

## Circular Dichroism and the Bouguer-Lambert-Beer Law

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Due to the fact that within their spectral ranges of circular dichroism optically active solutes exhibit different extinction coefficients,  $\epsilon_l$  and  $\epsilon_d$ , for left and right circularly polarized light, their solutions can not theoretically obey the Bouguer-Lambert-Beer law when studied in a normal spectrophotometer. The absorption law for such solutions is derived in the form

$$D = \epsilon l c - \log \cosh \left( \frac{\ln 10}{2} \right) g \epsilon l c$$

which may be approximated by

$$D = \epsilon l c - 0.288 g^2 \cdot (\epsilon l c)^2$$

where  $\epsilon \equiv (\epsilon_l + \epsilon_d)/2$  and  $g \equiv (\epsilon_l - \epsilon_d)/\epsilon$ . The deviation from the B-L-B law, being second order in the dissymmetry factor,  $g$ , rarely exceeds 1% of the absorption. Its presence is demonstrated experimentally for (+)<sub>D</sub>-camphor and the (+)<sub>D</sub>-tris(ethylenediamine) cobalt(III) ion.

*Derivation of the absorption law for circularly dichroic substances.* It is common usage to represent experimental data concerning absorption spectra in terms of the molar, decadic extinction coefficient,\*  $\epsilon$ , (or its logarithm). The validity of this representation depends upon the validity of the combined form of Bouguer-Lambert's and Beer's laws

$$D \equiv \log (I_0/I) = \epsilon l c \quad (1)$$

where  $D$  is the optical density,  $I_0$  and  $I$  the intensities of the light beam before and after transmission through the light path,  $l$ , in a medium containing the absorbing substance in the molar concentration  $c$ .

Under experimental conditions one often finds "deviations" from Bouguer-Lambert's ( $D \propto l$ ) as well as Beer's ( $D \propto c$ ) law, but as is well known these apparent deviations may usually be explained by the failure of the experimental situation to correspond to the theoretical conditions for the derivation of the laws. Both laws are, e.g., contingent upon the use of monochromatic light, and

\* or its equivalent, the molar absorptivity.

polychromacy as well as chemical changes caused by concentration changes (*e.g.* the displacement of complex equilibria) are responsible for many of the "deviations from Beer's law" so abundant in literature.

In the following we wish to call attention to the fact, hitherto apparently unrecognized by most chemists, that theoretically the light absorption in circularly dichroic solutions can not be represented by equation (1).<sup>\*</sup> Optically active substances might therefore be expected to show the first "real" deviation from Bouguer-Lambert-Beer law. (Linearly dichroic media would under suitable circumstances follow an absorption law similar to that of circular dichroic media, but linear dichroism does not occur within the realm of the Bouguer-Lambert-Beer law, *i.e.* isotropic solutions).

Eqn (1) is the integrated form of

$$dI = -\epsilon c I dl \quad (2)$$

which is based on the assumption that the only property of the (monochromatic) light influencing its absorption be its intensity. A circularly dichroic substance, however, is characterized by having different extinction coefficients,  $\epsilon_l$ , and  $\epsilon_d$ , for left and right circularly polarized light. Thus  $dI$  in a circularly dichroic medium depends upon the state of polarization of the light in addition to its intensity. For such a medium eqn. (2) may be rewritten as

$$dI = dI_l + dI_d$$

$$dI_l = -\epsilon_l c_{\text{act}} I_l dl \quad (3)$$

$$dI_d = -\epsilon_d c_{\text{act}} I_d dl \quad (4)$$

where  $I_l$  stands for the intensity of the left circularly polarized component of the light and  $c_{\text{act}}$  is the concentration of the circularly dichroic substance, *i.e.* that enantiomer which has the extinction coefficient  $\epsilon_l$  for left circularly polarized light. (Subscript *d* indicates right circularly polarized light). The light in spectrophotometers is generally partially polarized but usually so that the left and right circularly polarized components are of equal intensities. Under this assumption the equivalent of eqn. (1) for circularly dichroic substances becomes

$$D_{\text{act}} \equiv \log(I_0/(I_l + I_d)) = \epsilon l c_{\text{act}} - \log \cosh \left( \frac{1}{2} (\ln 10) g \epsilon l c_{\text{act}} \right) \quad (5)$$

where  $\epsilon \equiv (\epsilon_l + \epsilon_d)/2$  and  $g \equiv (\epsilon_l - \epsilon_d)/\epsilon$ .  $g$  is the dissymmetry factor and, ideally,<sup>\*\*</sup>  $\epsilon$  is equal to the extinction coefficient of the racemic substance. For small values of  $g \epsilon l c_{\text{act}}$  the last term in (5) may be approximated by  $-0.288(g \epsilon l c_{\text{act}})^2$  and setting  $\epsilon l c_{\text{act}} = D_{\text{rac}}$  we obtain

$$D_{\text{act}} = D_{\text{rac}} - 0.288 g^2 D_{\text{rac}}^2 \quad (6)$$

<sup>\*</sup> In particular, this fact is not recorded in standard texts on spectrophotometry.<sup>1,2</sup> The only mention we have found in chemical literature is by Velluz, Legrand and Grosjean, whose book<sup>3</sup> appeared after the conclusion of the experimental work reported in the present paper. Those authors arrive at the same absorption law as that given in the present paper as eqn. (5).

<sup>\*\*</sup> Ideality in this context implies that the transition probability involved in the absorption be independent of whether the molecule under consideration is surrounded in solution by molecules identical to itself or by a mixture of these and their mirror-images. In cases where a partial racemate formation occurs in solution ideality can not be assumed, and one may find  $\epsilon_{\text{rac}} \neq (\epsilon_l + \epsilon_d)/2$ .

in which the last term represents the "deviation" from the Bouguer-Lambert-Beer law caused by the circular dichroism.

If the light in the spectrophotometer happens to be partially circularly polarized (*i.e.*  $I_1 \neq I_a \neq I_0/2$ ) the deviation becomes smaller. In particular, if pure circularly polarized light is used eqn. (3) or (4) will be valid, and no deviation from the Bouguer-Lambert-Beer law (eqn. (1)) will be found.

In this context it should be mentioned that if pure circularly polarized light is used the Bouguer-Lambert-Beer law will apply to racemic as well as to circularly dichroic substances. This non-equivalence between the two experimental situations, "racemic light" interacting with enantiomeric molecules and "enantiomeric light" interacting with racemic mixtures, may be easily verified by the proper application of eqns. (3) and (4), *e.g.* for a racemic substance in left circularly polarized light

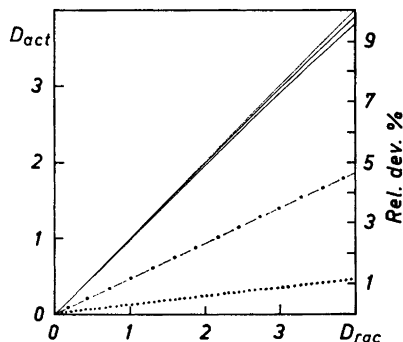
$$dI_1 = -\left[\varepsilon_1 \frac{c}{2} I_1 dl + \varepsilon_d \frac{c}{2} I_1 dl\right]$$

$$dI_1 = -\frac{\varepsilon_1 + \varepsilon_d}{2} c I_1 dl$$

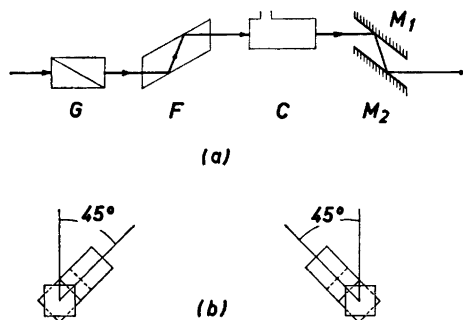
*i.e.*

$$D_1 (= D_d) = \varepsilon l c$$

where  $c$  is the total concentration of the racemic mixture, and  $\varepsilon_1$  and  $\varepsilon_d$  are the two extinction coefficients involved, these being by symmetry identical to those introduced above although here pertaining to the two different enantiomers (each present in the concentration  $c/2$ ).



*Fig. 1.* Relative deviation of the absorption of ordinary light (monochromatic, unpolarized or partially linearly polarized) by a circularly dichroic solution as a function of  $D_{rac}$  for the dissymmetry factors,  $g = 0.1$  (....) and  $g = 0.2$  (-.-.-). The absolute deviation may be judged by comparison of the three absorption curves (—) for  $g = 0$ ,  $g = 0.1$  and  $g = 0.2$ .



*Fig. 2.* CD attachment for double beam spectrophotometer. (a) Diagram of light path through linear polarizer (Glan prism, G), circular polarizer (Fresnel rhomb, F), sample cell (C) and parallel mirrors ( $M_1$  and  $M_2$ ) to bring the beam back to the ordinary light path of the spectrophotometer. The attachment consists of two such units, identical except for the relative orientation of the Fresnel rhomb and the Glan prism as viewed from the prisms in (b).

*Practical consequences.* The very considerable amount of circular dichroism data now available shows that the dissymmetry factor,  $g$ , rarely exceeds 0.1 for dissolved substances. The optical density of samples used for measuring absorption spectra may often be chosen with regard to the optimum range of the instrument to be used. In such cases it rarely exceeds unity and probably never exceeds 3.5. Even under these extreme conditions ( $g = 0.1$  and  $D = 3.5$ ) the correction term in eqn. (6), being equal to 0.035, only amounts to 1% of the total optical density. For most practical purposes the usual absorption law, eqn. (1), therefore represents a sufficient approximation. Fig. 1 shows the deviation as calculated from equation (6) as a function of  $D_{\text{rac}}$  for two selected values of  $g$ .

#### EXPERIMENTAL VERIFICATION OF THE DEVIATION

Spectrophotometric measurements were carried out at  $23 \pm 0.05^\circ\text{C}$  by means of a Cary Model 11 recording spectrophotometer using the ranges  $0 < D < 0.1$  and  $0.1 < D < 0.2$ .

Circular dichroism was measured by means of a Roussel-Jouan Dichrographe and by means of a CD attachment for the Cary spectrophotometer constructed by us.

The latter comprises two units, each consisting of a circular polarizer (Glan prism followed by Fresnel rhomb) as shown in Fig. 2. Inserted in the sample and reference beams of the spectrophotometer these units convert the light of the two beams to circularly polarized light of opposite rotations. When two identical samples of a circularly dichroic substance are introduced into the beams the spectrophotometer will record directly  $D_1 - D_2$ .

The calcite Glan prisms (aperture:  $15 \times 15$  mm, length: 20 mm; obtained from B. Halle Nachf., Berlin) permit the use of the wavelength range 800 nm (limit of Cary Model 11) to 228 nm (cut-off of prisms). The angle of the fused silica Fresnel rhombs (aperture:  $15 \times 15$  mm, length of long edge: 25 mm; made to order by Hilger & Watts, London) was  $v = 53.1^\circ$  which according to the relation between the index of refraction of the prism material,  $n$ , and the phase lag,  $2\varphi$ , produced by two consecutive total reflections

$$\tan \frac{\varphi}{2} = \frac{\cos v \sqrt{\sin^2 v - (1/n^2)}}{\sin^2 v}$$

results in the ellipticity-wavelength dependence indicated in Fig. 3. Although these rhombs will only produce exactly circularly polarized light (ellipticity =  $45^\circ$ ) at *ca.* 250 nm, the slight deviation in ellipticity ( $45^\circ \pm 3^\circ$ ) encountered within the wavelength range 800–200 nm only produces a very minor error in the CD measurements. As a simple calculation shows the maximum error caused by an ellipticity deviation of  $\pm 3^\circ$  from circularity gives rise to a relative error in the CD determination of  $-0.6\%$ , which is negligible in comparison with other sources of error.

The baseline of the CD recordings must be recorded at a total absorption similar to that prevailing during the CD recording itself. In the experiments reported below the baseline was obtained using two racemic samples of the same concentration as the active solution. (In cases where racemates are not available the samples could be introduced in the beam at points where it is not circularly polarized).

The recorder has been equipped with a special potentiometer slide-wire for the  $D$  ranges 0–0.1 and 0.1–0.2 so that the CD sensitivity is 0.1 absorbance unit per  $10^4$  (*ca.* 0.0004 abs. units per mm).

For our attempt at an experimental verification of eqn. (6) we selected the wavelengths indicated in Fig. 4 within two circularly dichroic absorption bands, *viz.* that of (+)-camphor at *ca.* 290 nm and that of (+)-tris(ethylenediamine)cobalt(III)bromide at *ca.* 465 nm. The CD was measured by means of the attachment described above as well as by means of the Dichrographe. The same samples were used in both measurements but whereas the Dichrographe values are read off a curve drawn during wavelength scanning, each Cary value represents the time average of a recording at fixed wavelength.

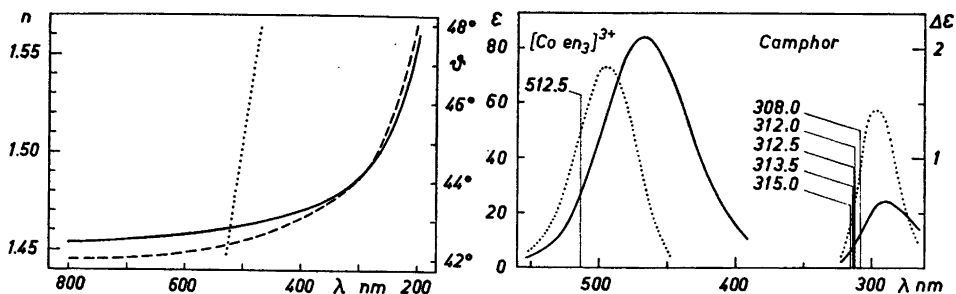


Fig. 3. Ellipticity,  $\theta$ , versus wavelength for fused silica Fresnel rhomb with angle  $53.1^\circ$  (---), index of refraction,  $n$ , for fused silica (—) as used in the calculation and, for comparison, the ellipticity produced by a quarter-wave plate for 500 nm (...).

Fig. 4.  $\epsilon$  (—) and  $\Delta\epsilon$  (...) for two optically active absorption bands of  $[\text{Co}(\text{en})_3]^{3+}$  (around 460 nm) and camphor (around 290 nm) with indication of the wavelengths at which the effect was studied.

The spectral slit width was smaller in the Cary measurements and this as well as a possible slight wavelength discrepancy between the two instruments may in view of the rather steep parts of the CD curves used be partially responsible for the deviation.

For comparison some of the CD data are presented in Table 1.

The experimental procedure aimed at the direct determination of the correction term

$$C = 0.288 (g/c_{\text{act}})^2 \quad (7)$$

In view of the relative minuteness of the effect we have refrained from varying the concentration of the samples as we felt that a deviation accompanying a concentration change would be far less convincing than a deviation caused by a variation in light path. The experiments therefore prove the presence of a deviation from the Bouguer-Lambert law only, the deviation from the Beer law being an obvious corollary of this.

The experimental determination of  $C$  was done by measuring the difference in optical density,  $\Delta D$ , between samples with equal light paths of a racemic and an active solution

Table 1. CD of (+)<sub>D</sub>-camphor (0.695 g/100 ml in 96 % ethanol) in 1 cm cell.

$\lambda$ nm	CD in absorbance units *		Relative deviation %
	Cary 11 with attachment (Av. of four measurements)	Dichrographe	
308.0	0.0401 $\pm$ 0.0006	0.0415	3
312.5	0.0255 $\pm$ 0.0005	0.0269	5
313.5	0.0222 $\pm$ 0.0005	0.0234	5
315.0	0.0183 $\pm$ 0.0006	0.0186	2

\* In the wavelength range under consideration  $d(\text{CD})/d\lambda$  is ca. 0.0033 abs. units per nm. Therefore differences in slit width and wavelength accuracy could produce relatively large discrepancies between instruments.

at two different light paths, *viz.* 1 cm and 10 cm. According to eqn. (5) these differences are \*

$$\Delta D_1 = \varepsilon_{\text{rac}} c_{\text{rac}} - \varepsilon_{\text{act}} c_{\text{act}} + 0.288 g^2 \varepsilon_{\text{act}}^2 c_{\text{act}}^2$$

$$\Delta D_{10} = \varepsilon_{\text{rac}} c_{\text{rac}} \times 10 - \varepsilon_{\text{act}} c_{\text{act}} \times 10 + 0.288 g^2 \varepsilon_{\text{act}}^2 c_{\text{act}}^2 \times 10^2$$

Multiplying the difference found for 1 cm light path,  $\Delta D_1$ , by ten and subtracting the result from  $\Delta D_{10}$  we obtain

$$\Delta D_{10} - 10\Delta D_1 = 0.9 (0.288 g^2 \varepsilon_{\text{act}}^2 c_{\text{act}}^2 \times 10^2)$$

whence the correction term  $C$  for the active solution in a 10 cm cell

$$C = (\Delta D_{10} - 10\Delta D_1) / 0.9 \quad (8)$$

In these experiments the following precautions against the occurrence of spurious  $\Delta D$  values were taken: The samples to be compared were introduced into the sample compartment only, (a 1 cm or 10 cm sample of the racemic solution being introduced into the standard compartment in order to compensate the absorption of the samples to be compared). The same 1 cm and 10 cm cells were used for the racemic and active solutions to be compared. The slit width was controlled manually so that comparisons between 1 cm and 10 cm samples could be made at identical slit width. (This may require different settings of the dynode voltage for measuring the 1 cm and 10 cm samples). The wavelength setting was left untouched during each set of measurements.

Obtaining one set of  $\Delta D$  values involved the following procedure:

1° Two 1 cm samples of the racemic solution were placed in the sample and standard compartments, respectively, and the pen of the recorder balanced at a convenient point of the scale,  $D_{\text{app},1}$ .

2° The sample in the cell in the sample compartment was replaced by a circularly dichroic solution of approximately the same concentration. New pen position:  $D_{\text{app},2}$ .  $\Delta D_1 = D_{\text{app},1} - D_{\text{app},2}$ .

3° Two 10 cm samples of the racemic solution were placed in the two compartments. Arbitrarily balanced pen position:  $D_{\text{app},3}$ .

4° The sample in the sample compartment was replaced by the same active solution as used under 2°. New pen position:  $D_{\text{app},4}$ .  $\Delta D_{10} = D_{\text{app},3} - D_{\text{app},4}$ .

Besides the experiments thus conducted for the purpose of determining the correction term,  $C$ , for dichroic solutions, a separate series of experiments was conducted in order to determine the precision of the method (in terms of the standard deviation,  $s$ , of the quantities determined in replicate experiments) and, in particular, to ascertain that within experimental accuracy  $C = 0$  for a racemic solution as a consequence of the validity of the Bouguer-Lambert-Beer law for such solutions.

Details of an experiment of the latter type are shown in Table 2. Solutions Nos. 1 and 2 in this type of experiment are racemic solutions of different concentrations of which the latter takes the place of the active solution in the four step experimental procedure described above. Before each measurement the sample cell was emptied, refilled and wiped clean.

The experimental evidence for the predicted deviation is reviewed in Table 3. The experimental  $C$  values presented were calculated from the  $\Delta D$  values shown by means of eqn. (8). The calculated  $C$  values were obtained from eqn. (7) by insertion of the  $g$  factors calculated from the CD of the active solutions measured by means of the Dichrographe and our CD attachment and the absorption spectra of the same solutions.

\* In the three following equations  $\varepsilon_{\text{rac}}$  indicates the extinction coefficient of the racemic solution whereas  $\varepsilon_{\text{act}}$  stands for  $(\varepsilon_1 + \varepsilon_d)/2$ . These may be equal or different depending upon whether the system is ideal in the sense of the footnote on page 1146. The distinction is made here in order to show that the validity of the method is independent on assumptions concerning ideality.

Table 2. Details of an experiment with two solutions of racemic camphor at  $\lambda = 310.0$  nm.

Light path	1 cm		10 cm	
	1	2	1	2
Sol. No.				
Apparent optical density *	2.91	3.89	2.7	12.8
$D_{app} \times 10^2$	2.78	3.99	2.6	12.7
	3.05	4.01	2.6	12.6
	3.05	3.95	2.6	12.6
	3.00	3.92	2.7	12.5
	3.02	4.07	2.5	12.7
	3.04	4.06		12.6
		3.95		
Av. of $D_{app}$	0.0298	0.0398	0.0262	0.1264
$\pm s$	$\pm 0.0004$	$\pm 0.0002$	$\pm 0.0003$	$\pm 0.0004$
$\Delta D$		0.0100		0.1002
$\pm s$		$\pm 0.0004$		$\pm 0.0005$
$(\Delta D_{10} - 10\Delta D_1)/0.9$			0.0002	
$\pm s$			$\pm 0.004$	

\* The true optical density (0.3 and 3 in 1 cm and 10 cm cells, respectively) was essentially compensated by a sample of solution No. 1 in the reference beam.

Table 3.

	$\lambda$ nm	Approx. opt. density of solutions in 1 cm cells	$\Delta D$		$C_{10 \text{ cm}}$	
			1 cm	10 cm	expt.	calc.
Standard deviation			$\sim 5 \times 10^{-4}$	$\sim 5 \times 10^{-4}$	$\sim 6 \times 10^{-3}$	$\sim 10\%$ rel.
(+)D-camphor	308.0	0.673	0.0014	0.0485	0.038	0.050
	312.0	0.395	-0.0188	-0.1663	0.024	0.018
	312.5	0.410	-0.0117	-0.0799	0.041	0.020
	313.5	0.357	-0.0141	-0.1182	0.025	0.016
	315.0	0.287	-0.0186	-0.1690	0.019	0.009
(+)D-[Co en <sub>3</sub> ] <sup>3+</sup>	512.5	0.383	0.0025	0.0300	0.006	0.006

## DISCUSSION

The main experimental result is that the  $C$  values have the expected sign, are of the expected order of magnitude, and are sufficiently large in comparison to their standard deviations to annihilate any doubt as to whether they be real.

A comparison between experimental and calculated  $C$  values shows that the former tend to be higher by somewhat more than one standard deviation. (In the only example of a substantial difference of the opposite sign the optical density of the solutions in the 10 cm cells had the unusually high value of *ca.* 6.7 so that even in the Cary spectrophotometer stray light may have

played a role). When judging the significance of this discrepancy one should keep in mind *pro primo* that CD is still difficult to measure accurately and *pro secundo* that the relative error in the  $g$  factor is doubled in the calculated  $C$  value due to the quadratic nature of the  $C$  term (equation (7)).

One possible source of error in the  $\Delta D$  measurements would be a dependence of the photomultiplier response upon the direction of polarization of the light incident on the photocathode. The light from the monochromator is partially polarized, and since the rotation of the 10 cm samples amounts to a few degrees such errors might be anticipated. Table 4 contains data from two experiments designed to check this possibility. The four step experimental procedure outlined above was again applied but pure water took the place of the racemic solution while the circularly dichroic solution was replaced by *ca.* 3.5 % (+)<sub>D</sub>-sucrose solutions possessing a rotation at the wavelength under consideration comparable to that of the formerly used circularly dichroic solutions (*ca.* + 3.2° at 10 cm light path) but void of their CD. In the first experiment a pure sucrose solution of colourless appearance was used; in the second experiment it was coloured by a content of racemic [Coen<sub>3</sub>]<sup>3+</sup>. On the basis of the results obtained the effect of the rotation of the sample can hardly be deemed significant.

#### CONCLUSION

Experiments have shown that circularly dichroic substances exhibit a deviation from the Bouguer-Lambert law and, therefore, from the combined Bouguer-Lambert-Beer law. In most practical cases the deviation does not exceed 1 % of the optical density.

The agreement between the deviation found and that calculated from CD data — although not entirely satisfactory in view of the apparent precision of the two methods — probably warrants the use of the expression derived (eqns. (5) and (6)) for the calculation of the light absorption of a circularly dichroic substance.

Consequently, both  $\varepsilon$  ( $\equiv(\varepsilon_1 + \varepsilon_a)/2$ ) and  $g$  ( $\equiv(\varepsilon_1 - \varepsilon_a)/\varepsilon$ ) are necessary for the description of the light absorption of a solution of a circularly dichroic substance, and the knowledge of these quantities permits the description of the absorption of light in any state of polarization.

Table 4. Effect of rotation without circular dichroism at  $\lambda = 512.5$  nm.

	Opt. density of active sol. in 1 cm cell	Opt. rotation of sol. in 10 cm cell	$\Delta D$		$C_{10\text{ cm}}$	
			1 cm	10 cm	expt.	theor.
(+) <sub>D</sub> -sucrose versus H <sub>2</sub> O colourless	0.001 <sub>s</sub>	3.2°	0.001 <sub>s</sub>	0.0202	0.002 ± 0.006	zero
(+) <sub>D</sub> -sucrose versus H <sub>2</sub> O coloured	0.016 <sub>s</sub>	3.2°	0.016 <sub>s</sub>	0.1580	-0.009 ± 0.006	zero



The “ $\varepsilon$ ” ( $\equiv D/(l \times c)$ ) where  $D$  is measured in an ordinary spectrophotometer) which appears in published absorption spectra of circularly dichroic substances is, strictly speaking, not a constant characteristic of the substance but a function of the  $l$  and  $c$  used in the experimental determination of  $D$ . As shown above the “ $\varepsilon$ ” determined in normal spectrophotometry usually represents a good numerical approximation to  $\varepsilon$ , but “ $\varepsilon$ ” in itself becomes a physically meaningful quantity only when accompanied by the pertinent values of  $l$  and  $c$ .

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#### REFERENCES

1. Kortüm, G. *Kolorimetrie, Photometrie und Spektrometrie*, Springer, Berlin 1955.
2. Meehan, E. T. *Optical Methods of Analysis*, Interscience, N. Y. 1964.
3. Velluz, L., Legrand, M. and Grosjean, M. *Optical Circular Dichroism*, Academic, N. Y. 1965, p. 25.

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