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# Reductive Dechlorination of Carbon Tetrachloride by Cobalamin(II) in the Presence of Dithiothreitol: Mechanistic Study, Effect of Redox Potential and pH

**Nada Assaf-Anid,† Kim F. Hayes,† and Timothy M. Vogel*,†,‡**

Department of Civil and Environmental Engineering, Environmental and Water Resources Engineering, The University of Michigan, Ann Arbor, Michigan 48109-2125, and Department of Safety and Environment, Rhône-Poulenc Industrialisation, 24 Avenue Jean-Jaurès, 69153 Décines-Charpieu, France

A mechanistic study of the reductive dechlorination of carbon tetrachloride by vitamin $B_{12}$ (cyanocobalamin) in the presence of dithiothreitol was conducted as a function of redox potential and pH. The solution redox potential decreased both with an increase in the total concentration of dithiothreitol present and with an increase in pH. The pseudo-first-order rate constant of carbon tetrachloride disappearance increased with decreasing redox potential. The predominant cobalt species present under the reaction conditions was cobalamin(II) (vitamin $B_{12r}$), as confirmed by spectrophotometric analysis, suggesting a one-electron reduction of vitamin $B_{12}$ and the involvement of two vitamin $B_{12}$ molecules per reacting carbon tetrachloride molecule. This study illustrates the role of Co(II) in reductive dechlorination by vitamin $B_{12}$.

## *Introduction*

A major degradation process for highly chlorinated organic compounds in anoxic environments is their reductive dechlorination to less chlorinated products (*1, 2*). Reductive dechlorination involves a net two-electron transfer from a donor compound to the chlorinated organic with the removal of the chlorine as chloride and its replacement with hydrogen (*1, 3*). A substantial amount of research has been conducted investigating the reductive dechlorination process under environmentally relevant yet slow reaction conditions (*4–7*). Experiments with heat-treated microorganisms capable of reductive dechlorination (*8, 9*) support the hypothesis that metal-containing bacterial coenzymes, such as vitamin $B_{12}$ and $NiF_{430}$, act as facilitators of the reductive dechlorination reaction *in vivo* (*1, 2*). Studies using cell-free systems of iron, cobalt, and nickel porphyrins and corrins, free or associated with proteins, have also shown the potential importance of metal ions and coenzymes in the reductive dechlorination process (*10–15*). These abiotic but biomimetic systems have provided information on possible dechlorination mechanisms of chlorinated aliphatics and aromatics with greatly enhanced reaction rates over *in vivo* systems.

In this study, the model chlorinated compound carbon tetrachloride (CT) has been chosen. It is a hazardous compound classified as a priority toxic water pollutant by the U.S. Environmental Protection Agency (*16*). Its release to the environment through spills and evaporation during handling (*17*), coupled with its persistence and reported health risks (*18*), make it critical to develop new technologies which can efficiently convert it and other similar compounds to less harmful products. Among the possible degradation pathways of CT in water, sediment, and soil, reductive dechlorination has the potential to completely reduce it to methane (*19*). However, under natural conditions, the reduction steps are generally slow (*20*), and hence, speeding up the process is desirable.

A recent study of the reductive dechlorination of CT by cobalt corrins, using the electron donors titanium(III) citrate or dithiothreitol (DTT) in excess, led to the proposal of an initial two-electron reduction of Co(III) to Co(I) by either titanium(III) citrate or DTT followed by a nucleophilic attack of CT by Co(I) to yield a cobalt trichloromethyl intermediate (*19*), similar to a mechanism proposed earlier for the reaction of Co(I) supernucleophiles with alkyl halides (*21*). In these studies, the +1 oxidation state of cobalt has been proposed to be the active species in reductive dechlorination. However, in the presence of DTT ($E_o'$ = −332 mV vs SHE) (*22*), the cobalt in vitamin $B_{12}$ ($B_{12}$) is probably in the +2 rather than the +1 oxidation state (*23*), suggesting that a different mechanism involving Co(II) might be in effect. The determination of the $B_{12}$ oxidation state and its influence on the reductive dechlorination of carbon tetrachloride in the presence of DTT was the purpose of this investigation. In particular, this study was conducted to determine the effects of changes in reducing agent concentration and pH on the redox potential of the system and, hence, on the reductive dechlorination rates and product distribution.

## *Materials and Methods*

**Chemicals.** Reduced dithiothreitol $S_2C_4H_{10}O_2$ ($HSCH_2$-CHOH-CHOH-$CH_2SH$) or (DTT), oxidized dithiothreitol (cyclic $S_2C_4H_8O_2$), and vitamin $B_{12}$ (cyanocobalamin; purity ca. 99%) were obtained from Sigma Chemical (St Louis, MO). Carbon tetrachloride (CT) (Fisher Scientific, Pittsburgh, PA), chloroform (CF) (Mallinckrodt, St Louis, MO), and dichloromethane (DCM) (Aldrich, Milwaukee, WI) were stored at 6 °C in methanol (J. T. Baker, Phillipsburgh, NJ). Chloromethane (CM) and methane were obtained from Aldrich. Tris buffer (Sigma Chemical Co.) for pH 8.5 and 7.3 and 1:1 sodium acetate/acetic acid (Mallinckrodt) buffer for pH 5.0 were used for these studies. The pH was measured with a Fisher Scientific pH meter (Fisher Scientific, Pittsburgh, PA) and an Orion Ross electrode (Fisher Scientific, Pittsburgh, PA). The absolute error on the readings was ±0.05 pH unit.

**Redox Potential Determination.** The redox potential of the $B_{12}$–DTT solutions used in the dechlorination experiments was measured with a Corning ion analyzer 250 and a Corning redox combination electrode (Corning, NY). The redox potential was reported with an absolute error of ±5 mV relative to the standard hydrogen electrode (SHE) by using the conversion of +202 mV, which is the

* Address correspondence to this author at The University of Michigan.
† The University of Michigan.
‡ Rhône-Poulenc Industrialisation.



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standard redox potential of the Ag/AgCl reference electrode relative to the SHE (*24*). All the measurements were conducted in an anaerobic chamber equipped with an oxygen sensor (Coy Lab Products, Ann Arbor, MI). Redox Fe(II)/Fe(III) standards were prepared according to ASTM Method D1498-76. The equilibrium time used was 55 min, which was the time needed for the redox potential readings to stabilize.

**Rate and Product Determination.** The reductive dechlorination of CT was assayed in buffered 1-mL reaction mixtures consisting of DTT, $B_{12}$, and CT (*9*). The DTT concentration was varied from 50 to 600 mM, and the buffer, vitamin $B_{12}$, and CT concentrations were fixed at 0.5 M, 0.05 mM, and 1.1 mM, respectively. Buffer solutions were rendered anoxic by flushing with nitrogen gas (99%) and then mixed with DTT in the anaerobic chamber, which contained a 90% $N_2$, 10% $H_2$ atmosphere. The redox potential of the buffered DTT–$B_{12}$ solutions was measured with the redox probe described above. The reaction vessels were 10-mL serum vials (Hewlett-Packard, Palo Alto, CA) wrapped with aluminum foil, capped with a Teflon-faced rubber septum (Alltech Assoc. Inc., Deerfield, IL), and sealed with an aluminum crimp seal. Reactions were started by adding CT through the septum with a Hamilton microliter syringe (Reno, NV). Five experiments, one for each DTT concentration (50, 100, 200, 400, and 600 mM), were performed at each pH value (5.0, 7.3, and 8.5), resulting in a total of 15 dechlorination experiments. All the experiments were performed in duplicate sets of samples with the maximum reaction time of 2 h for a given set of reaction conditions. DTT controls, in which $B_{12}$ was absent, were prepared and analyzed at the same time points as the samples. The amount of chloroform produced in these controls after 2 h was negligible compared to that produced in the samples. Losses of CT through the septum in another set of controls, in which both DTT and $B_{12}$ were absent, were not significant over the duration of the experiment. Concentrations of chlorinated reactant and products were determined by a modified headspace gas chromatography (GC) method with column switching using a Hewlett-Packard 19395A headspace autosampler and 5890 gas chromatograph equipped with a flame ionization detector. Initial separation was done on a DB-624 capillary column (30 m × 0.53 mm i.d. 3.0-mm film thickness, J&W Scientific), and further separation of compounds with a retention time smaller than 2.5 min was done on Haysep D 80 × 100 mesh packed-bed column (4 ft. × 1/8 in. stainless steel). Under these conditions, CT eluted at 8.6 min, CF at 7.9 min, DCM at 4.7 min, CM at 12.2 min, and methane at 1.9 min. Chromatographic peaks were recorded and analyzed using a HP 9000 Series 300 computer and HP 5895A GC Chemstation software. Eight reaction vessels with identical initial solution concentrations were prepared and placed in the automatic sampling carousel maintained at 35 °C. Automatic sampling at 15-min intervals of duplicate samples over a 2-hour period was used to monitor the rate of CT disappearance. Peak quantitation was done by reference to external standards. Pseudo-first-order rate constants ($k_{obs}$) were calculated for the disappearance of CT from linear regression analysis of plots of $\ln(C_t/C_o)$, the natural logarithm of CT concentration $C_t$ at time $t$, relative to the initial concentration $C_o$, versus time. Prior to the initiation of the dechlorination experiments by the addition of the chlorinated compound to the DTT–$B_{12}$ mixtures, the rate of reduction of the cobalt(III) of $B_{12}$ by DTT was determined by spectrophotometry at each DTT concentration. The dechlorination reaction was initiated only after the cobalt was completely reduced (>99%).

**Table 1. Variation of Pseudo-First-Order Rate Constant ($k_{obs}$) of CT Disappearance, Redox Potential in Volts ($E_v$), and Dichloromethane (DCM) Production with DTT Concentration and pH for [$B_{12}$] = 0.05 mM**

| [DTT] (mM) | pH | $k_{obs}$ ($h^{-1}$) | $r^2$ [a] | SE[b] ($\pm h^{-1}$) | $E_v$ [c] | [DCM] after 120 min (mM) |
|---|---|---|---|---|---|---|
| 50 | 5.0 | 0.24 | 0.93 | 0.025 | −0.198 | 0.020 |
| 100 | 5.0 | 0.26 | 0.80 | 0.050 | −0.218 | 0.030 |
| 200 | 5.0 | 0.31 | 0.94 | 0.030 | −0.228 | 0.050 |
| 400 | 5.0 | 0.41 | 0.99 | 0.013 | −0.228 | 0.090 |
| 600 | 5.0 | 0.43 | 0.97 | 0.031 | −0.248 | 0.120 |
| 50 | 7.3 | 0.80 | 0.85 | 0.090 | −0.378 | 0.130 |
| 100 | 7.3 | 1.00 | 0.89 | 0.115 | −0.398 | 0.135 |
| 200 | 7.3 | 1.60 | 0.98 | 0.113 | −0.413 | 0.137 |
| 400 | 7.3 | 2.19 | 0.94 | 0.116 | −0.415 | 0.139 |
| 600 | 7.3 | 2.26 | 0.92 | 0.122 | −0.420 | 0.140 |
| 50 | 8.5 | 2.38 | 0.99 | 0.120 | −0.428 | 0.140 |
| 100 | 8.5 | 2.60 | 0.99 | 0.120 | −0.468 | 0.160 |
| 200 | 8.5 | 3.69 | 0.98 | 1.225 | −0.468 | 0.230 |
| 400 | 8.5 | 4.01 | 0.96 | 0.446 | −0.468 | 0.320 |
| 600 | 8.5 | 5.79 | 0.97 | 0.488 | −0.468 | 0.390 |

[a] $r^2$ on the correlation between $\ln(C_t/C_o)$ and time $t$ linear regressions of slope $-k_{obs}$. [b] Standard error on $k_{obs}$. [c] The minimum recorded redox potential was −0.468 V.

**Spectrophotometric Determination.** Spectrophotometric determinations of cyanocobalamin absorbance were made using a Varian Cary 3 (UV–vis) specrophotometer interfaced to an IBM computer P. S./2 Model 50 (Cary E software). Quartz cuvettes (type 21; NSG Precision Cells Inc., Farmingdale, NY) with a 10-mm path length were filled with sample solutions in the anaerobic chamber to avoid oxygen contamination. Cyanocobalamin(III) has a characteristic spectrum with absorbances at 278, 361 (typically intense), and 550 nm (*25*). Cobalamin(II) has a distinctly different spectrum with absorbances at 311, 405, and 473 nm (*26*). Two sets of the experiments were performed (1) to determine the extent of reduction of $B_{12}$ by DTT at the different DTT concentrations specified above and (2) to identify possible reaction intermediates of the reductive dechlorination of carbon tetrachloride. In the first set, the solutions analyzed consisted of buffered DTT–$B_{12}$ mixtures where the $B_{12}$ concentration was fixed at 0.05 mM and the DTT concentrations were varied from 50 to 600 mM. In the second set, 40 mL (0.41 mmol) of CT were introduced to a DTT (600 mM)–$B_{12}$ (0.05 mM) solution buffered at pH 7.3. The reaction progress was followed at given time intervals by analysis of UV–vis absorbance of the sample solutions between 250 and 700 nm, referenced against a blank cuvette containing buffer and DTT, using a multiple scan mode.

## *Results and Discussion*

**Redox Potential.** The measured redox potential in volts ($E_V$) of the 15 different DTT–$B_{12}$ solutions (Table 1) is related (within 10%) to the pH and the ratio of oxidized to reduced DTT by the following equation:

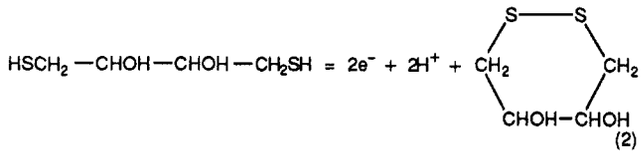
$$E_v = 0.288 + 0.030 \log \frac{(\text{ox DTT})}{(\text{red DTT})_T} - 0.082\ \text{pH} \quad (1)$$

For each DTT–pH combination of Table 1, the following

assumptions were verified by calculation of the concentrations using eq 1

$$(\text{red DTT})_T \simeq (\text{DTT})_T^\circ \text{ and } (\text{ox DTT}) > (B_{12})_T^\circ$$

where $(DTT)_T^\circ$ and $(B_{12})_T^\circ$ are the total initial concentrations of DTT and $B_{12}$, respectively, and (ox DTT) is the concentration of the cyclic product of DTT, formed in the following oxidation half-reaction:



(2)

$(\text{red DTT})_T$ is the total concentration of all the reduced DTT species, which may include the deprotonated forms with $pK_{r_1} = 8.3$, and $pK_{r_2} = 9.5$ (*27*), the first and second dissociation constants of the dithiol according to

$$(\text{red DTT})_T = (HSCH_2\text{-}CHOH\text{-}CHOH\text{-}CH_2SH) + (HSCH_2\text{-}CHOH\text{-}CHOH\text{-}CH_2S^-) + (^-SCH_2\text{-}CHOH\text{-}CHOH\text{-}CH_2S^-)$$

Under the experimental conditions, the total concentration of all the reduced DTT species was equal to the total DTT added, $(DTT)_T^\circ$, and the concentration of oxidized DTT exceeded that of the total $B_{12}$ added.

Equation 1 was developed in a separate study of the pe–pH behavior of DTT where pH and the ratio of oxidized to reduced DTT were varied independently (*28*). It predicts a standard redox potential (at 50% reduction) of −0.286 V for DTT at pH 7.0, which is higher than the reported value of −0.332 V (*22*) determined by equilibrating the oxidized–reduced DTT couple with a $DPN^+$–DPNH system, assuming the standard redox potential of $DPN^+$ was −0.330 V (*29*). The equation predicts a 2.7-fold decrease of the redox potential with unit increase of pH and a 1-fold decrease with each unit increase of log $(DTT)_T^\circ$, in accordance with the ionization equilibria of reduced DTT and the two-electron oxidation half-reaction.

**Rates of Reduction of $B_{12}$ by DTT.** Evidence that the cobalt of $B_{12}$ was reduced by DTT from the +3 to the +2 oxidation state was obtained spectrophotometrically (inset, Figure 5), which translates into a stoichiometry of two reduced $B_{12}$ molecules for each molecule of DTT that is oxidized. The rate of reduction of Co(III) to Co(II) was obtained by following changes in the UV–vis absorbance spectrum of cyanocobalamin over time. No evidence was found for the reduction of Co(III) by DTT to Co(I), which is in agreement with other recent findings (*8*). In this study, the complete reduction to Co(II) at pH 8.5 was almost instantaneous (99% after 1 min) at a very high DTT concentration (600 mM), but was much slower (99% after 30 min) at a low DTT concentration (50 mM) (Figure 1). At lower pH (5.0), the reduction was slower for a given DTT concentration, suggesting a role for the deprotonated DTT species in the reduction of Co(III) to Co(II). Reports on cyanocobalamin(III) reduction to cobalamin(II) by thiols (i.e., cysteine, ethanethiol) also show a dependence of reduction rates on pH, with faster reductions at higher pH values (*30*). The reduction of cyanocobalamin(III) by DTT, when relatively slow, may limit the rate of dechlorination reactions where $B_{12}$ is used as a mediator. Such limitations have not been previously described in studies using DTT as a reducing agent, since the electron transfer between reducing agent and facilitators like $B_{12}$ is usually assumed to be relatively fast compared to dechlorination.

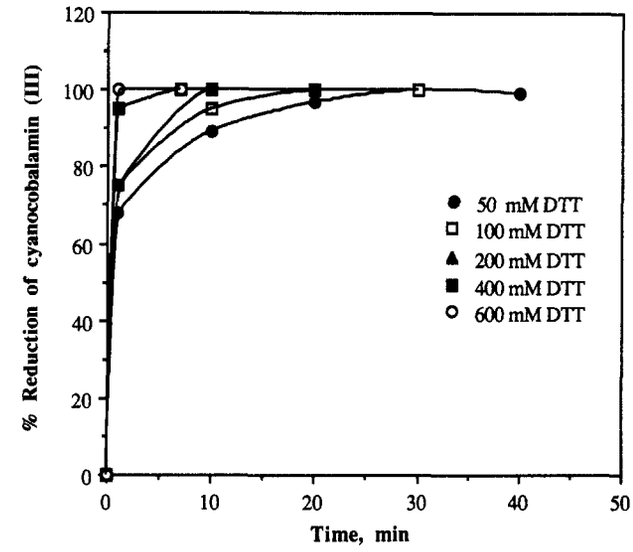


**Figure 1.** Percent reduction of vitamin $B_{12}$ by DTT as a function of time at pH 8.5 and at different DTT concentrations.

**Reductive Dechlorination Kinetics as Function of DTT Concentration and pH.** The reductive dechlorination of CT resulted in the formation of variable amounts of the mono- and di-dechlorination products CF and DCM, depending on DTT concentration and pH (Table 1). The quantity of products formed was not stoichiometric, thereby suggesting that a parallel pathway, such as carbon monoxide formation via carbenes, may have been competing with reductive dechlorination (*31*). For example, at pH 8.5 and after 120 min, only 0.14 mmol of DCM and 0.03 mmol of CF were formed from 1.1 mmol of CT, with 50 mM DTT present. However, only 0.39 mmol of DCM was formed from the almost complete dechlorination of the first dechlorination product CF at 600 mM DTT, pH 8.5, after 120 min (Figure 2). Pseudo-first-order rate constants ($k_{obs}$) increased with increasing DTT concentration (Figure 3) and pH. The slower reductive dechlorination rates at lower DTT concentrations and at a given pH are most likely due to rate limitations caused by the cyanocobalamin(III) reduction by DTT.

Higher $k_{obs}$ values were obtained at low redox potential (Table 1), and the following linear correlation between ln $k_{obs}$ and measured redox potential values $E_V$ was established (Figure 4):

$$\ln k_{obs} = -3.21 - 9.08(E_v) \qquad r^2 = 0.94$$

Although empirical relationships of this type are not generally reported (*32*), there are cases where high degradation rates have been shown to correspond to low system redox potentials. For example, in a study aimed at characterizing the physicochemical effects of organic matter on dechlorination rates, the rapid degradation of the pesticide DDT was related to the low redox potentials of urease-amended soils (*33*). In another example, measured electrode potentials were used as a predictor variable to explain the high degradation rates of methyl parathion in low $E_h$ aqueous systems (*34*). In addition, in studies where the redox potential was not measured, the rates of product formation from the dehalogenation of 1,2-dichloroethane by cobalamin (*8*) and of FREON 11 by aquo-

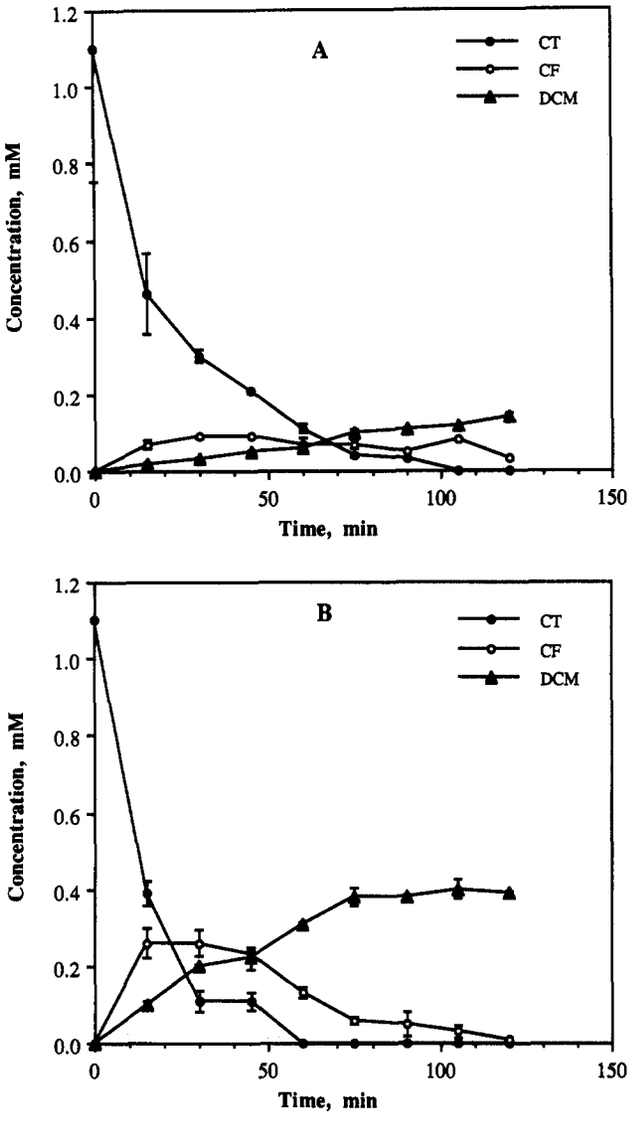



**Figure 2.** Reaction progress of the reductive disappearance of carbon tetrachloride by vitamin $B_{12}$ and the appearance of dechlorination products, at pH 8.5, and at the two DTT concentrations of 50 (A) and 600 mM (B). The concentrations are plotted versus time as the mean of two replicate measurements with standard deviation bars.

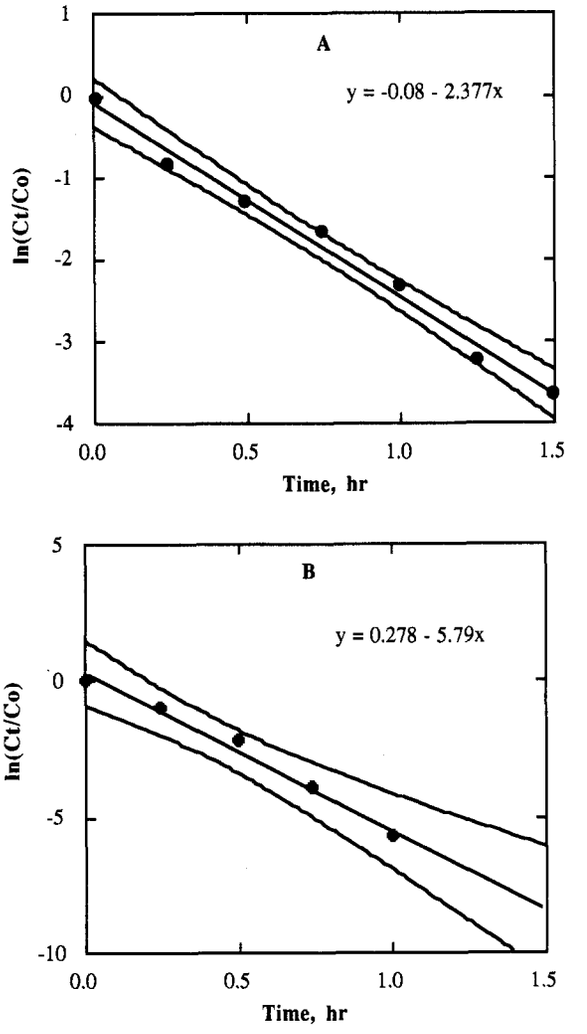



**Figure 3.** Linear regression plots with 90% confidence envelopes showing the correlation between time and the natural logarithm of the CT concentration at any time $C_t$ relative to the initial concentration $C_o$. The absolute value of the slope corresponds to $k_{obs}$ for the reductive disappearance of carbon tetrachloride by vitamin $B_{12}$, at pH 8.5, and at the two DTT concentrations of 50 (A) and 600 mM (B). Each $C_t$ value is the average of two values obtained from two experimental replicates.

cobalamin (*31*) have been shown to increase with the amount of reducing agent. Methane production rates from the reaction of methyl(aquo)cobaloxime with DTT have also been found to be directly proportional to DTT concentrations (*35*).

**Determination of Co-alkyl Intermediates.** Evidence suggesting the direct participation of the cobalt(II) center of the DTT-reduced cyanocobalamin in the reductive dechlorination of CT was obtained from UV–vis spectra recorded before and at 10-min intervals after the addition of CT to the reacting mixture. The addition of CT resulted in a color shift from amber to pink, with a decrease of the typical Co(II) peaks at 311, 405, and 475 nm and a simultaneous increase in absorbance at 280, 360, and 520 nm and distinct isobestic points (Figure 5). The spectra resulting from these increases are similar to those of methyl-, chloromethyl-, dichloromethyl-, and trichloromethylcobalamin(III) with reported absorbances at about 280, 360, and 520 nm (*36, 37*) and indicate the formation of a direct carbon–cobalt $\sigma$ bond between CT and Co(II) (Figure 6A) (*38*). The type of bond detected is the same as that resulting from the nucleophilic substitution reaction of Co(I) corrins with chloromethanes (*19*) and tetrachloroethylene (*12*). However, Co(I) was not detected in our experiments, which rules out its direct participation as shown in reaction B of Figure 6. Moreover, its transient formation through disproportionation of Co(II) (Figure 6C) (*39, 40*) is unlikely in the pH range of interest (*23, 41*). The only Co(II) compounds that have been reported to yield the same type of bond are cobaloximes(II) in their reaction with CT (*38*) and pentacyanocobaltate(II) anion with organic halides (*42, 43*). Both types of Co(II)-containing complexes are considered to be models of the vitamin $B_{12}$–corrin system. The bond was also detected in trace amounts in the reaction of cobalamin(II) with CT in methanol (*44*).

Exposure of the reaction mixture to light destroyed the photolabile carbon–cobalt bond, which has been described in the case of methylcobalamin (*30, 45*). The resulting spectrum was identical to that of the starting DTT-reduced cyanocobalamin. Similar lysis of the carbon–cobalt has been reported when reaction mixtures containing chloroform and (cyanoaquo)cobinamide were subjected to light (*19*). The bond reappeared when the cuvette was protected from light. Such a reversed photolytic reaction has also

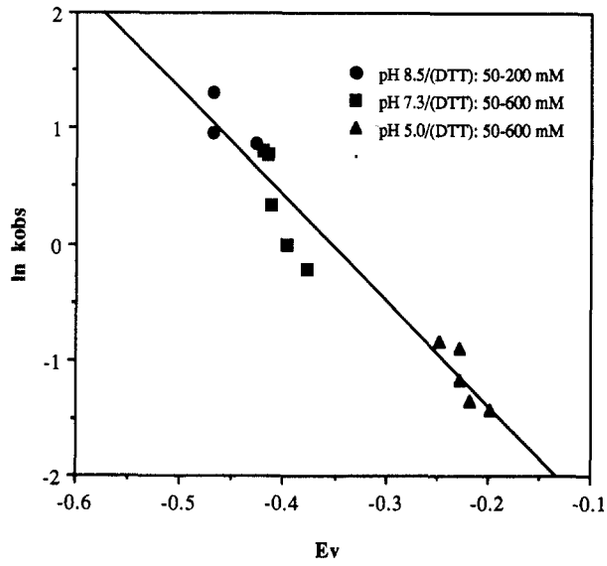



**Figure 4.** Linear correlation between ln $k_{obs}$ of carbon tetrachloride disappearance with measured redox potential.

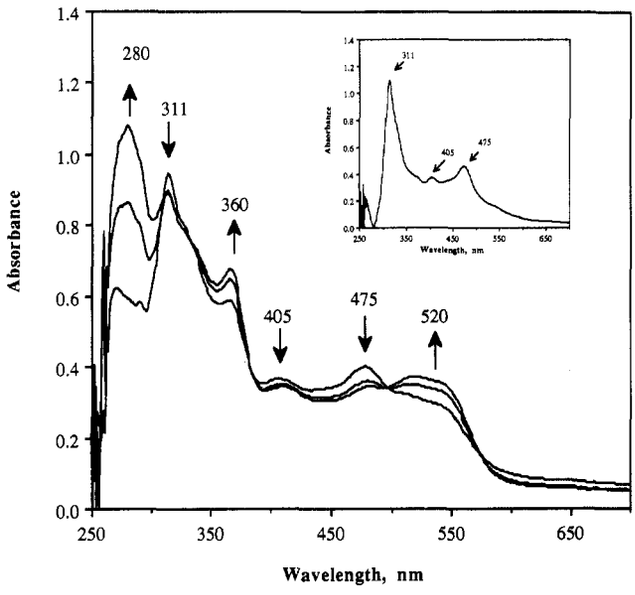



**Figure 5.** Absorption spectra of the reacting DTT–vitamin $B_{12}$–CT mixture recorded at the beginning of the reaction (time 0) and at 10 and 20 min. The direction of the arrows indicates increasing or decreasing peak absorbance relative to time 0. Inset: Absorption spectrum of DTT-reduced cyanocobalamin showing characteristic Co(II) peaks for 311, 405, and 475 nm.

been observed with methylcobalamin (*30*) and provides additional evidence for the formation of the chlorocarbon–cobalt intermediates.

**Reaction Mechanism.** The above results are consistent with a reaction mechanism that involves, as a first step, a one-electron reduction of cyanocobalamin(III) to cobalamin(II) coupled to the oxidation of DTT (Figure 7). The rate of this reduction is greatly dependent on the concentration of DTT present and the pH as described earlier:

$$\text{red DTT} + 2[\text{Co(III)}] = \text{ox DTT} + 2[\text{Co(II)}] \quad (3)$$

In view of its paramagnetic property, the Co(II) species formed is able to induce and terminate free radical reactions (*40*). Free radical formation through a halogen transfer as proposed for the reaction of pentacyanocobaltate(II) with organic halides (*42*, *43*), could have

**A**

One-electron reduction of vitamin $B_{12}$ followed by atom transfer:

$$[\text{Co(III)}] + 1e = [\text{Co(II)}]$$

$$2\,[\text{Co(II)}] + \text{Cl-CCl}_3 = [\text{Co(III)-CCl}_3] + [\text{Co(III)-Cl}]$$

**B**

Two-electron reduction of vitamin $B_{12}$ followed by nucleophilic substitution:

$$[\text{Co(III)}] + 2e = [\text{Co(I)}]$$

$$[\text{Co(I)}] + \text{Cl-CCl}_3 = [\text{Co(III)-CCl}_3] + \text{Cl}^-$$

**C**

One-electron reduction of vitamin $B_{12}$ followed by disproportionation:

$$[\text{Co(III)}] + 1e = [\text{Co(II)}]$$

$$2\,[\text{Co(II)}] = [\text{Co(III)}] + [\text{Co(I)}]$$

$$[\text{Co(I)}] + \text{Cl-CCl}_3 = [\text{Co(III)-CCl}_3] + \text{Cl}^-$$

**Figure 6.** Possible reactions of vitamin $B_{12}$ with carbon tetrachloride leading to the formation of the cobalt–trichloromethyl intermediate $[Co^{III}CCl_3]$.

occurred in our experiments:

$$[\text{Co(II)}] + CCl_4 = [Co^{III}Cl] + {}^{\bullet}CCl_3 \quad (4)$$

Evidence that this step is rate-determining is provided by experiments (not shown) that show a first-order dependence of the reaction rate on cyanocobalamin concentration in systems in which DTT concentrations are high and not rate limiting. Based on such a dependence, it is unlikely that this step involves the supernucleophile Co(I) as the intermediate, because its formation via disproportionation would imply a second-order rate dependence on cyanocobalamin(III) concentration (*30*). Although eq 4 is a net halogen atom transfer from $CCl_4$ to the Co(II) cobalamin, it might involve some degree of electron transfer via a halogen-bridged transition state of the type $[Co^{\delta+} \ldots Cl^{\delta-} \ldots CCl_3]$ (*46*), as proposed for the reaction of Co(II) complexes with alkyl halides (*41*) and benzyl bromides (*40*). The radical is unlikely to extract a hydrogen atom from the reaction medium since carbon–cobalt intermediates are detected. However, based on the property of Co(II) to terminate free radical reactions and on known alkylation reactions of Co(II) complexes (*40*, *41*), it seems plausible that the radical is trapped by another cobalamin(II) to yield the trichloromethyl–cobalamin intermediate $[Co^{III}CCl_3]$ as shown in the following reaction step:

$$[\text{Co(II)}] + {}^{\bullet}CCl_3 = [Co^{III}CCl_3] \quad (5)$$

Reactions 2 and 3 can be summed up to the overall atom-transfer reaction (Figure 6A) during which the carbon–chlorine bond of $CCl_4$ is cleaved homolytically (*38*). The trichloromethyl–cobalamin intermediate formed would then be reduced further to yield an unstable radical anion $[Co^{III}CCl_3]^{\cdot-}$, the fate of which would most probably be heterolytic cleavage (*47*) generating a trichloromethyl carbanion $:CCl_3^-$:

$$[Co^{III}CCl_3] + 0.5\text{red DTT} = [Co^{III}CCl_3]^{\bullet-} + 0.5\text{ox DTT} \quad (6)$$

$$[Co^{III}CCl_3]^{\bullet-} = [\text{Co(II)}] + :CCl_3^- \quad (7)$$

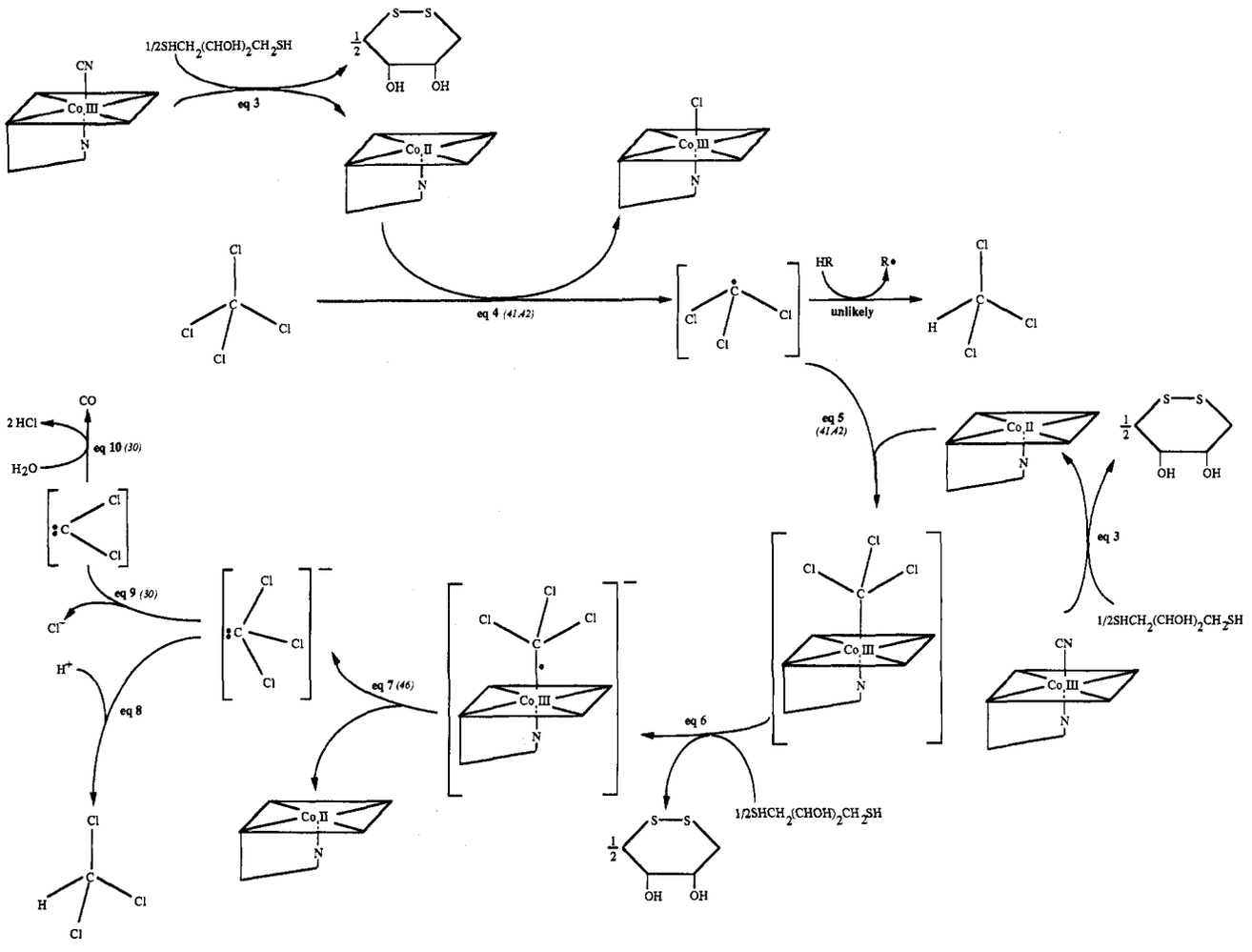



**Figure 7.** Proposed reaction pathway of carbon tetrachloride reduction to chloroform in the DTT–vitamin $B_{12}$ system.

The cleavage could involve the participation of DTT through ligand exchange between the conjugate base of DTT and the axial base on the corrin (*35*). The exchange would precede the carbon–cobalt bond cleavage resulting in the formation of the carbanion, $:CCl_3^-$, and of an unstable Co(III)–DTT complex, which would decompose yielding Co(II) and oxidized DTT. The likely fate of the carbanion would then be the abstraction of a proton from water and the formation of chloroform:

$$:CCl_3^- + H^+ = HCCl_3 \qquad (8)$$

The mechanism is similar to that proposed for the analogous reactions of chromium(II) (*48–50*), of alkylnickel(II) complexes (*51*), and of Ni(II) $F_{430}$ (*8*). A free-radical mechanism has also been proposed for the reductive dechlorination of alkyl halides by iron(II) porphyrins (*52*). The disappearance of CT through reductive dechlorination pathways such as the one proposed above accounts for less than 50% of the total observed CT conversion to chlorinated products. Therefore, competing pathways which consume CT but lead to products other than dechlorination products must exist. One possible pathway might involve the decomposition of the carbanion to chloride ion $Cl^-$ and dichlorocarbene $:CCl_2$, followed by hydrolysis of the latter to carbon monoxide and hydrochloric acid (*31*):

$$:CCl_3^- = Cl^- + :CCl_2 \qquad (9)$$

$$:CCl_2 + H_2O = CO + 2HCl \qquad (10)$$

Direct evidence for this pathway was not obtained during this study.

## *Conclusions*

This work demonstrates the participitation of Co(II) in reductive dechlorination reactions and suggests that Co(II) probably has an important role in vitamin $B_{12}$-dependent reactions in general. In past studies, the active species has been assumed to be the Co(I) species $B_{12s}$. The redox potential and the rate of reduction of Co(III) to Co(II) were found to be interrelated and directly correlated to the rate of the reductive dechlorination of CT. The chemical control of the redox potential by changing reducing agent concentration and pH appears to be a useful way of systematically investigating the effect of redox potential on reductive dechlorination rates and may serve as a potentially useful approach for studying reductive dechlorination mechanisms. In addition, DTT was shown to be an effective reductant and might be a good model of biologically important thiols that are involved in the reduction of naturally occurring cobalamins and, by analogy with this study, in reductive dechlorination pathways. Hence, the results provide strong support for the influence of an adequate reductant supply on the oxidation state of $B_{12}$ for a given pH and, consequently, on the kinetics and pathways of environmental reductive dechlorination reactions.

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