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Differential Scatter of Left and Right Circularly Polarized Light by Optically Active Particulate Systems

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Abstract. Because of the many heterogeneous systems which are of interest to the chemist and biochemist, the problems of distortions in circular dichroism patterns have been investigated. Specifically in this communication it is shown with a relatively well-characterized particular system (a suspension of α-helical poly-L-glutamic acid) that there is a measurable differential scatter of left and right circularly polarized light by suspensions of the optically active particles. This is a specific example of Perrin's assertion in 1942 (Perrin, F., *J. Chem. Phys.*, **10**, 415 (1942)) that the polarization characteristics of scattered light would differ depending on whether or not the scattering particle was optically active.

Differential scatter is included with the concentration obscuring effects to demonstrate that distorted circular dichroism spectra on poly-L-glutamic acid suspension can be calculated with satisfying accuracy. The approach should be applicable to correcting the circular dichroism spectra for the many particles of biological interest, e.g., membranes, viruses, mitochondria, and insoluble proteins and polypeptides, and for small crystals in an effort to answer the crystal solution problem.

Many dissymmetric systems of interest to the chemist, and especially to the biochemist, are particulate. For the biochemist it is important to determine the structures of membranes by first assessing the conformation of the molecules within the membrane; it is important to know the conformation of molecules in viruses, and to assess the three-dimensional shapes of polymers in insoluble systems, such as β-structures and elastin. For the chemist as well as the biochemist it is significant to be able to utilize fully the data on the atomic coordinates of molecules in crystals. Absorption and circular dichroism data on suspensions of randomly-oriented crystals of micron or submicron dimensions would be most valuable if it could be obtained free of distortions. The above are but a few reasons why it would be of consequence to understand the nature of distortions which occur when studying heterogeneous systems and to be able to calculate and correct for such distortions.

We take the position here that the presence of artifacts in optical rotation spectra of heterogeneous systems has been amply demonstrated[1–4] and that the major distortions in spectra are due to concentration obscuring effects. The

obscuring of chromophores is derived from two sources: one is the absorption flattening of Duysens[5] wherein a particle casts a shadow and obscures the chromophores in the light path behind the particle; the second is the probability that a photon scattered from a particle would have been absorbed had the sample been molecularly dispersed. In the latter case the concentration of obscured chromophores is proportional to the product of the probabilities of absorption and scatter. Treating these as additive effects the relationship between a suspension and a correct ellipticity curve takes on the simple form[3, 4]

$$[\theta]_{susp} = [\theta]_{corr}(Q_A - A_S) = \frac{3300}{C_0 l}(A_L - A_R)(Q_A - A_S), \quad (1)$$

where Q_A is the flattening quotient of Duysens[5] and A_S is the absorption due to light scattering as measured by the phototube. It has been shown, when the ellipticity and absorption measurements are obtained simultaneously on the same phototube with a single beam-sample-phototube configuration, that the values of Q_A and A_S which are calculated from the experimental absorption curves are those required to calculate the distorted spectra.[4]

We wish here to add an interesting refinement to the above considerations which had been properly anticipated.[1, 3] In addition to the concentration obscuring effects there is a differential scatter of left and right circularly polarized light by an optically active particle. Equation (1) then becomes

$$[\theta]_{susp} = \frac{3300}{C_0 l}[Q_A(A_L - A_R) - (A_{SL}A_L - A_{SR}A_R) + (A_{SL} - A_{SR})], \quad (2)$$

where A_{SL} and A_{SR} are the measured absorptions due to light scattering for the left and right circularly polarized beams, respectively. When $A_{SL} = A_{SR}$ equation (2) reduces to equation (1). This situation is obtained at wavelengths where the optical rotatory dispersion of the particle is zero, i.e., where the indices of refraction of the particles for left and right circularly polarized light are equal. It is the purpose of this communication to demonstrate that, with the index of refraction of the poly-L-glutamic acid particle, equation (2) may be used to calculate the experimental curve for poly-L-glutamic acid suspensions. The agreement of calculated and experimental curves are almost within experimental error. In these calculations there are no parameters; one simply constructs the particle index of refraction, employs the Rayleigh-Gans or Mie dependence of scatter on particle and solvent refractive indices and utilizes the experimental values for the absorption due to light scattering.

As circular dichroism is a difference-absorption measurement, the information contained in the absorption curve obtained on the same phototube as the circular dichroism measurement can be most valuable. In the case of the polypeptides, poly-L-alanine and poly-L-glutamic acid, there is no appreciable absorption at wavelengths greater than 240 mμ. Any measured absorbance in this wavelength range for a pure sample is due to light scattering. The expression for the apparent absorption due to light scattering is

$$A_S = -0.434 \ln\left(\frac{I'}{I_0}\right), \tag{3}$$

where $I' = I_0 - I_s$ with I_s being the total intensity of scattered light. By observing the wavelength region outside of true absorption, one can assess the magnitude of A_S and get an estimate of its wavelength dependence. By utilizing the intersection point between the molecularly dispersed and suspension absorption curves, A_S can be approximated through the true absorption region.[4] This means that one need not assume the λ^{-4} wavelength dependence of the Rayleigh-Gans and Mie equations which can be seen as inadequate for the systems of interest here. Equation (3) may be written as

$$A_S(\lambda) = 0.434 \ln[1 - K'(\lambda)(n_p^2 - n_s^2)^2], \tag{4}$$

where the refractive index dependence of the Rayleigh-Gans expression has been used. n_p is the index of refraction of the particles and n_s is that of the solvent. Table 1 contains the experimentally derived values of Q_A, A_S, and K' as a function of wavelength.

TABLE 1. *Flattening quotients, Q_A, and absorption due to scatter, A_S, for a suspension of poly-L-glutamic acid.*

$\lambda(m\mu)$	Q_A*	A_S*	K'
240	0.992	0.159	0.231
238	0.992	0.169	0.234
236	0.981	0.165	0.220
234	0.981	0.183	0.230
232	0.972	0.180	0.218
230	0.965	0.176	0.206
228	0.948	0.182	0.205
226	0.939	0.185	0.201
224	0.914	0.172	0.184
222	0.900	0.176	0.181
220	0.883	0.177	0.176
218	0.873	0.184	0.174
216	0.858	0.189	0.169
214	0.841	0.178	0.152
212	0.813	0.187	0.148
210	0.774	0.190	0.144
208	0.732	0.188	0.141
206	0.694	0.192	0.147
204	0.664	0.188	0.154
202	0.641	0.177	0.159
200	0.612	0.173	0.171
198	0.571	0.186	0.199
196	0.530	0.186	0.220
194	0.497	0.200	0.270
192	0.471	0.206	0.340
190	0.463	0.203	0.446

* Values from the data of Urry, Hinners, and Masotti.[4]

Differential Scatter by an Optically Active Particle. In 1942 Perrin appreciated that the polarization characteristics of scattered light would differ depending on whether or not the particle was optically active.[6] Within our formalism the effect of the optically active particle on scattering left and right circularly polarized light may be written

$$A_{SL}(\lambda) = -0.434 \ln[1 - K'(\lambda)(n_{PL}^2 - n_s^2)^2], \quad (5)$$

$$A_{SR}(\lambda) = -0.434 \ln[1 - K'(\lambda)(n_{PR}^2 - n_s^2)^2], \quad (6)$$

$$n_{PL} = n_p + \frac{(n_L - n_R)_p}{2}, \quad (7)$$

$$n_{PR} = n_p - \frac{(n_L - n_R)_p}{2}, \quad (8)$$

$$(n_L - n_R)_p = \frac{[m]\lambda\rho_{PGA}}{18mw}, \quad (9)$$

where $[m]$ is the mean residue rotation for the α-helical polypeptide, ρ_{PGA} is the density of the poly-L-glutamic acid particles, λ is the wavelength in centimeters, and mw is the residue molecular weight. The differential scatter by the particle of the left and right circularly polarized beams will be treated by the monitoring system as a true difference absorbance. As such it will be recorded as an ellipticity. From equations (5)–(9) it is apparent that this distortion will resemble an optical rotatory dispersion curve. That such a distortion is contained in the circular dichroism spectra on suspensions has already been demonstrated.[4]

Index of Refraction of the Poly-L-glutamic Acid Particle. It would be best if it were possible to directly measure the index of refraction of the poly-L-glutamic acid particle. These values are not readily obtained, particularly in the absorption region of interest. Reasonable values can be obtained, however, by approximating the background contributions and by using the Kronig-Kramers transforms to add in the partial refractive indices of local bands. The index of refraction for the particle is written

$$n_p = 1 + n'(bkg) + n'(190)_{PLA} + n'(204)_{PLA} + n'(216)_{PLA}, \quad (10)$$

where

$$n'(bkg) = \rho'_{PGA}\left[\frac{n'(HOAc)}{\rho'(HOAc)} + \frac{n''(DMF)}{\rho'(DMF)}\right], \quad (11)$$

by ρ'_{PGA} is meant the density of the poly-L-glutamic acid particle divided by the mean residue molecular weight. Similarly $\rho'(HOAc)$ and $\rho'(DMF)$ are the densities for acetic acid and *N*,*N*-dimethyl-formamide divided by their respective molecular weights. The data for acetic acid and dimethyl-formamide were obtained from the International Critical Tables. The long wavelength values were fitted to the equation

$$n = 1 + \frac{a\lambda^2}{\lambda^2 - C} \quad (12)$$

by a least-squares method to give $n(HOAc)$ and $n(DMF)$. These quantities were related to those in equation (11) by the relations

$$n'(HOAc) = n(HOAc) - 1 \quad (13)$$

and

$$n''(DMF) = n(DMF) - 1 - n'(196)_{DMF}, \quad (14)$$

where the partial refractive index of the 196 mμ band of DMF was removed and the long wavelength values were again fitted to give $n''(DMF)$. The partial refractive indices due to the α-helix bands $n'(190)_{PLA}$, $n'(204)_{PLA}$, and $n'(216)_{PLA}$ were calculated using the Kronig-Kramers transforms[8, 9*] and the resolved data on poly-L-alanine.[7]

Calculation of the Suspension Ellipticity Curve and the Differential Scatter Curve. The density of the poly-L-glutamic acid particle has been extensively studied and well determined. Vinograd and Hearst,[11] using the density gradient technique, reported a value of 1.50 gm/ml. Ifft *et al.*[12] have followed the density as a function of pH in a thorough study. And in this laboratory we have examined the samples used in the present study and obtained a density of 1.50 gm/ml. Calculating from group molar volumes, this density indicates the absence of appreciable water in the particle.[13] With the density of the poly-L-glutamic acid particle one may use equation (9) to obtain $(n_L - n_R)_p$ and this value may be used in equations (7) and (8) to obtain n_{PL} and n_{PR}. These quantities are given in Table 2. The experimentally derived values for K' in connection with n_{PL}, n_{PR}, and n_s are all that are required to calculate A_{SL} and A_{SR}. The latter values are also included in Table 2.

TABLE 2. *Quantities for calculating the differential scatter of left and right circularly polarized light.*

λ(mμ)	$\frac{(n_L - n_R)_p}{2} \times 10^4$	n_{PL}	n_{PR}	n_s	A_{SL}	A_{SR}
240	−0.96	1.75177	1.75197	1.38462	0.158965	0.15919
238	−1.10	1.75881	1.75903	1.38583	0.168951	0.169223
236	−1.23	1.76654	1.76678	1.38709	0.164366	0.164656
234	−1.31	1.77471	1.77497	1.38838	0.182344	0.182689
232	−1.30	1.78286	1.78312	1.38972	0.179942	0.180274
230	−1.15	1.79061	1.79085	1.3911	0.17558	0.175861
228	−0.867	1.7979	1.79808	1.39253	0.181825	0.18043
226	−0.452	1.8049	1.805	1.394	0.185429	0.185544
224	0	1.81186	1.81186	1.39553	0.172048	0.172048
222	0.400	1.81899	1.81891	1.39711	0.176406	0.176313
220	0.836	1.82673	1.82657	1.39875	0.177082	0.176889
218	1.13	1.83585	1.83563	1.40045	0.18407	0.183799
216	1.43	1.84718	1.8469	1.40221	0.189003	0.188658
214	1.63	1.86064	1.86032	1.40403	0.178529	0.178171
212	1.87	1.87446	1.87408	1.40593	0.186721	0.186297
210	2.10	1.8852	1.88478	1.4079	0.190697	0.190215
208	2.91	1.88939	1.88881	1.40994	0.188498	0.187842
206	3.59	1.88527	1.88455	1.41207	0.191933	0.191097
204	4.04	1.87415	1.87335	1.41428	0.188578	0.187634
202	4.45	1.85949	1.85861	1.41659	0.177094	0.176099
200	4.72	1.8445	1.84356	1.41899	0.173146	0.172082
198	4.68	1.82981	1.82887	1.42149	0.186927	0.185726
196	3.53	1.81298	1.81228	1.4241	0.186025	0.185082
194	2.33	1.79049	1.79003	1.42683	0.200443	0.199718
192	0.768	1.76006	1.7599	1.42968	0.205698	0.205428
190	0	1.72285	1.72285	1.43265	0.203327	0.203327

As may be seen in Table 2 ($A_{SL} - A_{SR}$) at 234 mμ is 3.45×10^{-4}. The mean residue concentration in this study was 1.31×10^{-2} moles/liter and the path length was 2.18×10^{-2} cm. This gives 1.6×10^{7} for the coefficient, $3300/C_0 l$, in equation (2). Therefore the contribution of the differential scatter to the ellipticity at 234 mμ is 4×10^{3}. This is an appreciable magnitude. Relative to the significance of this effect in circular dichroism of membrane fragments, it should be noted that the values of A_S for the poly-L-glutamic acid suspension curve are considerably lower than those observed in membrane fragments.[2] The difference in mean residue ellipticity because of differential scatter, $\Delta[\theta]_{DS}$, which arises from the optically active α-helices in the poly-L-glutamic acid suspension is plotted in Figure 1. Note that $\Delta[\theta]_{DS}$ is of the form of an α-helix type

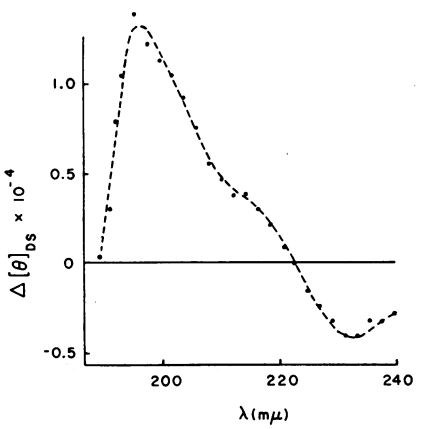

FIG. 1.—The calculated contribution of the differential scatter of left and right circularly polarized light, $\Delta[\theta]_{DS} = (3300/C_0 l)(A_{SL} - A_{SR})$.

optical rotatory dispersion curve. This is the main reason for the red shift[14–16] or broadening[17] observed in the circular dichroism membranes. It does not reflect helix-helix interaction or hydrophobic bonding within the membrane.

The calculated values of A_{SL} and A_{SR} are used in equation (2) together with Q_A of Table 1 to calculate the suspension curve. A_L and A_R are, of course, obtained from the molecularly dispersed reference state for α-helical poly-L-glutamic acid at pH 3.9 where the extent of aggregation is about ten helical rods.[18] Figure 2 contains the molecularly dispersed circular dichroism curve and the suspension curve which corresponds to the values of Q_A and A_S of Table 1. Plotted along with the experimental suspension curve is the calculated curve. The correspondence is satisfying. It should be emphasized that there are no parameters and that these values were directly calculated with terms which sum to give n_p. It should also be mentioned that small, reasonable adjustments in the components which comprise n_p would allow a perfect superposition of calculated and experimental suspension curves. The limitation, however, is not so much in the values for n_p, but in the experimental values for A_S. In the above calculations the Rayleigh-Gans refractive index dependence was used. If the Mie dependence[19] is used, i.e., $(n_p^2 - n_s^2)^2$ is replaced by $[(n_p^2 - n_s^2)/(n_p^2 + 2n_s^2)]^2$ in equations (5) and (6), a satisfactory fit is still obtained although it is not quite as good as that obtained using the Rayleigh-Gans expression.

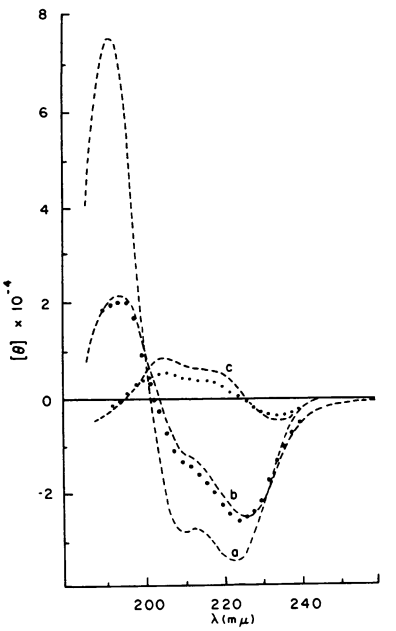

FIG. 2.—Calculation of the distorted suspension poly-L-glutamic acid ellipticity curve. Curves *a* and *b* are from the work of Urry, Hinners, and Masotti.[4] Curve *c* is the difference curve between values calculated neglecting differential scatter (i.e., with Eq. (1)) and the experimental suspension curve *b*. Curve *c* is the total effect of differential scatter. The plotted points near curves *b* and *c* are the calculated curves and demonstrate that even the features of the sensitive-difference curve *c* are well reproduced. See text for discussion.

The direct effect of differential scatter of left and right circularly polarized light can be seen by subtracting the curve calculated by equation (1) from the experimental curve. This difference is also included in Figure 2 along with the calculated curve which is the difference between equations (1) and (2). It is seen that the difference curve grossly resembles the optical rotatory dispersion curve for the α-helix. Comparison of what may be considered the experimental differential scatter of curve C and the calculated differential scatter curve shows that all of the features of this sensitive difference curve are reproduced. We take these results to indicate that in addition to the concentration-obscuring distortions in the circular dichroism patterns of suspensions there is a differential scatter of left and right circularly polarized light which is also included in the circular dichroism measurement. The latter is the major source of what has been referred to as the red shift[14-16] or broadening[17] in the circular dichroism spectra on membranes.

The above considerations must now be applied to the many optical rotation studies on particulate systems and films that have already appeared and to future studies.

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* A 13-term Fourier sine series was least squares fitted to numerical values[10] of the quantity $e^{-x^2}\int_0^x e^{y^2}dy$. See refs. 8 and 9 for definitions.

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