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A CRITICAL STUDY ON CIRCULAR DICHROISM MEASUREMENT IN LONGER SIDE OF VISIBLE REGION

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A test sample method revealed large instrumental deviations of molar ellipticities, [θ], up to ±35% in circular dichroism(CD) in the 500-800 nm region, even after (+)-10-camphorsulfonic acid(CSA) calibration at 290 nm. Use of the nickel(II)tartrate solution and the tentative [θ] values, -101.2 at 718 nm and -110.7 at 777 nm(degree $dm^3 \cdot mol^{-1} cm^{-1}$), were recommended to calibrate the wavelengths and magnitudes of the CD extrema in this region.

Although molar ellipticity, [θ], is one of the most useful physical constant for optically active compounds, calibration on the magnitudes of circular dichroism (CD) has not been well established.1) Previously a critical study was made for the CD magnitudes in three wavelength regions using (−)-pantolactone(222 nm), (+)-10-camphorsulfonic acid(CSA, 290 nm), and (+)-tris(ethylenediamine)cobalt(III)iodide monohydrate(490 nm) as CD standards.2) This study revealed that the CSA calibration at 290 nm was not sufficient at 222 nm and 490 nm. Recently CD measurements have been extended to 1000 nm. However, the reported CD magnitudes sometimes gave large deviations from our traced values in spite of careful CSA calibrations. In this report a critical study was made on the CD measurements in the wavelength region of 500-800 nm by a test sample method. An aqueous solution of nickel(II)tartrate was used for the purpose, since the solution gives CD extrema around 720 nm and 780 nm. The suitability of the standard was discussed.

The nickel(II)tartrate solution was prepared by mixing the equal volumes of the aqueous solutions A and B prior to the CD measurements: (A) nickel(II)sulfate (0.24 mol dm^{-3}) and (B) potassium sodium-(+)-tartrate(Rochelle salt, 0.36 mol·dm^{-3}). After the solutions were subdivided and sealed into brown test tubes, the test sample solutions were mailed to the laboratories in Japan, the United States, France, Italy, and England. Standard CSA solution was also sent to check the CD magnitudes of the instrument at 290 nm.3) The CD instruments were 10 JASCO, 3 CARY, and 3 JOBIN YVON ones. The sealed test sample solutions were stable during the period of the survey. All the CD spectra were measured in the same manner in the experimental section.

The molar ellipticities observed by the contributors, after the CSA calibra-

Table The [Θ] values and wavelengths of the CD extrema observed for the nickel(II)tartrate solution after the CSA calibration at 290 nm.

Instruments	CSA[2,3)]	nickel(II)tartrate, [Θ]/degree $dm^3 mol^{-1} cm^{-1}$ (nm)*					
A	+7780(290)	+48.5(371)	-47.3(400)	+34.3(428)	+8.0(472)	-99.1(718)	-108.3(777)
B	+7780(290)	+50.7(371)	-46.7(399)	+36.0(428)	+8.5(470)	-100.9(721)	-109.8(779)
C	+7780(290)	+51.5(372)	-47.9(400)	+36.6(428)	+7.9(470)	-101.8(718)	-111.4(777)
D	+7780(290)	+50.0(372)	-46.7(402)	+35.7(429)	+8.3(472)	-100.0(719)	-112.4(776)
E	+7780(290)	+50.7(372)	-44.9(399)	+36.5(427)	+8.8(470)	-104.2(720)	-111.7(780)
F	+7780(290)	+37.1(371)	-35.1(399)	+26.4(428)	+6.4(470)	-	-
G	+7780(290)	+48.7(372)	-46.1(400)	+33.7(428)	+7.7(472)	-83.2(730)	-93.9(780)
H	+7780(290)	+54.1(370)	-49.7(398)	+40.1(426)	+9.6(469)	-	-
I	+7780(290)	+50.5(366)	-43.7(392)	+35.7(421)	+9.4(460)	-95.7(702)	-103.4(758)
J	+7780(290)	+47.3(399)	-43.7(399)	+33.2(428)	+7.6(270)	-	-
K	+7780(291)	+50.1(370)	-47.4(400)	+35.4(429)	+8.8(473)	-93.0(729)	-101.3(793)
L	+7780(290)	+50.5(372)	-46.5(399)	+35.6(428)	+8.3(470)	-94.6(702)	-104.2(757)
M	+7780(290)	+53.4(369)	-53.3(398)	+23.4(428)	+8.4(470)	-109.4(700)	-121.7(753)
N	+7780(290)	+50.6(370)	-47.8(400)	+36.1(428)	+8.3(470)	-	-
O	+7780(290)	+47.2(369)	-40.7(398)	+36.3(426)	+10.0(470)	-	-
P	+7780(290)	+41.1(370)	-36.4(400)	+26.3(427)	+5.7(470)	-44.9(718)	-45.8(777)
Mean	+7780	+48.88	-45.24	+33.83	+8.23	-93.35	-102.17
SD		4.16	4.50	4.36	1.05	16.62	19.09
D%		±16.68%	±19.50%	±25.26%	±25.00%	±34.89%	±36.62%

*Wavelengths in nm are described in the parentheses. SD: standard deviation
D% = ±(1.96 x SD)100/Mean, Temperature: 16.5-25 °C.

tion, are shown in the Table. The significant results are summerized as follows.

(1) The deviations of molar ellipticity became considerably large with increasing the wavelengths from 400 nm to 800 nm. For the CD extrema at 718 nm and 777 nm, the deviations of the [Θ] values reached to ±35% and ±37% of the mean at 95% reliability. In an extreme case the [Θ] values of the instrument M were about three times larger than those of the instrument P.

(2) The five instruments(G, I, K, L, and M) showed large shifts of the CD extrema at 718 nm and 777 nm. For example, the instrument G gave a red shift of 12 nm and 3 nm, and the instrument M gave a blue shift of 18 nm and 24 nm for the extrema at 718 nm and 777 nm.

Here the wavelengths of CD extrema of the nickel(II)tartrate solution were calibrated with a standard filter composed of neodymium glass, referring the absorption to the HT-voltage and/or the DC-current of the instruments C and L. Furthermore, the absorption maxima of the neodymium glass filter were calibrated in the wavelengths with the line spectrum of D_2-lamp(at 656.10 nm and 486.00 nm) on a HITACHI MODEL 200-10 SPECTROPHOTOMETER having a grating. Corrected wavelengths of the CD extrema of the nickel(II)tartrate solution were 371, 399, 428, 470, 718, and 777 nm.

These results show that the CSA calibration at 290 nm has not been sufficient for the CD observation in the 500-800 nm region and the use of a standard solution should be necessary for the CD measurements in this region. For this purpose we propose to use the nickel(II)tartrate solution as a standard for the CD observation in this wavelength region.

In order to get the tentative [θ] values, mean values of the data made on the five instruments(A, B, C, D, and E) were calculated. The calculation gave +50.3 (at 371 nm), -46.7(399 nm), +35.8(428 nm), +8.3(470 nm), -101.2(718 nm), and -110.7(777 nm) for the CD magnitudes at each extrema respectively. On getting the mean values, other 11 instruments were eliminated because of the following reasons. (a) The instruments in which the CD data were not measured in the 500-800 nm region(F, H, J, N, and O). (b) The instruments whose molar ellipticities were out of 95% reliability(F, M, and P). (c) The instruments which gave large wavelength errors up to 4 nm in the 700-800 nm region(G, I, K, L, and M). Therefore, the above mean values might be regarded as absolute values. They should be regarded as the most probable mean values in the literatures measured on the present instruments. However, if all the [θ] values are calibrated with the standard solution, the present confusion on the CD magnitudes can be minimized. The values will be easily re-calibrated when the absolute value will be established in future. In addition, it is very important to use the commercially available substances like nickel(II)tartrate in this region as a CD standard.

In conclusion, we recommend to use the tentative [θ] values of the nickel(II)-tartrate solution, -101.2 at 718 nm and -110.7 at 777 nm(degree $dm^3mol^{-1}cm^{-1}$), as a CD standard. All authors should describe in their papers that these [θ] values for the CD spectra in the 500-800 nm region have been calibrated on the bases of the Konno's procedure recommended here.

Experimentals: the preparation of the nickel(II)tartrate solution and CD measurement are as follows.

(1) Preparation of solution A: nickel(II)sulfate·$6H_2O$ (MW=262.87) 6.309g/100 ml of redistilled water(a reagent grade, from Yoneyama Chemical Ind. Ltd.). Although nickel(II)sulfate·$6H_2O$ is slightly hygroscopic, water uptake during weighing was negligibly small. Nickel(II) concentration of the commercial reagent is more than 99.5%, but concentration determined by the titration method using Cu-PAN as a indicator gave 99.0%. Therefore, nickel(II) concentration of the solution A was normalized to 0.2376 mol dm^{-3}.

(2) Preparation of solution B: potassium sodium-(+)-tartrate·$4H_2O$(Rochelle salt, MW=282.22) 10.160g/100 ml of redistilled water(a reagent grade, from Wako Pure Chemical Ind. Ltd.). This reagent is nonhygroscopic.

(3) Preparation of the nickel(II)tartrate solution: the equal volumes of the solutions A and B were mixed prior to CD measurements. The concentration of the nickel(II) in this solution is 0.1188 mol dm^{-3}. The solution can be checked by the absorbances of 1.45(at 399 nm), 0.647(666 nm), and 0.600(726 nm). The pH of

the nickel(II)tartrate solution was 5.36. The solutions A and B are stable at least for three months at room temperature, when they were sealed into brown test tubes. The mixed solution was also stable for three months under the same conditions.

(4) CD measurement: CD spectra were measured with a wavelength expansion of 10 nm/cm. Quartz cells of 1 cm were used in order to minimize errors due to the cell length. Molar ellipticities of the nickel(II)tartrate solution were calculated on the bases of the nickel(II) concentration after the mixing.

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