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Simple Approximate Approaches for the Determination of Circular Dichroism Spectra in Multiple Scattering Systems

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A convenient method for the determination of CD spectra in multiple scattering systems is demonstrated. The formula of approximation, which gives the molar CD in terms of the values observable with commercial spectrometers by using a plate of opal glass, is presented based on some assumptions. The resulting solid-state CD spectrum of Λ-[Co(en)$_3$]Br$_3$·H$_2$O is reported and compared with the solution CD spectrum.

Circular dichroism (CD) spectra have proved very useful in the studies of the structure of molecules. The measurements of CD spectra have recently been extended to the suspensions of particulate biological matters.[1,2] However, the CD spectra obtained are contributed by unequal scattering of left- and right-circularly polarized light, and this effect has not been well elucidated.

In the preceding paper[3] we demonstrated the method for determining the approximate molar CD in a multiple scattering system, a Nujol mull of cobalt(III) complex. In our method, the ellipticity (Θ) and the transmittance (T) are measured with conventional spectrometers by only setting a plate of opal glass on the unilluminated sides of a sample and a blank cell. The molar CD ($\Delta\varepsilon$) of a particulate sample is evaluated based on appropriate assumptions and approximations from the values of Θ and T by somewhat lengthy calculations. The resulting molar CD is well reproducible. The $\Delta\varepsilon$ of Λ-[Co(en)$_3$]Br$_3$·H$_2$O obtained by our method at the peak position of the first absorption band is almost equal in magnitude to that in aqueous solution.

In order to obtain the CD spectrum in such a multiple scattering particulate system, lengthy calculating operations, which are inconvenient for rapid analysis of spectra, need to be carried out at every wavelength. In this paper a simple approximation method for such a CD spectrum will be described, and the resulting CD spectrum of solid-state Λ-[Co(en)$_3$]Br$_3$·H$_2$O will be shown.

Calculation Methods

The variation of the intensity and polarization state of a light beam in a multiple scattering medium is written by the equation of radiative transfer in the vector form.[4] In the preceding paper the intensity of light transmitted through the plane-parallel scattering medium containing optically active particles has been analyzed by using the Kubelka-Munk model.[5] When the optical activity of the scatterers is weak, as is often the case with the d-d transition of cobalt(III) complexes, the transmittance constituents for the left (L) and right (R) circularly polarized light are approximately given by[3]

$$T_i = 4\beta_i(I_0)_i/[(1+\beta_i)^2e^{\alpha_i l} - (1-\beta_i)^2e^{-\alpha_i l}]I_0;\ i=\mathrm{L,R}, \quad (1)$$

where

$$\alpha_i = [k_i(k_i+2s_i)]^{1/2}, \quad (2)$$

$$\beta_i = [k_i/(k_i+2s_i)]^{1/2}, \quad (3)$$

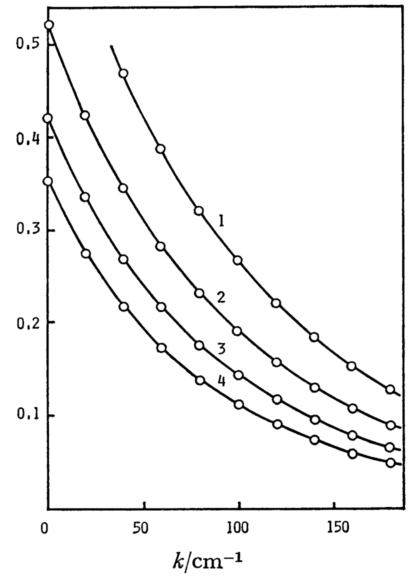

Fig. 1. Variation of T with k.
The value of s: (1) 50 cm^{-1}, (2) 100 cm^{-1}, (3) 150 cm^{-1}, and (4) 200 cm^{-1}.

and

$$I_0 = \Sigma_i(I_0)_i. \quad (4)$$

The letters k and s denote the absorption and scattering coefficients, respectively, l the path length, and I_0 the intensity of incident light. Hereinafter, l is always taken to be 9.15×10^{-3} cm (path length of our sample cell). Figure 1 shows the variation of T with k plotted when s remains constant.

The angular dependence of the scattering contribution to CD has been investigated by Bohren[6] and Gitter-Amir *et al.*[7] It has been shown that the difference between scattering of left- and right-circularly polarized light by optically active spheres is significant only in the forward scattering directions. The difference in scattering is negligible in the backward directions. In the Kubelka-Munk model, s is the backscattering coefficient. It is, therefore, assumed in this study that the scattering coefficients s_L and s_R are equal, and hence their value is equal to that for natural light:

$$s_L = s_R = s. \quad (5)$$

In a conventional Grosjean-Legrand type CD instrument,[8] the polarization state of incident light varies with time alternately between left- and right-circular polarizations. As shown in the preceding paper,[3] the recorded ellipticity Θ in radians is related to the trans-

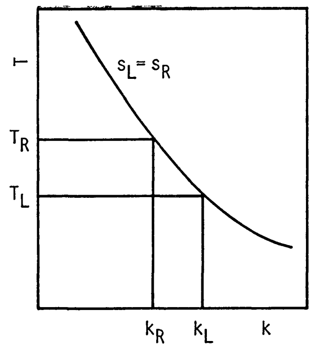

Fig. 2. Diagram showing the relation between ΔT and Δk in the case of $s_L = s_R$.

mittances for the periodical incidence of left- and right-circularly polarized light, T_L and T_R, by

$$\Theta = -(1/4)\Delta T/T, \tag{6}$$

where

$$\Delta T = T_L - T_R, \tag{7}$$

and

$$T = (T_L + T_R)/2. \tag{8}$$

Consequently, T_L and T_R are separately given by

$$T_L = (1 - 2\Theta)T, \tag{9}$$

and

$$T_R = (1 + 2\Theta)T. \tag{10}$$

Thus the values of T_L and T_R which are defined in the Kubelka-Munk model can be determined by measuring Θ and T with conventional spectrometers using a plate of opal glass.

Since the scattering coefficients s_L and s_R are considered equal in the Kubelka-Munk model, the T_L and T_R are given by the ordinates of two points on a certain k-T curve (see Fig. 2). Then the distance between the two points along the horizontal axis gives the experimental value of circular dichroism:

$$\Delta k = k_L - k_R. \tag{11}$$

The experimental value of ΔT being generally small, about the order of 10^{-3} in our measurements, the ratio $\Delta T/\Delta k$ approaches the differential coefficient $\mathrm{d}T/\mathrm{d}k$, *i.e.*, the slope of the k-T curve. In practice we may let

$$\Delta T/\Delta k = \mathrm{d}T/\mathrm{d}k. \tag{12}$$

Rearranging this equation we have

$$\Delta k = (\mathrm{d}k/\mathrm{d}T)\Delta T. \tag{13}$$

According to Eq. 6 we can rewrite Eq. 13 in the form

$$\Delta k = -4\Theta(\mathrm{d}k/\mathrm{d}T)T. \tag{14}$$

Thus, in order to obtain the CD spectrum, we must evaluate $\mathrm{d}k/\mathrm{d}T$ for the set of T and s values found at every wavelength. But this is a big operation.

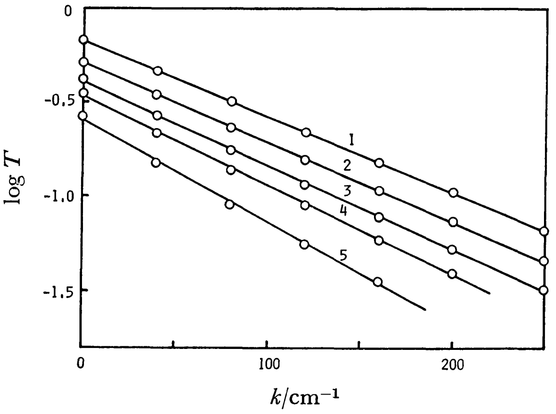

Fig. 3. Plots of log T against k.
The value of s: (1) 50 cm^{-1}, (2) 100 cm^{-1}, (3) 150 cm^{-1}, (4) 200 cm^{-1}, and (5) 300 cm^{-1}.

Now we consider the value of $(\mathrm{d}k/\mathrm{d}T)T$. In the Kubelka-Munk model, k, s, and T are associated with one another as has been represented by Eqs. 1—4. When s remains constant, log T is plotted against k in Fig. 3. Figure 3 shows these plots are approximately aligned in a straight line. In other words, the slopes of the plots are not exactly but nearly constant regardless of the value of k. Therefore, as a first approximation, we may let

$$\log T = Ak + B, \tag{15}$$

where A and B are constants.

Differentiating log T with respect to k, we have

$$(1/2.303T)(\mathrm{d}T/\mathrm{d}k) = A. \tag{16}$$

Rearranging this equation we have

$$(\mathrm{d}k/\mathrm{d}T)T = 1/2.303A. \tag{17}$$

Since A is a constant in the above approximation, the value of $(\mathrm{d}k/\mathrm{d}T)T$ can be considered constant, too.

It is also found from Fig. 3 that the slopes of the lines are not sensitive to their s values. In particular, when the values of s are not so different from each other, the slopes are nearly equal.

The scattering coefficient usually increases in magnitude with decreasing wavelength, but its variation should be small in the region of the d-d transitions of cobalt(III) complexes,[9] which we are now concerned about. Consequently, as a first approximation, the value of $(\mathrm{d}k/\mathrm{d}T)T$ can be taken as constant in such a region, though k, s, and T may vary with wavelength. In our studies a certain value of $(\mathrm{d}k/\mathrm{d}T)T$ associated with the first absorption maximum will be chosen for convenience.

In the preceding paper the molar CD of a microcrystalline sample has been determined based on the assumption that the molar absorbance is equal both in crystal and in solution in the region of d-d transitions. By using the molar absorbance ε(soln) and the absorption coefficient k at a peak position, which will be marked by asterisks, the molar CD in crystal is related to the experimentally determinable Δk as follows:

$$\Delta\varepsilon(\text{cryst}) = \varepsilon^*(\text{soln})\Delta k/k^*. \tag{18}$$

Substituting Eq. 14 in Eq. 18 we have

$$\Delta\varepsilon(\text{cryst}) = -4\Theta(\mathrm{d}k/\mathrm{d}T)_{s^*,T^*}T^*\varepsilon^*(\text{soln})/k^*, \tag{19}$$

where the subscripts s^* and T^* denote the restriction of $(\mathrm{d}k/\mathrm{d}T)$ to the value for the case of $s = s^*$ and $T = T^*$. The values of Θ, ε^*(soln), and T^* are measurable by conventional instruments. Further, the values of k^* and $(\mathrm{d}k/\mathrm{d}T)_{s^*,T^*}$ can be calculated if s^* and T^* are fixed. Although s^* cannot be measured directly, but estimated from the data of transmittance

Table 1. Spectral properties of Λ-[Co(en)$_3$]Br$_3$·H$_2$O

Absorption (soln)		CD (soln)		Solid-state CD (Nujol mull)	
$\sigma/10^3$ cm^{-1}	log (ε/mol^{-1} dm^3 cm^{-1})	$\sigma/10^3$ cm^{-1}	$\Delta\varepsilon$/mol^{-1} dm^3 cm^{-1}	$\sigma/10^3$ cm^{-1}	$\Delta\varepsilon$/mol^{-1} dm^3 cm^{-1}
21.5	1.95	20.4	+1.89	20.2	+1.83
		22.4	0	22.1	0
		23.3	−0.14	23.0	−0.26
25.9	0.99	25.8	0	25.7	0
29.6	1.91	28.8	+0.24	28.9	+0.24

in the wavelength region without absorption.[3] Thus the quantities other than Θ are fixed irrespective of wavelengths, and hence the CD curve can be readily obtained by only multiplying Θ by a definite value associating with an absorption maximum:

$$-4(\mathrm{d}k/\mathrm{d}T)_{s*,T*}T^*\varepsilon^*(\mathrm{soln})/k^*. \qquad (20)$$

Experimental

Materials. Λ-[Co(en)$_3$]Br$_3$·H$_2$O was prepared by the method of Werner.[10] Commercial Nujol was used as the mulling agent.

Measurements. The principles and the validity of our measurements have been described in the preceding paper.[3] The transmittance and the absorbance were measured by a Shimadzu UV-200 spectrophotometer, and the ellipticity by a JASCO MOE-1 spectropolarimeter(detector: end-window photomultiplier tube, HTV-R376). Measurements on Nujol mulls were carried out by the opal glass technique,[11] where a plate of opal glass was placed just after the sample cell, and the diffused light penetrating behind the plate was detected by a photomultiplier.

A quartz cell with demountable window (path length 9.15×10^{-3} cm) was used in the measurements on mulls. A mull was prepared by grinding the complex salt (5—11 mg) and mixing it with Nujol (40—50 mg) in an agate mortar.

Since the intensity of light after passage of a plate of opal glass was very weakened in the wavelength region shorter than *ca.* 320 nm, the CD measurements were unable to be carried out in this region.

Results and Discussion

The CD spectra of Λ-[Co(en)$_3$]Br$_3$·H$_2$O in Nujol and in water are shown in Fig. 4. Table 1 gives the intensities of $\Delta\varepsilon$ at peak positions. Both the CD curves are roughly similar in pattern and intensity. This shows the effects of molecular orientation and crystal anisotropy, which are usually observed in the single-crystal CD, are cancelled out in a randomly oriented particulate system, microcrystallines in Nujol. The solid-state CD spectrum obtained by our method is, therefore, regarded as an isotropic CD spectrum, and hence can be compared directly with a solution CD spectrum.

Figure 4 shows the solid-state CD spectrum is slightly changed in intensity compared to the solution CD spectrum. There are a decrease in the rotational strength, proportional to the area of a CD peak, of E$_a$ band and an increase in that of A$_2$ band. These changes are similar to the changes well-known as the ion-pairing effects,[12] which are usually observed in the CD spectrum of Λ-[Co(en)$_3$]$^{3+}$ on addition of anions. Thus the solid-state CD measurements may provide some significant informations regarding the ion-pairing effects.

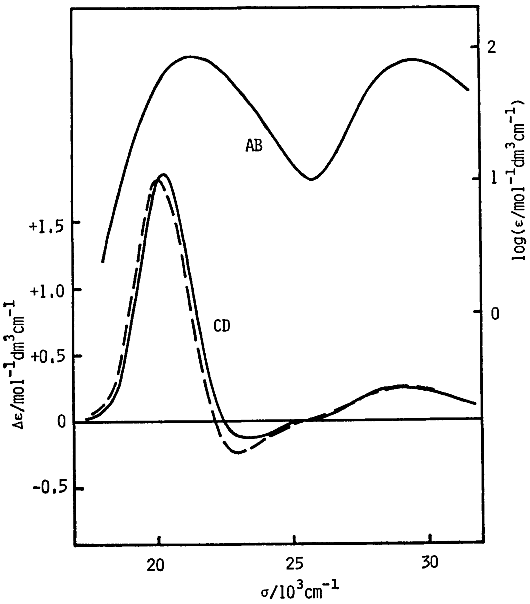

Fig. 4. Absorption (AB) and CD spectra of Λ-[Co(en)$_3$]Br$_3$·H$_2$O.
- - -: Nujol mull, ——: aqueous solution.

Single-crystal CD spectra are generally attended by experimental difficulties owing to the crystal anisotropies.[13] Solid-state CD spectra are readily obtained by the KBr method, but there are some questions in the resulting CD spectra. Even if disks are clear, there may exist optical artifacts more or less by the effects of light scattering arising from optical fluctuation in disks. The counter ions of complex ions may be substituted by the matrix ion K$^+$ or Br$^-$ in disks; so essential informations may not be obtained.

On the other hand, there is no fear of the exchange of counter ions in Nujol mulls. A Nujol mull method will give good results in this connection. Another advantage of our Nujol mull method is that the isotropic solid-state CD spectrum can be obtained by easy procedures of experimentation and calculation.

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9) The scattering coefficient of a colored particle depends on the complex refractive index. When its absorption is weak, as is often the case with the d-d transition bands, the scattering coefficient is mainly governed by the real part of the complex refractive index. The variation of the real part should not be so large in the d-d absorption region because the normal dispersion of the real part is almost determined by far apart intense bands in the ultraviolet region. The anomalous dispersion should be small in the d-d absorption region, because the absorption in such a region is generally small. Therefore, we can suppose the scattering coefficient is not so changed in such a d-d absorption region.

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