



## CIRCULAR DICHROISM IN COORDINATION COMPOUNDS

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### Abstract

Circular dichroism (CD) spectroscopy is widely used to study biological systems and non-biological systems, being an invaluable tool in inorganic chemistry, particularly in coordination chemistry. In this work it is described how CD may be used empirically to obtain structural and analytical information on transition metal complexes with optically active ligands, as well as relevant information on the interaction of metal ions with large bio-molecules such as proteins and DNA. Particular relevance is given to oxovanadium (IV) compounds.

**Keywords:** circular dichroism; coordination compounds; oxovanadium complexes; chiral complexes; protein-metal binding; DNA-metal binding.

### Resumen

La espectroscopía de dicroísmo circular (CD) es ampliamente usada para estudiar sistemas biológicos y no-biológicos, siendo una herramienta útil para la química inorgánica y en particular para la química de coordinación. En este trabajo se describe como la CD puede emplearse empíricamente para obtener información estructural sobre complejos de metales de transición con ligandos

ópticamente activos, así como también información relevante acerca de la interacción de iones metálicos con biomoléculas grandes tales como las proteínas y el ADN. Una relevancia particular poseen los compuestos de oxovanadium (IV).

**Palabras clave:** compuestos de coordinación; complejos de oxovanadium (IV); complejos quirales; enlace metal-proteína; enlace metal-DNA.

## Introduction

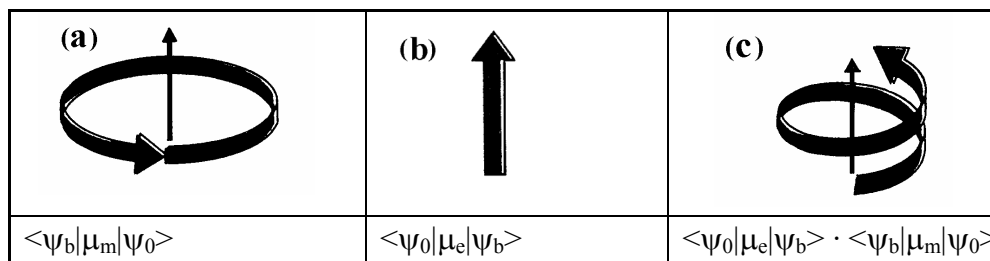
In a typical circular dichroism instrument left and right circular polarized light are produced and pass alternately through the sample compartment. In the detector the intensities of the two beams are measured and compared. If the sample compartment contains an optically active medium, the extinction coefficients for the left and right circular polarized light,  $\epsilon_L$  and  $\epsilon_D$ , respectively, may differ depending upon the wavelength ( $\lambda$ ) of the polarized incident beam. Therefore the difference between the extinction coefficients,  $\Delta\epsilon = \epsilon_L - \epsilon_D$ , may not be zero and  $\Delta\epsilon$  is called circular dichroism.  $\Delta\epsilon$  values depend on the wavelength of the radiation used, and the representation of  $\Delta\epsilon$  as a function of the wavelength is called the circular dichroism (CD) spectrum of the sample.

Plane polarized light is equivalent to two circular polarized beams with equal amplitudes, one circular polarized to the right and another circular polarized to the left. When plane polarized radiation passes through an optically active sample that absorbs to some extent light of a certain  $\lambda$ , three effects may then be measurable: optical rotation ( $\alpha$ ), circular dichroism ( $\Delta\epsilon$ ) and Faraday rotation. Together, they comprise the Cotton effect, though often the term is employed for any one of them. [1] Although the other two effects have their own impact on other fields, herein we focus on circular dichroism, and will address it further.

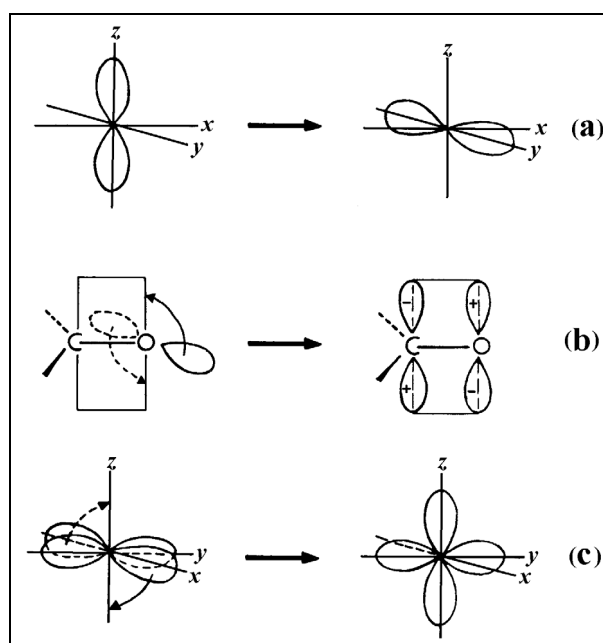
$\Delta\epsilon$  values at a certain wavelength can only be different from zero if the sample is optically active and absorbs light of that wavelength. Therefore CD spectra are observed at the same wavelengths as the absorption spectra of the sample, the latter sometimes being called isotropic absorption spectra. CD spectra are usually measured in the UV or visible range, but with some instruments the measurements are made in other wavelength regions (e.g. IR).

It is probably true that circular dichroism spectroscopy is more widely used to study biological systems than non-biological systems. CD is however an invaluable tool in inorganic chemistry as well, particularly in coordination chemistry. The technique swiftly provides not only structural information without the need of full X-ray diffraction determinations, but also information on the electronic origin of the absorption of radiation and of optical activity. It may also be used in a simple way to probe the interaction of metal ions with chiral ligands, these being either small compounds such as amino acids, or complex bio-molecules such as proteins.

The selection rules for CD transitions differ from those of isotropic absorption spectra. Pictorially the condition for an allowed CD transition is represented in Fig. 1: a magnetic-dipole-allowed transition is one in which both rotation and translation of electric charge occurs. The  $\mu_m$  (magnetic dipole operator, transforming as a rotation) combining with  $\mu_e$  (electric dipole operator, transforming as a translation) gives rise to a screw or helix, which is an enantiomorphous object. In other terms, the magnetic dipole (an axial vector, Fig.1(a)) combining with the electric dipole (a polar vector, Fig.1(b)) gives rise to a helix (Fig.1(c)). In Fig. 2 it is shown how this occurs for  $p_z \rightarrow p_y$ ,  $n \rightarrow \pi^*$  (in carbonyl groups) and in some  $d \rightarrow d$  transitions.



**Figure 1** - Physically the magnetic-transition moment can be regarded as charge movement of type (a); the electric-transition moment can be regarded as charge movement of type (b) and the observation of a CD band requires a charge movement of type (c). The probability of an isotropic absorption transition from  $\psi_0$  to  $\psi_b$  depends on  $\langle \psi_0 | \mu_e | \psi_b \rangle$ , while the probability of a CD transition depends on  $\langle \psi_0 | \mu_e | \psi_b \rangle \cdot \langle \psi_b | \mu_m | \psi_0 \rangle$ .

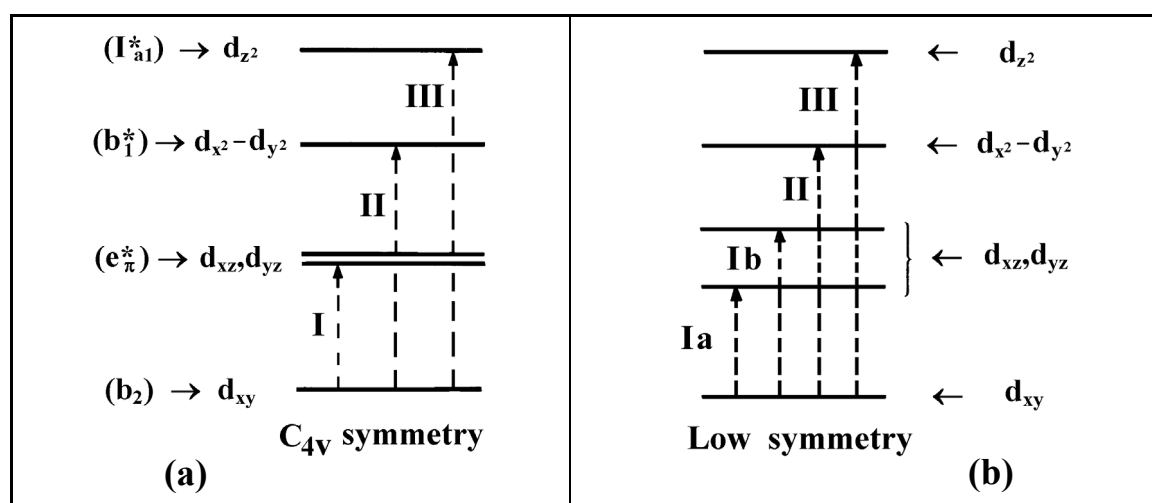


**Figure 2.** Magnetic-dipole-allowed transitions: (a)  $p_z \rightarrow p_y$ ; (b)  $n \rightarrow \pi^*$ ; (c)  $d_{xy} \rightarrow d_{yz}$  transitions.

The literature available on the CD of coordination compounds runs into thousands of references, and theoretical studies of transition metal complexes have focused mainly on tris-chelated complexes of  $\text{Co}^{\text{III}}$  and  $\text{Cr}^{\text{III}}$ , particularly with saturated amines, [2 and references therein]. General theory of circular dichroism and/or more practical aspects related to CD or chirality of coordination compounds can be accessed in many publications for example [1-8]. We will focus herein on how CD may be used empirically to obtain a) structural and analytical information on transition metal complexes with optically active ligands; b) relevant information on the interaction of metal ions with large bio-molecules such as serum proteins and DNA. In this work more relevance will be given to oxovanadium ( $\text{V}^{\text{IV}}\text{O}$ ) compounds with special emphasis on work conducted by our workgroup.

In the case of coordination compounds absorption in the visible range is particularly informative, and the CD due to electronic transitions between d orbitals is usually measured. Therefore the symmetry and nature of the ligand field is highly relevant information. In the case of

$d^1 V^{IV}O$ - compounds, often the unpaired electron is located in an essentially non-bonding  $d_{xy}$  orbital and the ordering of the d levels is as depicted in Fig. 3. [9]



**Figure 3.** Ordering of d levels in  $V^{IV}O$ -compounds. (a) compounds with  $C_{4v}$  symmetry (e.g.  $[V^{IV}O(H_2O)_5]^{2+}$ ); (b) Compounds with low symmetry [e.g.  $V^{IV}O(lactato)_2$ ]. [9]

Three or four d-d transitions are thus expected for  $V^{IV}O$  complexes depending upon their symmetry.

## Experimental

**Instrumentation.** CD spectra were recorded with a JASCO 720 spectropolarimeter, either with a red-sensitive photomultiplier (EXEL-308) suitable for the 400-1000 nm range, or with the photomultiplier suitable for the 200-700 nm range. Unless otherwise stated, by visible (Vis) or circular dichroism (CD) spectra we mean a representation of  $\epsilon_m$  or  $\Delta\epsilon_m$  values vs.  $\lambda$  [ $\epsilon_m$  = absorption/( $bC_M$ ) and  $\Delta\epsilon_m$  = differential absorption/( $bC_M$ ) where  $b$  = optical path and  $C_M$  = total metal concentration]. All measurements and operations of the spectropolarimeter were computer controlled. In some cases ellipticity (in mdeg) is represented.

**Reagents, solutions and methods.** All oxovanadium (IV)-containing solutions were manipulated in an inert atmosphere. Spectra of solutions containing amino acids and  $V^{IV}O^{2+}$  salts were recorded at 25 °C with solutions containing 2.25 M  $NaNO_3$ . Other spectra were recorded in media specified in the corresponding figures.

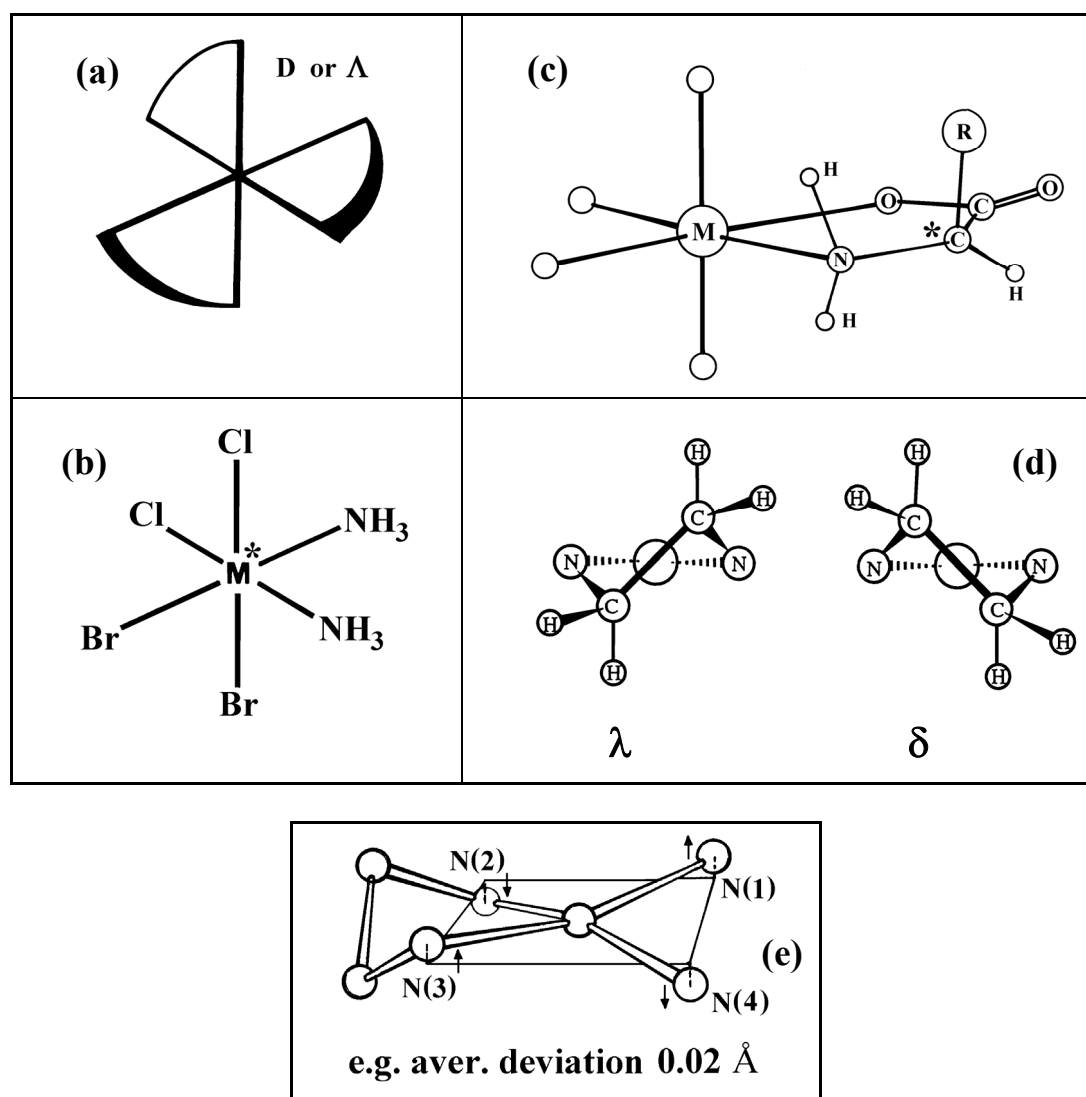
CD spectra of complexes in the solid state (dispersed in Nujol mulls or in KBr disks) were prepared as described in refs. [10,11].

## Discussion

### Background

A theoretical approach to the CD technique is a laborious and highly complex task. To simplify the understanding of CD spectra of coordination compounds the origin of the global signal measured may be considered as the sum of several (considered additive) effects: (a) configuration effect; (b) inherent dissymmetry; (c) vicinal effect; (d) conformational effect and (e) symmetry

distortion effect (see Fig. 4). The order of importance of these effects often decreases from (a) to (e), but the symmetry distortion effect may be as important as the configuration effect. This approach is very rewarding in the sense that is simple and quite informative, but it must be emphasized that this is no more than an artificial reasoning that helps in understanding the intensity of the spectrum recorded.



**Figure 4.** Effects contributing to an overall CD signal measured for a coordination compound. The configuration effect (a) is due to the chiral arrangement of chelate rings around the metal centre. The inherent dissymmetry (b) is the equivalent in coordination compounds of the chiral carbon atoms in organic compounds. The vicinal effect (c) may exist if the ligand is optically active and if its chirality is induced into electronic transitions involving mainly the metal centre. The conformational effect (d) may contribute if the ligand preferentially assumes a chiral conformation (e.g.  $\lambda$  or  $\delta$ ). The symmetry distortion effect (e) may be operating if chiral distortions occur in the coordination compound. In a particular case several of these effects may be operating simultaneously.

The mere presence in the same solution of an asymmetric substance and a chromophore separately do not give rise to Cotton effects. [12] The essential feature is that the chromophore itself

must be situated in an asymmetric field, that is, the chromophore and the asymmetric substance must be somehow connected. The d-d bands of a transition metal may be rendered optically active by introducing a chiral ligand. For example, in  $[\text{Co}(\text{en})_3]^{3+}$  compounds (en = ethylenediamine), effect (a) may operate. In  $[\text{Co}(\text{S-pn})_3]^{3+}$  (S-pn = S-2-methyl-1,2-ethylenediamine) or  $[\text{Co}(\text{L-Ala})_3]$ , besides (a), effect (c) [and also (d)] may be relevant. This induced asymmetry (or induced chirality) – effect (c) – is important in coordination compounds, particularly in d-d transitions, as a circular dichroism spectrum may be measured in wavelength ranges where the chiral ligands do not absorb (and only the complex is active) which highly simplifies data interpretation.

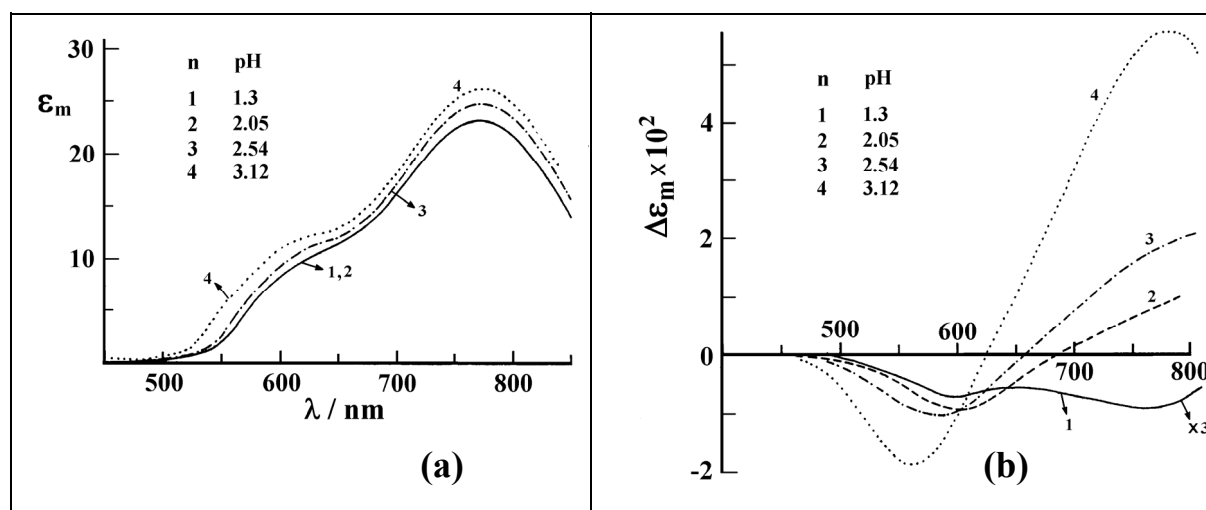
### **Circular dichroism vs. isotropic absorption spectra**

A CD spectrum can only be measured for a coordination compound if at least one of the effects mentioned above is operating, i.e. if there is some source of chirality in the system. In this case circular dichroism spectra may present advantages over isotropic absorption spectra.

### **Interaction of a chiral ligand with a metal ion in solution. Speciation studies**

When a metal ion and a chiral compound (such as an amino acid) are dissolved in an aqueous solution and a circular dichroism spectrum is observed in the visible range (due to d-d transitions), this means that the metal ion is undoubtedly to some extent in the form of a complex with the amino acid. If no chiral ligand is bound to the metal ion the CD is zero. Such clear information cannot be obtained from the isotropic absorption spectrum.

Fig. 5 further emphasizes this advantage of CD. It depicts spectra of solutions containing  $\text{V}^{\text{IV}}\text{O}^{2+}$  and D-penicillamine at low pH. [13] The visible isotropic spectra of the solutions at pH 1.3 and 2.05 coincide, while for pH 2.54 and 3.12 they show some changes (mainly an increase of  $\epsilon$  values). In contrast, CD spectra show enormous changes both in the pattern and in intensity. While the observation of CD spectra indicate that at least three optically active  $\text{V}^{\text{IV}}\text{O}$ -amino acid complexes must form in the pH range 1.3-3.1, the Vis spectra indicate a maximum of three species, one of them being  $[\text{V}^{\text{IV}}\text{O}(\text{H}_2\text{O})_5]^{2+}$ .



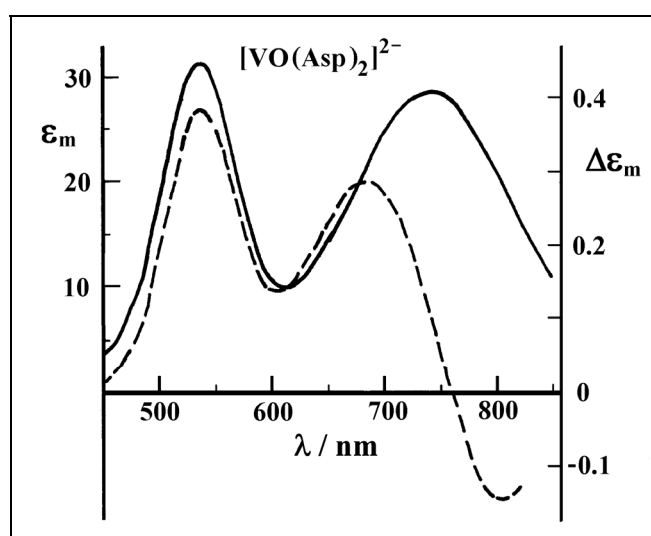
**Figure 5.** Spectra of solutions containing  $\text{V}^{\text{IV}}\text{O}^{2+}$  and D-penicillamine at low pH. (a) isotropic absorption spectra; (b) the corresponding CD spectra.  $C_{\text{VO}} \approx 0.01 \text{ M}$  and  $C_{\text{Pen}} \approx 0.237 \text{ M}$ . [13]

A second example is provided by the system  $V^{IV}O$ -GSH (GSH = Glutathione in the reduced form,  $\gamma$ -Glutamyl-L-Cysteinyl Glycine), in which a complex could be identified in a pH range where the metal-ion hydrolysis was precluding the use of potentiometry, EPR and isotropic absorption to obtain reliable information. At pH~8-10 CD spectroscopy allowed the clear detection a mixed-hydroxo complex and enabled the calculation of its stability constant. It was thus possible to extend the reliability of the  $V^{IV}O$ -GSH speciation model from pH 7 up to pH~10. [14]

These two examples emphasize not only the higher typical sensitivity of CD to changes in the coordination geometry when chiral ligands are involved, but also the greater possibility of using CD spectra for speciation studies, namely for identifying complex species and to enable the calculation of the corresponding stability constants. This particular advantage has been applied in many systems to establish the equilibrium models, to detect interactions of metal ions with biological molecules such as sugars, oligopeptides, DNA or proteins (see examples below), to follow reactions of coordinated ligands, etc. [13-21].

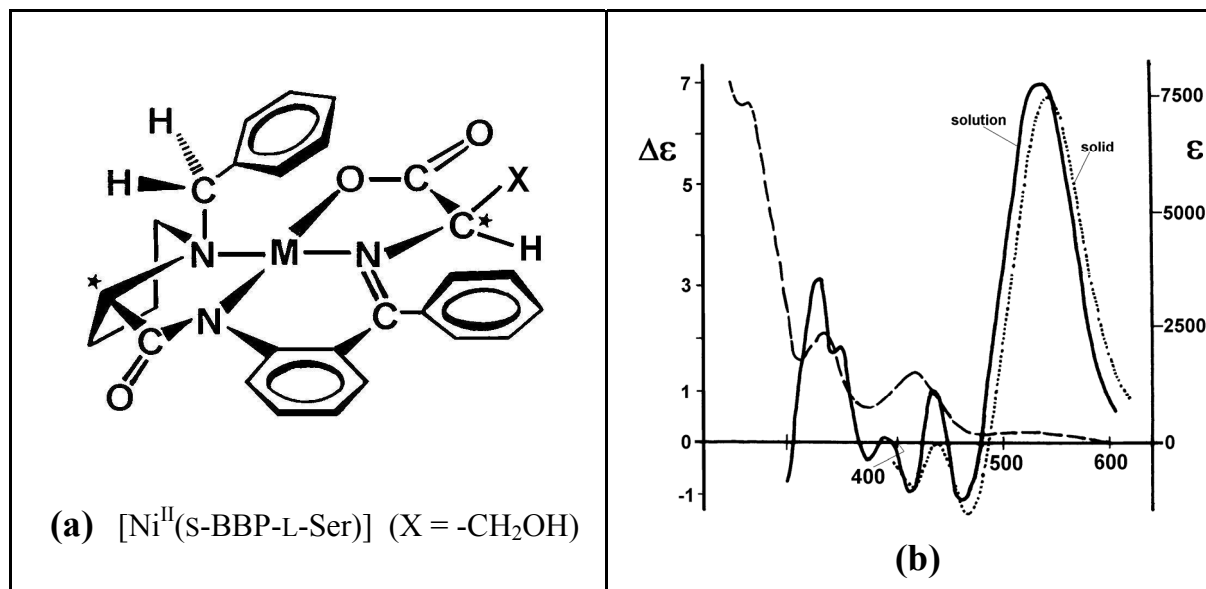
### Information on structure/symmetry of coordination compounds

In  $V^{IV}O$ -compounds with  $C_{4v}$  symmetry three d-d transitions are expected, which in a simplified model (Fig. 3) can be regarded as I:  $d_{xy} \rightarrow d_{xz}, d_{yz}$ ; II:  $d_{xy} \rightarrow d_{x^2-y^2}$ ; III:  $d_{xy} \rightarrow d_{z^2}$ . In the visible region often band III is overlapped by charge transfer bands in the near UV range not being easily identified in absorption spectra (and also in CD), and normally only two bands (I and II) are observed/recorded. Even in cases where it is expected that the  $d_{xz}, d_{yz}$  orbitals do not have the same energy, band I is quite broad and only one band is observed as in Fig. 5a and Fig. 6. In the corresponding CD spectra, particularly if bands Ia and Ib have Cotton effects of opposite signs, the non-degeneracy of the  $d_{xz}, d_{yz}$  orbitals is more clearly seen. For example, in solutions containing  $V^{IV}O^{2+}$  and L-aspartic acid at pH ~7.0, where the main species formed corresponds to  $[V^{IV}O(L-Asp)_2]^{2-}$ , the isotropic absorption spectra exhibits only one band corresponding to transition I in the visible range, while in the CD spectrum two bands are recorded at ca. 680 and 800 nm (see Fig. 6). Clearly the CD spectrum gives more information on the structure of the complex, namely that the broad band in the Vis spectrum at 750 nm corresponds in fact to two distinct transitions. As such the symmetry of the complex present in solution is neither  $C_{4v}$  nor  $C_{2v}$ , but lower.



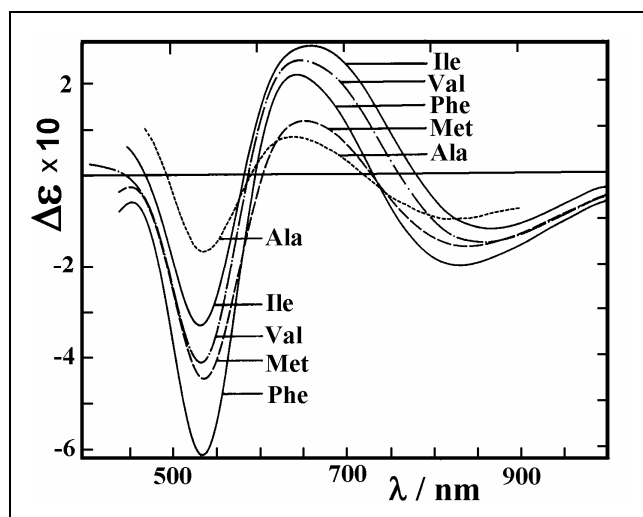
**Figure 6.** Spectra of solutions containing  $V^{IV}O^{2+}$  and L-aspartic acid at pH = 7.0. Full line: isotropic absorption spectrum; dashed line: CD spectrum.  $C_{VO} \approx 0.011$  M and  $C_{Asp} \approx 0.141$  M. [16]

Similar information can be obtained for other metal ions. For example in the  $\text{Ni}^{\text{II}}$ -complex with the optically active ligand derived from the reaction of *S*-*o*-[N-(N-benzylpropyl)amino] benzophenone (*S*-BBP) and L-Ser, the two bands at ca. 350 and 430 nm observable in the isotropic absorption spectrum correspond to at least seven distinct transitions, which can be detected in the CD.



**Figure 7.** (a) Structure of  $[\text{Ni}^{\text{II}}(\text{S-BBP-L-Ser})]$  and (b) isotropic absorption spectrum in dichloromethane solution (dashed line), CD spectrum (full line) and CD spectra of solid sample of the same complex dispersed in a KBr disk (····). [11]

In most of the examples given the main effect operating for the observation of the CD signal is the vicinal effect (c). The conformational effect (d) is usually much less important, but the symmetry distortion effect (e) may play some role in  $\text{Ni}^{\text{II}}$ -complexes such as the example given in Fig. 7. In Fig. 8 the importance of the vicinal effect is clearly seen in the spectra of a set of  $[\text{V}^{\text{IV}}\text{O}(\text{sal-L-Ama})(\text{H}_2\text{O})]$  complexes (sal-L-Ama = N-salicylidene-L-aminoacidato), the CD signal intensity increasing as the size of the amino acid side group also increases.



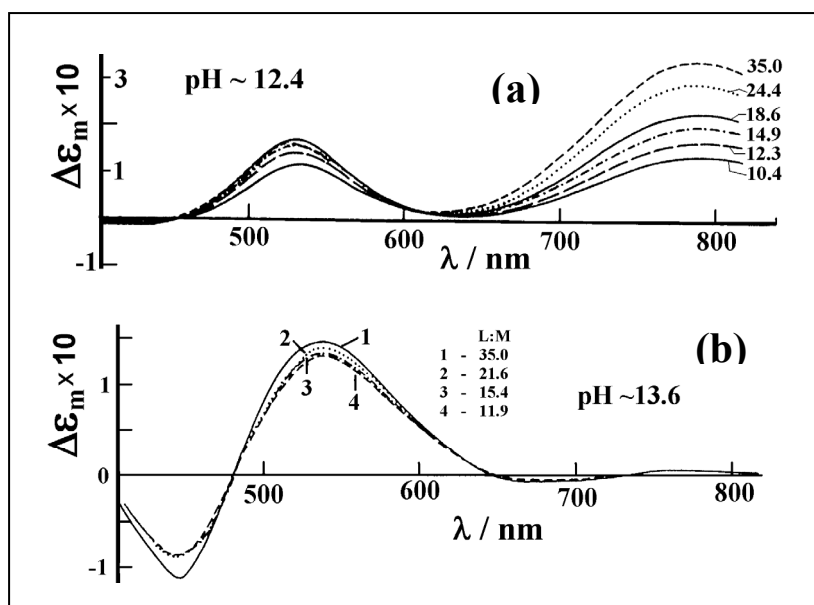
**Figure 8.** CD spectra of  $[\text{V}^{\text{IV}}\text{O}(\text{sal-L-Ama})(\text{H}_2\text{O})]$  complexes (sal-L-Ama = N-salicylidene-L-aminoacidato) in methanolic solution. [22]. For band II the influence of the vicinal effect is clear, i.e. the CD signal intensity increases with the size of the amino acid side group. The pattern of all spectra is the same, indicating that the binding modes and the main features of the molecular structure are the same for all complexes. The spectra of these complexes in the solid state (dispersed in Nujol mulls - not shown) exhibited in all cases an identical pattern, indicating that the molecular structure is the same in the solid state and in solution.



### Measuring informative CD spectra in conditions not viable for isotropic absorption spectra

In some cases the isotropic absorption spectra measured cannot give useful information on the metal-ligand interactions. This is frequently the case in the interactions of metals in media mimicking biological conditions where too many species are absorbing (see below) or when the hydrolysis of the metal ion is too extensive.

For example, in solutions containing oxovanadium(IV) at high pH, hydrolysis is very extensive and the Vis spectra is dominated by the charge-transfer bands that arise from the  $[(V^{IV}O)_m(OH)_n]$  species present. As such, variations in concentration of  $V^{IV}O$ -complexes with other ligands are hardly detected by conventional UV-Vis spectroscopy. Fig. 9 shows CD spectra of solutions containing  $V^{IV}O^{2+}$  and L-Thr at pH 12.4 (a) and 13.6 (b). At pH 12.4, the experiment proceeded by varying the metal concentration, while keeping the L-Thr concentration approximately constant, and this yielded CD spectra with exactly the same pattern, but different intensity. This means that, as the total  $V^{IV}O$  concentration increases, the concentration of oligomeric species  $\{[(V^{IV}O)_m(OH)_n]\}$  also increases, but the nature of the optically active  $V^{IV}O$ -Thr complex present is always the same. In Fig.9(b) the CD signal is intense at pH  $\approx$  13.6, therefore there is a significant concentration of  $V^{IV}O$ -Thr complexes present (but distinct from those at pH 12.4), but the increase in the  $V^{IV}O$  concentration does not change the CD signal much (neither in pattern nor intensity). In these experimental conditions (high ligand-to-metal ratio) this means that the equilibrium only involves species with the same degree of oligomerization, here only monomeric species: the  $V^{IV}O$ -Thr complex(es) and  $[V^{IV}O(OH)_3]^-$  [15].



**Figure 9.** CD spectra of solutions containing L-Thr and  $V^{IV}O^{2+}$  with (a)  $C_{VO} = 8.5$  to  $25 \times 10^{-3}$  M and (b)  $C_{VO} = 6.8$  to  $18 \times 10^{-3}$  M. The solutions were prepared by varying the volume of the added  $V^{IV}O$  stock solution. For each group of spectra, the total L-Thr concentration is kept approximately constant [15].

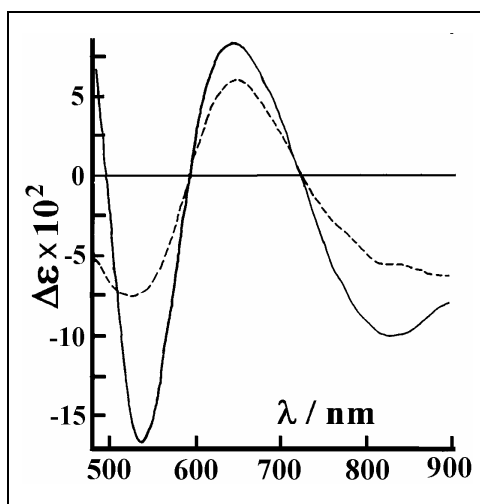
### Comparison of the structures in solution and in the solid state

It is frequently possible to obtain CD spectra of solid samples of optically active coordination compounds either in KBr disk or in Nujol mulls, much more easily and with better

signal-to-noise ratio than the corresponding absorption spectra. Due to the high sensitivity of CD spectroscopy to the coordination environment of metal complexes and to the spatial arrangement of atoms around the metal centre, the comparison of the CD spectrum in the solid state and in solution may provide information on whether the structure remains the same upon dissolving the compound or not.

Recalling the example in Fig 7b, it is clear that complex  $[\text{Ni}^{\text{II}}(\text{s-BBP-L-Ser})]$  has a CD spectrum quite similar either in the solid state or in solution, so it may be concluded that the molecular structure determined by X-ray diffraction [11] will be maintained upon dissolution of the complex in dichloromethane. The same was found for several other similar  $\text{Ni}^{\text{II}}$ -BBP-Ama complexes, but not for  $[\text{Ni}^{\text{II}}(\text{s-BBP-Gly})]$ .

In the case of Fig. 10, the pattern of the spectra of  $[\text{V}^{\text{IV}}\text{O}(\text{sal-L-Ala})(\text{bipy})]$  in the solid state and in methanolic solution is the same, indicating that the main features of the molecular structure of this complex are preserved in solution. This is another case when CD is highly preferred over conventional absorption: while in the isotropic absorption spectrum of the same complex in solution only two bands (II and I) are detected ([10], not shown) three bands are clearly observed in the corresponding CD spectrum: II, Ib and Ia.



**Figure 10. (a)** CD spectra of  $[\text{V}^{\text{IV}}\text{O}(\text{sal-L-Ala})(\text{bipy})]$  (sal-L-Ala = N-salicylidene-L-Alaninato) in methanolic solution (full line) and in a Nujol mull (dashed line). [10] The similarity of spectra indicates identical molecular structures for the compound in the solid state and in solution.

### ***Interaction of metal ions with high molecular weight bioligands***

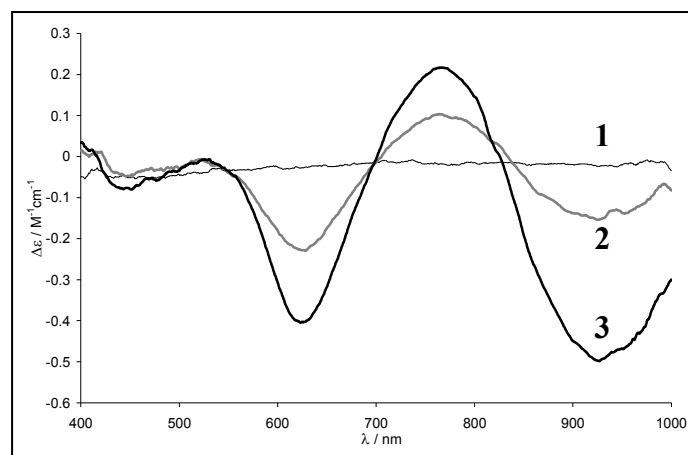
#### ***Binding to serum proteins***

The binding of metal ions and that of their complexes to bioligands is of utmost importance when considering applications in Medicinal Inorganic Chemistry. In this scope special emphasis is given to binding to proteins with well-known transport functions as a route to deliver a therapeutically active metal compound. Protein binding plays a major role in the compound speciation in *in vivo* conditions. Albumin is by far the main “transporter vehicle” and has focused most of the interest in this regard. Although with a different primary function, transferrin has proven relevant for the bio-speciation of metal complexes, and in their delivery to cells and also received attention.

The non-functional binding of vanadyl and vanadate to proteins is well known and has been mainly studied by UV-Vis, EPR and NMR. For example Chasteen [23] studied the binding of  $V^{IV}O$  to transferrin by EPR and UV-Vis, and Butler and co-workers reported the binding of vanadate by  $^{51}V$  NMR. [24] Vanadium (in both IV and V oxidation states) binds to transferrin at the two specific iron-binding pockets.

Circular dichroism has long been used to provide information on protein structure, and simple methods have been developed to analyse the protein secondary structure in terms of its content in  $\alpha$ -helix,  $\beta$ -strands and random coil. CD has also been used to detect adduct formation by analysing how the protein secondary (and tertiary) structure is affected upon the binding of a therapeutic agent. These studies have been focused on the UV range where the protein absorbs radiation. When metal-ion complexes are involved, circular dichroism can also be used to extend the study of protein binding to the visible range, and provide additional information in a simple way, namely the possibility of calculating conditional formation constants for protein-metal ion adducts. [25, 26]

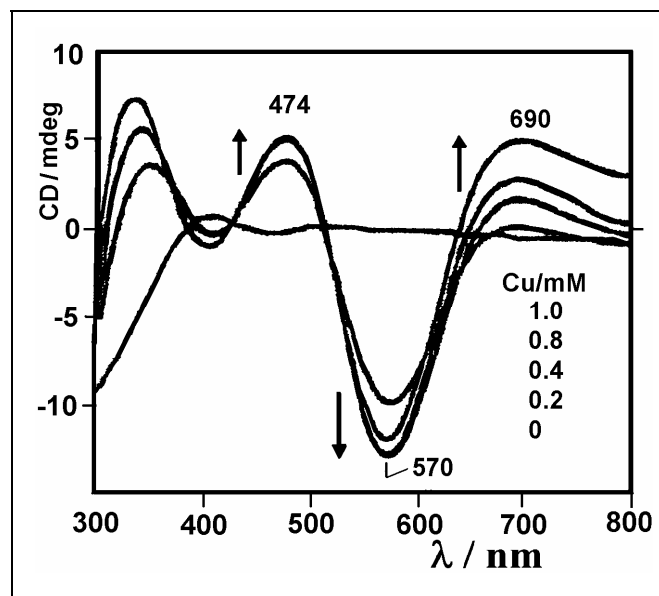
Fig. 11 depicts CD spectra in the visible region of solutions containing  $V^{IV}O$  and human apo-transferrin, and Fig. 12 of solutions containing bovine serum albumin (BSA) and  $Cu^{II}$ . [27] The fact that in Fig. 11 a CD signal is observed in the visible region indicates undoubtedly the binding of the metal ions to donor groups of the proteins in a chiral environment (neither the metal ion nor the protein alone are CD-active in this wavelength range). These CD spectra correspond to an induced CD signal as the bands observed are due to d-d electronic transitions. The slightly different CD signals 2 and 3 seen in Fig. 11, *i.e.* spectrum 3 is not the double of spectrum 2 (though the vanadyl content is), indicates that the two binding sites of apo-transferrin are similar but not identical.



**Figure 11.** CD spectra in the visible region of solutions containing (1) human apo-transferrin 750  $\mu$ M (in HEPES buffer pH = 7.4, containing 25 mM of  $HCO_3^-$ ); (2) after addition of 1 equivalent of  $V^{IV}O$ ; (3) after addition of a total of 2 equivalents of  $V^{IV}O$ . The bands at ca. 620, 770 and 925 nm correspond to transitions II, Ib and Ia (see Fig. 3).

In the  $Cu^{II}$ -BSA system, monitoring the CD spectral intensities at 474 and 570 nm, upon the first additions of  $CuCl_2$ , the CD signals indicate the formation of 1:1  $Cu^{II}$ :BSA adduct, with  $Cu^{II}$  binding with the N-terminal Asp-Thr-His moiety of BSA [27]. The spectral intensity at 690 nm

developed mainly when  $\text{CuCl}_2$  was added above the equimolar concentration of BSA, suggesting the presence of a second binding site of  $\text{Cu}^{\text{II}}$  in BSA. [27]



**Figure 12.** CD spectral titrations of 0.5 mM solutions of BSA with  $\text{CuCl}_2$  at pH 7.4, 0.1 M  $\text{NaClO}_4$  and 37 °C. The final concentrations of  $\text{Cu}^{\text{II}}$  are indicated [27].

This analysis (that has focused on uncomplexed metal ions *i.e.*, their inorganic salts) can be further extended to the study of metal complexes binding in general. If a non-optically active ligand is added to the solution containing the metal ion and the protein, for example the solution of spectrum 2 in Fig. 11, this may or may not change the CD spectrum recorded for the metal alone. If a new pattern develops, this means that the ligand participates in the binding of the metal to the protein, probably forming ternary complexes. If the CD spectrum does not differ from the previously recorded one, this means that the ligand does not bind to the metal ion. This type of analysis is presently being carried out to evaluate the formation of ternary complexes of metal ions with transferrin and with albumin [25, 26]

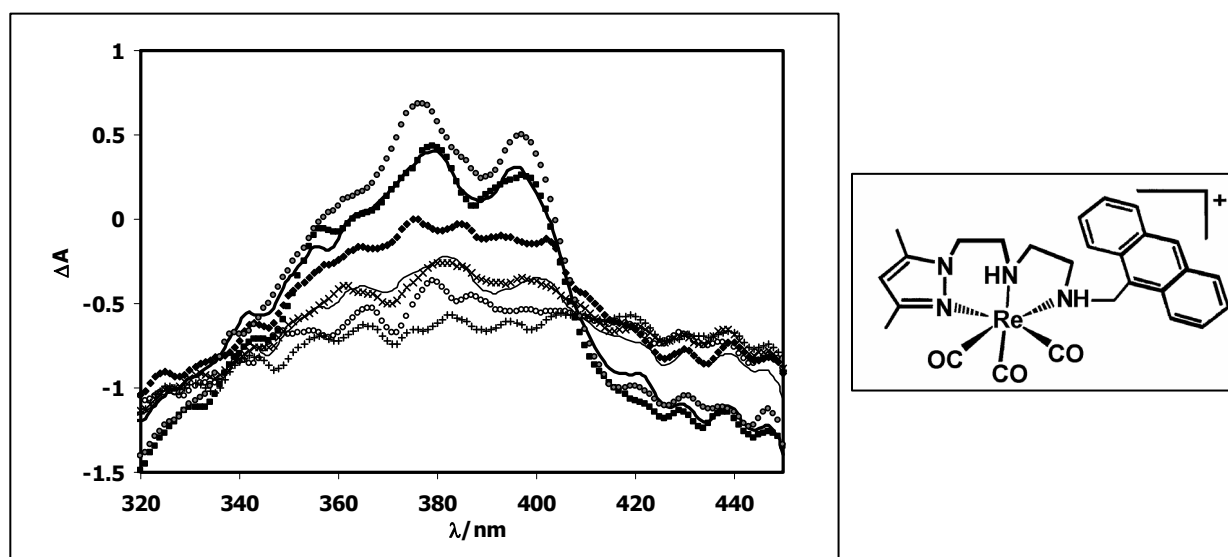
### **Binding to DNA.**

The binding of metal ions and/or their complexes to DNA has also been studied by CD spectroscopy.

Modifications in the CD signal in the 200-300 nm range have been used to follow changes in the conformation of the several forms of the DNA chains, [28-30] or to obtain information on the cleavage/damage [31] of the chain by the action of a metal ion (or metal complex). If no changes at all are seen in the plain DNA CD spectrum in the 200-300 nm range, this is an indication that the conformation of the DNA chain has not changed, but is not a clear proof that the metal ion does not bind. In contrast, the detection of e.g. LMCT or d-d bands in spectral regions where the DNA molecule does not absorb is much less frequently reported, but when observed are clear indications of the metal binding to donor groups close to chiral centres of the DNA molecule.

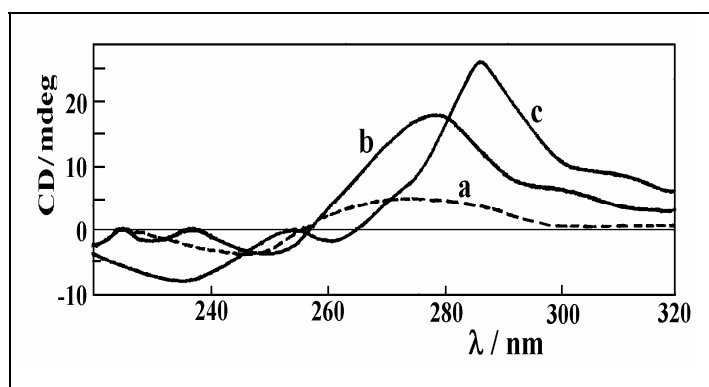
Fig. 13 shows CD spectra corresponding to additions of amounts of Calf Thymus DNA (0 to 25.5 eq.) to a solution of  $[\text{Re}(\text{CO})_3(\text{PzA})]^+$ . The signals observed corresponds to induced CD spectra: the complex is originally in a non-chiral environment and by intercalation of the aromatic part of the PzA ligand between the base pairs of DNA, chirality is induced in its  $\pi$ - $\pi^*$  bands, thus

allowing a CD spectrum to be recorded. This has frequently been used to prove binding of aromatic groups to DNA and to calculate binding constants for the process.



**Figure 13.** Induced CD spectra of  $[\text{Re}(\text{CO})_3(\text{PzA})]^+$  ( $8 \times 10^{-5}$  M) upon increasing amounts of added Calf Thymus DNA (from 0 to 25.5 eq.). The spectra were recorded at 28°C, with DMSO (20%)/aqueous (80%) of TRIS buffer (0.01M, pH=7.4, 0.05M NaCl) solutions. [32]

In Fig. 14a the CD spectrum of Calf Thymus DNA shows bands at 275 nm (positive) and 245 nm (negative) typical of the B-form. Upon addition of  $\text{Cu}^{\text{II}}$ -complexes, changes due to partial intercalative interaction of the heterocyclic rings of the complexes are seen [Fig.14(b) and (c)]. Moreover, induced CD bands that correspond to those of the corresponding UV isotropic absorption bands of the complexes were observed, undoubtedly proving the formation of adducts between DNA and  $\text{Cu}^{\text{II}}(\text{tdp})$  complexes.



**Figure 14.** CD spectra of CT DNA in 10%  $\text{CH}_3\text{CN}/\text{Tris}$  (5 mM Tris-HCl, 50 mM NaCl, pH 7.1) at 25°C in the absence (a) and presence of equimolar concentrations of (b)  $[\text{Cu}(\text{tdp})(\text{phen})](\text{ClO}_4)$  and (c)  $[\text{Cu}(\text{tdp})(\text{tmp})](\text{ClO}_4)$  [30]. {Htdp = 2-[(2-(2-hydroxyethylamino)-ethylimino)methyl]phenol; phen = 1,10-phenanthroline; tmp = 3,4,7,8-tetramethyl-1,10-phenanthroline}.

## Conclusions

The empirical use of Circular Dichroism spectroscopy to obtain structural and analytical information on transition metal complexes with optically active ligands as well as to access relevant information on the interaction of metal ions with large bio-molecules is revised with emphasis on oxovanadium(IV) compounds. It is shown that highly relevant information may be obtained in a simple manner from the observation of induced CD spectra of bands of transition metal complexes.

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