30,36]

$[CoX_6]^{3-/3+}$ X = *C*N^- > *P*$(OCH_2)_3CCH_3$ > *C*NO^- > NH_3 > H_2O > N_3^-[30,33] and *P*$(OCH_2)_3CCH_3$ > *P*$(OCH_3)_3$ > *N*H_3 (where *P*$(OCH_2)_3CCH_3$ is a cage phosphite),[37]

the relationship to the cobalamin series based on the NIR deconvoluted band is even more striking.

SCN-cbl in aqueous solution[14,15] and presumably in methanol is a mixture of the two isomers *N*CS-cbl and *S*CN-cbl and the wavelength of band A in the cobalamin series is thus the average for the two isomers. The slightly lower than expected position of *N*O_2-cbl, with respect to the spectrochemical series, could possibly be due to the presence in solution of a small amount of ON*O*-cbl. For the ligand OCN^- three isomers are possible, with bonding through C, N or O. The position of OCN-cbl in the cobalamin series (≈N_3-cbl) suggests that N is the donor atom and this is consistent with the positions of the αβ and γ bands[3,17] but small amounts of the O and C bonded isomers could possibly be present in solution.

Origin of band A

Band A is clearly not a π–π* transition because the shifts upon changing the axial ligand and solvent are different to those of the αβ and γ bands. Band A cannot be a spin-allowed d–d transition because the unsaturated corrin ligand, together with the unsaturated N of the dbzm ligand is expected to have a slightly stronger ligand field than five NH_3 ligands and a somewhat weaker field than five CN^- ligands. Thus, the lowest-energy spin-allowed d–d transition of I-cbl (low-field ligand) should be approximately between those of $[Co(NH_3)_5I]^{2+}$ (λ_{max}/nm 584 (ε/dm^{3}mol^{-1}cm^{-1} 79))[38] and $[Co(CN)_5I]^{3-}$ (435 (200)),[30] and those of CN-cbl (high-field ligand) between $[Co(NH_3)_5CN]^{2-}$ (441 (56))[34] and $[Co(CN)_6]^{3-}$ (312 (243)).[36] The spin-allowed d–d transition, with an expected low ε value, is probably hidden under the much more intense π–π* transitions. Also, band A is extremely unlikely to arise from a spin-forbidden d–d transition because it is far too intense and too broad and because only one band is seen in all cases. Ligand field theory predicts a pair of spin-forbidden d–d transitions ($^1A_{1g} \rightarrow {}^3T_{1g}$ and $^1A_{1g} \rightarrow {}^3T_{2g}$) for Co(III) complexes, and this is seen in the series of complexes $[Co(NH_3)_5X]^{2+}$, where X^- = Cl^- (λ_{max}/nm 877 nm, 657 (ε/dm^{3}mol^{-1}cm^{-1} 0.4, 0.8)), Br^- (922, 676 (4.7, 3.2)) and I^- (936, 725 (30.2, 18.6)).[38] Thus, the spin-forbidden d–d absorption bands are probably hidden underneath band A in the same way that the spin-allowed d–d absorption bands are hidden under the π–π* absorption bands. The most likely origin for band A is a LMCT transition and this would fit in with the large shifts in wavelength upon changing the solvent (see above).

Two possibilities should be considered for LMCT transitions in cobalamins: LMCT from a lone pair on the axial ligand to the cobalt and LMCT from a π orbital on the corrin ligand to the cobalt. The dbzm ligand is expected to give LMCT only in the far UV.[30] Jorgensen has observed that for LMCT transitions ($X^- \rightarrow M$) of octahedral hexahalides, MX_6 (X = F, Cl, Br, I), the difference in wavenumber ($\Delta\tilde{\nu}$) between each pair of halogens is proportional to the corresponding difference in electronegativity ($\Delta\chi$).[39] For band A in the series of cobalamins, the values of $\Delta\tilde{\nu}$ are, in methanol, F to Cl (3122 cm^{-1}), Cl to Br (719 cm^{-1}), Br to I (863 cm^{-1}) and, in water, Cl to Br (752 cm^{-1}), Br to I (423 cm^{-1}). These values show no relationship to Jorgensen's values, F to Cl (28 000 cm^{-1}), Cl to Br (6000 cm^{-1}), Br to I (10 000 cm^{-1}),[39] and it is thus very unlikely that band A corresponds to a $X^- \rightarrow$ Co(III) LMCT transition. LMCT transitions between 300–380 nm are expected for cobalamins with polarizable axial ligands such as I^- and $S_2O_3^{2-}$,[3] and bands of this type are almost certainly present in the γ region. For example, I-cbl is expected to have a LMCT band in the γ region, since $[Co(NH_3)_5I]^{2+}$ has an $I^- \rightarrow$ Co(III) LMCT band at 383 nm (ε 2960)[38] and $[Co(CN)_5I]^{3-}$ has an $I^- \rightarrow$ Co(III) LMCT band at 330 nm (ε 2690)[36] and the corresponding band in I-cbl should be between 383 and 330 nm. Gaussian deconvolution of the spectrum of I-cbl (300–700 nm, this work) shows four Gaussians in the γ region, whereas all other cobalamins so far can be deconvoluted using three Gaussians in the γ region.[9] Thus, since cobalamins contain low-spin Co(III) in a tetragonally-distorted octahedral environment, band A is assigned as a corrin π → Co $3d_{z^2}$ transition.

Comparison with DFT theory

Brunold and co-workers[7] found a weak positive feature, at a lower energy than the αβ band, in the CD spectrum of H_2O-cbl$^+$ at 17 000 cm^{-1}/588 nm and assigned this to a corrin π → Co $3d_{z^2}$ transition, using DFT. This feature was noted to have "no obvious counterpart in the absorbance spectrum" and to be "predominantly magnetic dipole in character." I propose that this feature corresponds to band A in H_2O-cbl$^+$, which is found by the Gaussian deconvolution of the visible spectrum in water at a wavelength of 656 nm and in acetonitrile and methanol at 649 nm and 642 nm and, further, that band A moves to longer wavelengths and increases in intensity for Cl-cbl, Br-cbl and I-cbl as well as *S*CN-cbl, $(NH_2)_2$C*S*-cbl and *Se*CN-cbl.

In the DFT study of H_2O-cbl$^+$ and CN-cbl,[7] the *x* and *y* axes were rotated by 45° from the usual orientation along the metal–ligand bonds so that they pass through the four equatorial nitrogen atoms of the corrin ring. This rotation does not affect the d_z and d_{z^2} orbitals but changes the labels of the d_{xz}, d_{yz} and $d_{x^2-y^2}$ orbitals, giving the order $d_{x^2-y^2} < d_{xz}$, $d_{yz} < d_{z^2} < d_{xy}$ (instead of the more usual d_{xz}, $d_{yz} < d_{xy} < d_{z^2} < d_{x^2-y^2}$) for a tetragonal field with the ligands on the *z* axis further out than those on the *x* and *y* axes. This corresponds to the situation in cobalamins, where the axial cobalt–ligand and cobalt–nitrogen bonds are longer than the equatorial cobalt–nitrogen bonds. I have used this convention for Fig. 5 and the following discussion.

For H_2O-cbl$^+$, the (mainly) corrin π → (mainly) Co $3d_{z^2}$ transition in the molecular orbital diagram based on DFT (Fig. 10 and S8 in Ref. 7) is either the HOMO → LUMO transition, or very close to it.[7] However, for CN-cbl, the Co $3d_{z^2}$ orbital is considerably raised in energy (because CN^- is a much better σ-donor than H_2O) and the corrin π orbital is only slightly raised in energy (because the contribution from the formally unoccupied

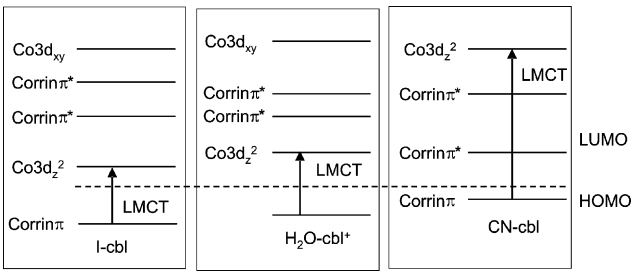

Fig. 5 Schematic molecular orbital diagram for I-cbl, H_2O-cbl$^+$ and CN-cbl (not to scale, based on Fig. 10 and S8).[7] LUMO = lowest unoccupied molecular orbital and HOMO = highest occupied molecular orbital. Note, that the x and y axes are rotated by 45° from the usual orientation along the metal–ligand bonds.[7] This rotation gives $d_{x^2-y^2} < d_{xz}$, $d_{yz} < d_{z^2} < d_{xy}$.

Co $3d_{z^2}$ orbital increases only slightly (from 0.2% for H_2O to 1.5% for CN^-) and the corrin π–π* transition becomes the HOMO → LUMO transition.[7] If these trends continue for ligands with smaller σ-donor abilities than H_2O, such as I-cbl, then the Co $3d_{z^2}$ orbital (LUMO) will rapidly decrease in energy but the corrin π orbital energy will decrease only slowly, thus decreasing the HOMO–LUMO gap. This is represented schematically for I-cbl, H_2O-cbl$^+$ and CN-cbl in Fig. 5. Thus, as the σ-donor power of the upper axial ligand decreases, Co(III) becomes more electropositive, the energy of the LMCT corrin π → Co $3d_{z^2}$ transition decreases and band A is found in the NIR (*e.g.* I-cbl). Conversely, as the σ-donor ability of the upper axial ligand increases, the energy of the LMCT transition increases rapidly (while the corrin π–π* transition energies increase more slowly) and band A is found in the visible region (*e.g.* F-cbl). Presumably, for the best σ-donors (*e.g.* CN-cbl), the LMCT transitions would be found in the UV region.

Experimental

Hydroxocobalamin hydrochloride (vitamin B_{12a}) was obtained from Roussel, CN-cbl from Fluka and CH_3-cbl from Sigma. Solvents used were as follows: methanol and DMSO (Saarchem Unilab), acetic acid (Labchem), acetonitrile (BDH HiPerSolv). Salts and compounds used as ligands for cobalamins were as follows: KOCN and thiourea (BDH Laboratory Reagent), $Na_2S_2O_3{\cdot}5H_2O$ (Labchem AR), Na_2SO_3, $NaNO_2$ and urea (Merck AR), KF, KSeCN and $Na_2Cr_2O_7{\cdot}2H_2O$ (Riedel-de Haën), LiCl, KI, and $K_2Cr_2O_7$ (Saarchem AR), NaBr and KSCN, (Saarchem CP), NaN_3 (Sigma) and diethyl phosphite (Aldrich 98%). Deionized water was purified to 18 MΩ using a MilliQ system. All experiments were done at least in duplicate.

UV-visible-NIR spectra

UV-visible-NIR spectra were determined on a Perkin-Elmer λ 25 spectrophotometer (1 nm slit, scan speed 240 nm min^{-1}, resolution 0.1 nm), using a matched pair of 1 cm quartz cells. The spectra were determined from 400–1100 nm, at a concentration (~2×10^{-4} mol dm^{-3}) high enough to easily see the NIR band if present, and from 300–700 nm at a lower concentration (~3.8×10^{-5} mol dm^{-3}), in order to include the intense γ band. For aqueous solutions, small positive and negative peaks, due to changes in absorption in the NIR when solutes were added to water,[40,41] were seen at 975 nm and 1050–1100 nm.

The concentrations of the cobalamins in water, methanol, methanol–water and acetonitrile–water were determined by the addition of excess KCN to the solution in order to give dicyanocobalamin ($\varepsilon_{367} = 3.04 \times 10^4$ L mol^{-1} cm^{-1}).[42] The dicyanocobalamin ε_{367} was assumed to be the same in methanol, methanol–water and acetonitrile–water as in water. The exact concentrations of cobalamins in DMSO and acetic acid could not be determined because KCN is insoluble in these solvents.

Gaussian deconvolution

The position of band A in the spectrum of each cobalamin (concentration 2.0×10^{-4} mol dm^{-3}) was determined by Gaussian deconvolution of the 400–900 nm (25 000–11 111 cm^{-1}) region of the spectrum[9,14] (Table 2 and Table S1, ESI†). The spectrum of each cobalamin was converted to a list of wavenumbers and absorbances (5000 points) and fitted to the sum of the minimum number of Gaussian components using the equation:

$$A_T = \sum_{i=1}^{n} \alpha_i \exp\left[\frac{-(\bar{\nu} - \bar{\nu}_i)}{2\Delta_i^2}\right] \quad (1)$$

where A_T is the total absorbance, $\bar{\nu}$ the measured wavenumber, $\bar{\nu}_i$ the wavenumber at which the ith Gaussian component reaches a maximum absorbance, α_i the absorbance of the ith component and Δ_i the half-width of the ith component at 0.607 of the maximum absorbance.[9,14] Band A was modelled by one Gaussian, the αβ band by three Gaussians and the D/E region of the spectrum by one or two Gaussians, giving a total of five or six Gaussians.

Preparation of cobalamins

H_2O-cbl$^+$, CH_3OH-cbl$^+$, CH_3COOH-cbl and $(CH_3)_2SO$-cbl$^+$ (Table 1) were prepared by dissolving vitamin B_{12a} in water, methanol, acetic acid and DMSO, respectively. H_2O-cbl$^+$ was also prepared by dissolving vitamin B_{12a} in 80 : 20 v/v methanol–water and 80 : 20 v/v acetonitrile–water. Vitamin B_{12a} was insoluble in pure acetonitrile.[25]

F-cbl, Cl-cbl, Br-cbl, I-cbl, SCN-cbl, $(NH_2)_2CS$-cbl$^+$, SO_3-cbl$^-$, S_2O_3-cbl$^-$, SeCN-cbl, OCN-cbl, N_3-cbl and NO_2-cbl (Table 1) were prepared by substitution of H_2O in H_2O-cbl$^+$ and/or CH_3OH in CH_3OH-cbl$^+$. A solid salt containing the relevant ligand, or the neutral ligand (for thiourea), was added to a solution of B_{12a} in water or methanol. The volume change (if any) was taken into account when calculating the final concentration of the cobalamin and salt. For aqueous solutions, sufficient salt or compound to ensure complete formation of the cobalamin, based on its formation constant,[8,17] was used. The pH values of the solutions were in the neutral region, whereas the pK_a values for protonation and removal of the dbzm base were ≤ 2, except for CH_3-cbl (pK_a 2.7).[5,44] Since high concentrations of the ligand were sometimes needed for complete formation in aqueous solution, the salts used were the most soluble available. For cobalamins prepared in methanol, the formation constants were generally much lower than in water but were not quantitatively determined. It was thus possible to obtain complete formation of F-cbl in methanol, but not in water. However, some salts such as NaN_3, $NaNO_2$, $Na_2S_2O_3$

$5H_2O$ and Na_2SO_3 were insufficiently soluble in methanol for complete formation to take place.

$(NH_2)_2CO$-cbl$^+$, $SC(NH_2)_2$-cbl$^+$, SeCN-cbl and I-cbl were prepared by reacting vitamin B_{12a} with urea, thiourea, KSeCN and KI, respectively, in DMSO but complete formation in this solvent did not occur. Urea apparently did not react with vitamin B_{12a} in water or methanol, with only dilution of H_2O-cbl$^+$ or CH_3OH-cbl$^+$ being observed.

OH-cbl was prepared by adding sodium hydroxide to H_2O-cbl$^+$ in water to give pH 10–11 (pK_a for H_2O-cbl$^+$/OH-cbl = 8.1[43]). CN-cbl, CH_3-cbl and $P(OC_2H_5)_2O$-cbl[21] were prepared by dissolving the respective crystalline cobalamins in water in dim light. $P(OC_2H_5)_3$-cbl$^+$ was prepared by the addition of triethyl phosphite to H_2O-cbl$^+$ in water.[21]

The visible spectrum and formation constant of CrO_4-cbl$^-$ were determined as follows: small amounts of solid vitamin B_{12a} were added to 2.5 mL of four separate solutions of $Cr_2O_7^{2-}$ in water, 0.0057 mol dm^{-3}, 0.027 mol dm^{-3}, 0.16 mol dm^{-3} ($K_2Cr_2O_7$) and 2.5 mol dm^{-3} ($Na_2Cr_2O_7$) in a spectrophotometric cell (with the same solution in the reference cell) and the spectra run from 600–1100 nm.

Conclusion

Visible-NIR spectroscopy, together with Gaussian deconvolution of absorption bands, was found to be a useful technique for studying LMCT transitions in cobalamins. The wavenumber of the corrin $\pi \rightarrow$ Co $3d_{z^2}$ LMCT transition (band A) was determined for a series of cobalamins: *P*$(OC_2H_5)_3$-cbl$^+$, *P*$(OC_2H_5)_2O$-cbl, *C*N-cbl, CH_3-cbl > OH-cbl > *S*O_3-cbl$^-$ > *N*O_2-cbl > OCN-cbl > N_3-cbl > *S*$_2O_3$-cbl$^-$ > CH_3OH-cbl$^+$ > *S*CN/*N*CS-cbl ≈ H_2O-cbl$^+$ ≈ $(CH_3)_2SO$-cbl$^+$ > CH_3COOH-cbl$^+$ > *S*$C(NH_2)_2$-cbl$^+$ > Cl-cbl ≈ *Se*CN-cbl > Br-cbl > I-cbl > *O*CrO_3-cbl$^-$ and F-cbl > H_2O-cbl$^+$. This differs from the order of the π–π transitions (αβ and γ bands) and correlates with the spectrochemical series, rather than the nephelauxetic series. As the σ-donor power of the axial ligand of cobalamins decreases, band A shifts to lower energies (into the NIR for I^- and *O*CrO_3^{2-}) and becomes the HOMO → LUMO transition for ligands with smaller σ-donor power than H_2O.

Acknowledgements

I thank the Faculty Research Committee (Health Sciences, University of the Witwatersrand) for financial support, my colleagues in the School of Therapeutic Sciences and the Department of Pharmacy and Pharmacology for support during my sabbatical leave, Dr C. B. Perry for the use of his Gaussian program and Dr P. R. Johnson for reading the manuscript.

References

1 K. Folkers, in *Vitamin B_{12}*, ed. B. Zagalak and W. Friedrich, W. de Gruyter, Berlin, 1979, pp. 7–18.
2 B. Ellis, V. Petrow and G. F. Snook, *J. Pharm. Pharm. Sci.*, 1949, **1**, 60–61.
3 J. M. Pratt, *Inorganic Chemistry of Vitamin B_{12}*, Academic Press, London, 1972.
4 C. Gianotti, in *B_{12}*, ed. D. Dolphin, Wiley-Interscience, New York, 1982, pp. 393–430.
5 J. M. Pratt, in *Chemistry and Biochemistry of B_{12}*, ed. R. Banerjee, Wiley, New York, 1999, pp. 73–112.
6 J. M. Pratt, in *Chemistry and Biochemistry of B_{12}*, ed. R. Banerjee, Wiley, New York, 1999, pp. 113–164.
7 T. A. Stich, A. J. Brooks, N. R. Buan and T. C. Brunold, *J. Am. Chem. Soc.*, 2003, **125**, 5897–5914.
8 J. M. Pratt and R. G. Thorp, *J. Chem. Soc. A*, 1966, 187–191.
9 C. B. Perry and H. M. Marques, *S. Afr. J. Chem.*, 2005, **58**, 9–15.
10 R. A. Firth, H. A. O. Hill, B. E. Mann, J. M. Pratt, R. G. Thorp and R. J. P. Williams, *J. Chem. Soc. A*, 1968, 2419–2428.
11 S. M. Chemaly and J. M. Pratt, *J. Chem. Soc., Chem. Commun.*, 1976, 988–989.
12 L. Randaccio, S. Geremia, M. Stener, D. Toffoli and E. Zangrando, *Eur. J. Inorg. Chem.*, 2002, 93–103.
13 L. Randaccio, S. Geremia, G. Nardin, M. Šlouf and I. Srnova, *Inorg. Chem.*, 1999, **38**, 4087–4092.
14 C. B. Perry, M. A. Fernandes, K. L. Brown, X. Zou, E. J. Valente and H. M. Marques, *Eur. J. Inorg. Chem.*, 2003, 2095–2107.
15 D. Thusius, *Chem. Commun. (London)*, 1968, 1183–1184.
16 G. Garau, S. Geremia, L. G. Marzilli, G. Nardin, L. Randaccio and G. Tauzher, *Acta Crystallogr., Sect. B: Struct. Sci.*, 2003, **59**, 51–59.
17 R. A. Firth, H. A. O. Hill, J. M. Pratt, R. G. Thorp and R. J. P. Williams, *J. Chem. Soc. A*, 1969, 381–386.
18 P. Mayer, W. Ponikwar, K. Feldl, P. Swoboda and W. Beck, *Z. Anorg. Allg. Chem.*, 2000, **626**, 2038–2039.
19 W. Beck, *Eur. J. Inorg. Chem.*, 2003, 4275–4288.
20 D. P. Fairlie, M. Turner, K. A. Byriel, J. A. McKeon and W. G. Jackson, *Inorg. Chim. Acta*, 1999, **290**, 133–138.
21 S. M. Chemaly, *J. Inorg. Biochem.*, 1991, **44**, 1–15.
22 C. Brink, D. C. Hodgkin, J. Lindsey, J. Pickworth, J. H. Robertson and J. G. White, *Nature*, 1954, **174**, 1169–1170.
23 J. A. Hill, J. M. Pratt and R. J. P. Williams, *J. Theor. Biol.*, 1962, **3**, 423–445.
24 R. Moreno-Esparza, M. Lopez and K. H. Pannell, *J. Chem. Soc., Dalton Trans.*, 1992, 1791–1795.
25 S. Balt, A. M. van Herk and W. E. Koolhaas, *Inorg. Chim. Acta*, 1984, **92**, 67–74.
26 *CRC Handbook of Chemistry & Physics*, ed. R. C. Weast, CRC Press, Cleveland, Ohio, 55th edn, 1974/5.
27 J. T. Slusher, *Mol. Phys.*, 2000, **98**, 287–293.
28 H. Yilmaz, S. Güler and Ç. Güler, *Phys. Scr.*, 1999, **59**, 77–80.
29 J. M. Pratt and R. G. Thorp, in *Advances in Inorganic Chemistry and Radiochemistry*, ed. H. J. Emeléus and A. G. Sharpe, Academic Press, New York, 1969, vol. 12, pp. 375-427.
30 A. B. P. Lever, *Inorganic Electronic Spectroscopy*, Elsevier, Amsterdam, 2nd edn, 1984.
31 J. G. Verkade and T. S. Piper, *Inorg. Chem.*, 1963, **2**, 944–947.
32 J. E. Huheey, *Inorganic Chemistry*, Harper and Row, New York, 3rd edn, 1983.
33 W. Beck and K. Feldl, *Z. Anorg. Allg. Chem.*, 1965, **341**, 113–123.
34 R. A. D. Wentworth and T. S. Piper, *Inorg. Chem.*, 1965, **4**, 709–714.
35 P. Kofod, *Inorg. Chem.*, 1995, **34**, 2768–2770.
36 V. M. Mizkowski and H. B. Gray, *Inorg. Chem.*, 1975, **14**, 401–405.
37 J. G. Verkade, *Coord. Chem. Rev.*, 1972/3, **9**, 1–106.
38 M. Linhard and M. Weigel, *Z. Phys. Chem., Neue Folge*, 1957, **11**, 308–317.
39 C. K. Jorgensen, *Orbitals in Atoms and Molecules*, Academic Press, London, 1962.
40 M. Chaplin, http://www.lsbu.ac.uk/water/vibrat.html, accessed 17/1/2008.
41 C. L. Braun and S. N. Smirnov, *J. Chem. Educ.*, 1993, **70**, 612–617.
42 J. A. Hill, J. M. Pratt and R. J. P. Williams, *J. Chem. Soc.*, 1964, 5149–5153.
43 H. M. Marques, K. L. Brown and D. W. Jacobsen, *J. Biol. Chem.*, 1988, **263**, 12378–12383.
44 S. M. Chemaly, *J. Inorg. Biochem.*, 1991, **44**, 17–25.