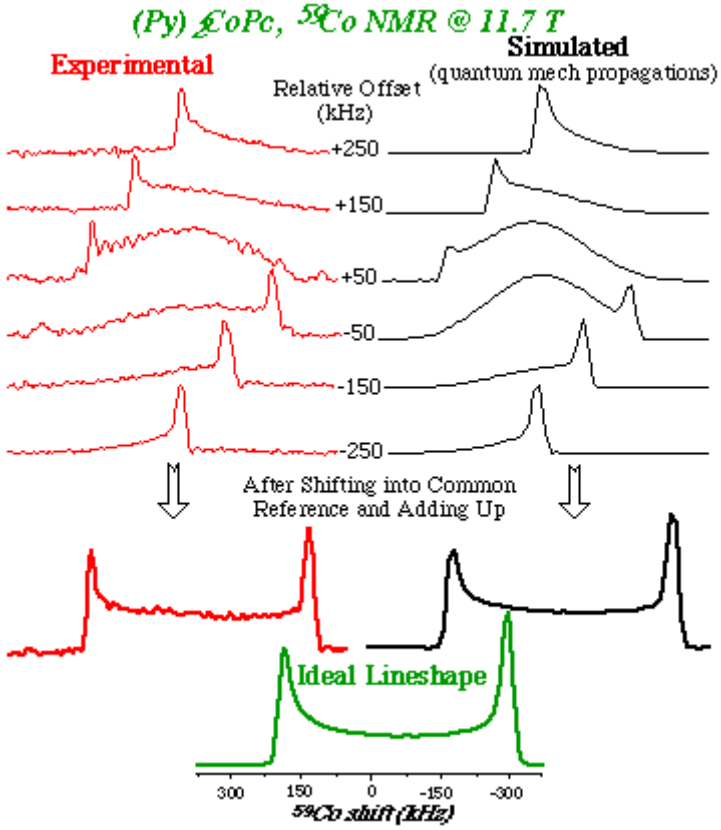
SOLID STATE NMR OF COBALAMINS AND DERIVATIVES

General Objectives

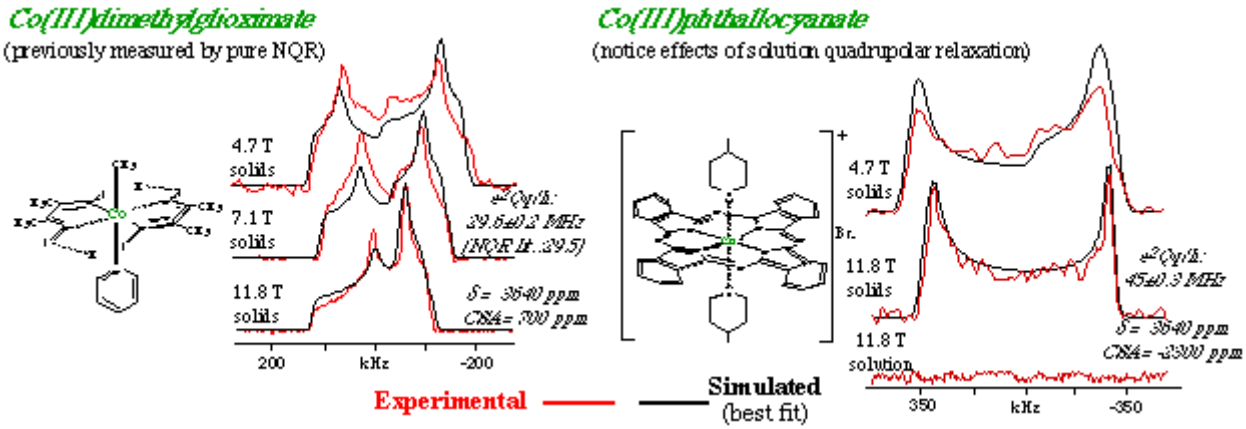
We attempt to develop reliable protocols for the acquisition of powder NMR spectra from half integer quadrupole nuclei even in the presence of very large quadrupolar couplings (e^2qQ/h comparable to the Larmor frequency). So far we have managed to acquired undistorted, ideal powder lineshapes from sites where e^2qQ/h exceeds 100 MHz. As application of these methods, we have began a series of static NMR analyses of diamagnetic metals bound to biomolecules. We summarize here some of our findings about the cobalamin family of compounds.

Spectroscopic Strategy:

The approach that we employ to characterize the central transitions of static quadrupolar samples involves the use of tuned spin echo sequences which employ very low radiofrequency fields (to avoid nutation distortions), and the acquisition of a series of data sets acquired as a function of the irradiation offset. Although each of these data sets presents highly distorted lineshapes, it can be shown that after these are added up suitably shifted with respect to a common reference frequency the resulting powder lineshape is indistinguishable from the ideal powder pattern that could have been expected from theoretical calculations:

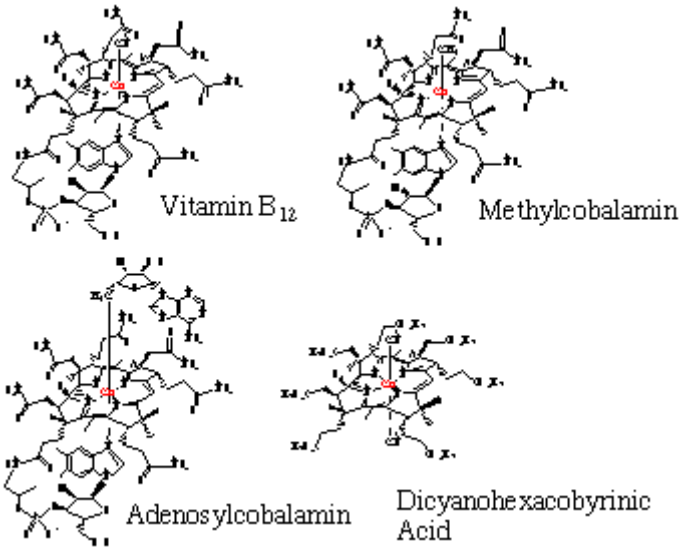


Because up to eight parameters (3 chemical shift coupling constants, 2 quadrupolar, 3 Euler angles) can affect these central transition lineshapes, ambiguities usually arise in their indidual determination. These can be lifted by carrying out variable-magnetic-field acquisitions, which scale the shielding and quadrupolar contributions in opposite ways. **Some pertinent ^{59}Co NMR examples**:

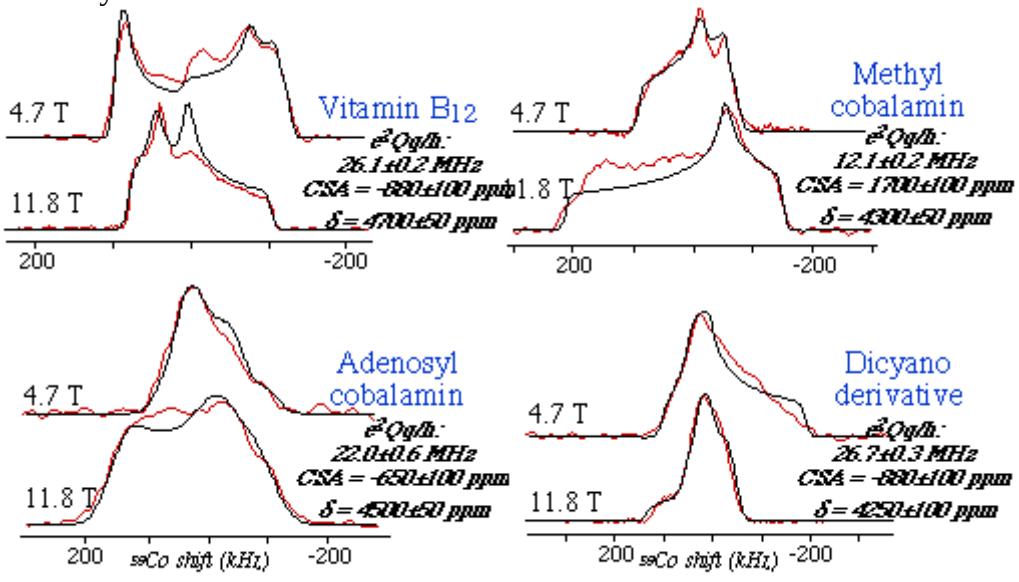


Results: Cobalamins and Cobaloximes

We all carry small amounts of cobalt distributed throughout our body's cells, as this element is essential in the catalysis of numerous enzymatic reactions. This is so because of cobalt's unique role in establishing covalent bonds with carbon, such as the ones occuring in the cobalamins:

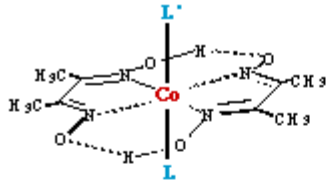


We have employed the approach described above to monitor the diamagnetic environment of these complexes as viewed by cobalt NMR. Some of the observed results:



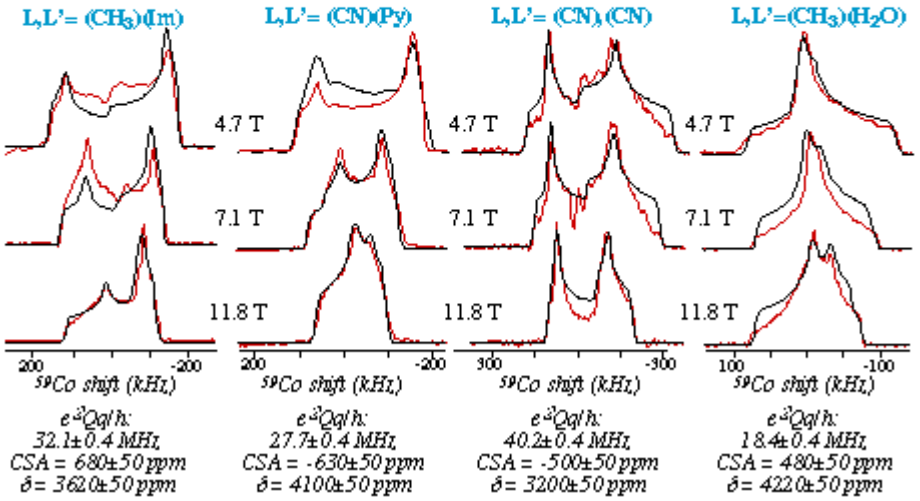
The parameters of vit B_{12} agree well those recently reported by Power et al (*JACS 1998*).

We have began to analyze these tensor coupling parameters by comparing them with the behavior displayed by structural analogues of B_{12}. One such family: the Cobaloximes.



L'LCo(DH)$_2$: Analogues of cobalamins developed by Schrauzer (1968)

- they can exhibit a pattern of axial ligation comparable to the corrinoids (e.g. Co-C covalent bonds)
- they are crystallographically very well characterized

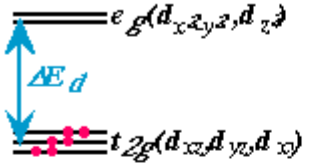


Further Analysis of the Cobalamin, Cobaloxime and Cobaltoporphyrin Coupling Parameters

^{59}Co shielding parameters have traditionally been rationalized in terms of the Freeman-Murray-Richards model of paramagnetic contributions. According to the Jameson & Gutowsky rendering of this model

$$\sigma^p_{\alpha\beta} = \frac{\mu_0 e^2}{4\pi 2m^2} \left[\frac{\langle r^{-3} \rangle_d (D)_{\alpha\beta}}{\langle \Delta E \rangle_d} + \text{p-orbital cont.} \right]$$

For spin-paired d^6 systems where $\langle r^{-3} \rangle_d$ and $(D)_{\alpha\beta}$ are more or less constant, shielding ends up determined by the crystal splitting $\langle \Delta E_d \rangle$



We can thus understand the cobalamins' shielding trend $\delta_{His_{base}} < \delta_{MeCN} < \delta_{AdoCN} < \delta_{CNCN}$ and the cobaloximes' shielding trend $\delta_{His_{base}} < \delta_{MeIm} \approx \delta_{MePy} < \delta_{PyCN} < \delta_{MeH_2O} < \delta_{imino}$ in terms of the spectrochemical ligand field series

$$\text{-CH}_3 > \text{-CH}_2\text{-ribose} > \text{-CN} > \text{=N-} > \text{-O-}$$

Furthermore,

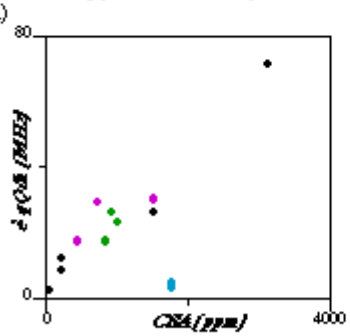
- for every pair of analogues (dicyano, cyano-base, alkyl-base) we see $\delta_{Cblx} < \delta_{Cblm}$ => **glyoximates are stronger in-plane ligands than the natural corrinoids**
- Euler angles between shielding and quadrupole tensors more regular in cobaloximes than in cobalamines (β=90°)=> **reflecting a higher symmetry?**

At a similar level of complexity: **Townes-Dailey EFG model**

$$(e^2qQ/h)_{\alpha\beta} = \frac{1}{7\pi\varepsilon_0} \frac{e^2Q}{h} \left[(1-R) \langle r^{-3} \rangle_d (D)_{\alpha\beta} + \text{p-orbital cont.} \right]$$

=> correlations can be expected between the local anisotropy measured by the shielding and quadrupolar tensor elements (Spiess et al, Au-Yeung et al)

This trend is fulfilled by **literature data** as well as by **cobalamines** and **cobaloximes**, but not by the **cobaltoporphirins**

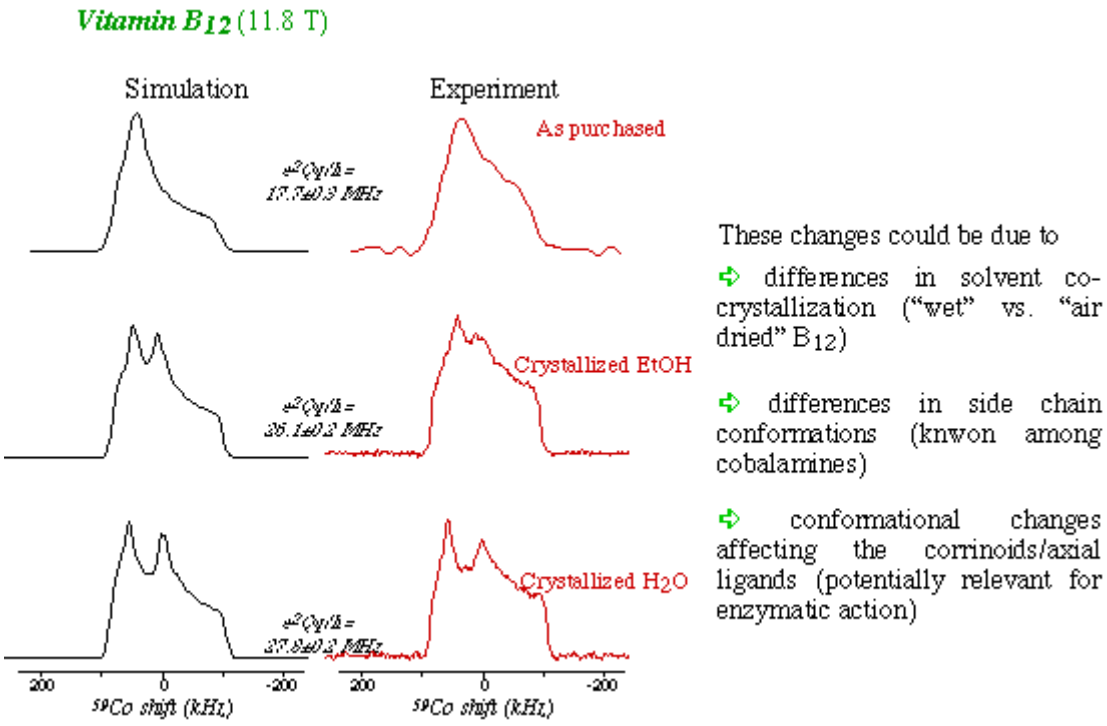


A possible explanation: cobaltoporphyrins might possess much smaller $\langle \Delta E_d \rangle$'s than the other complexes. ZINDO calculations, however, suggest similar crystal field splittings for all the derivatives (12,000-16,000 cm-1)

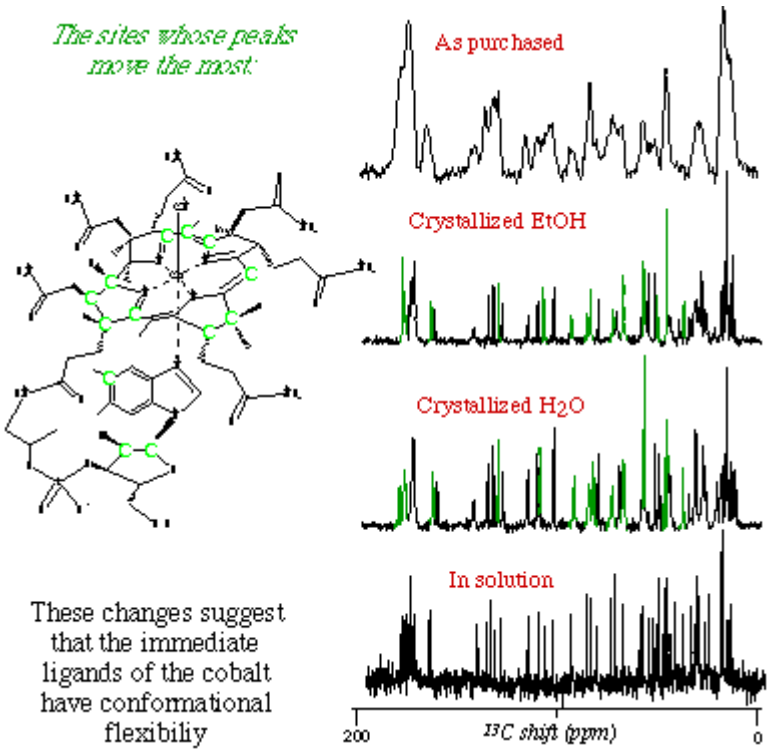
Another explanation: ZINDO also shows an extensive delocalization of the *d*-orbitals over the aromatic system; **the smaller $\langle \Delta E \rangle$ gap of this system might then be governing the ^{59}Co CSA observed in cobaltoporphyrins**

Dependence of the Cobalamin Spectra on Crystallization Conditions

We have observed that the ^{59}Co NMR spectra of the cobalamins are quite sensitive to the crystallization conditions, as well as to temperature.



To further elucidate the nature of these changes we have also carried out a series of ^{13}C, ^{15}N and ^{31}P NMR analyses. The carbon NMR are the most revealing, as they show the sites that are most affected by the crystal phase changes.



We are currently expanding these investigations with solution phase analyses and studies of cofactors bound to protein systems.