Vacuum-Ultraviolet Circular Dichroism Spectrophotometer Using Synchrotron Radiation: Optical System and Off-line Performance

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With the aim of measuring the circular dichroism (CD) spectra of biomaterials in aqueous solutions in the vacuum ultraviolet region of 310–140 nm, the optical and sample cell system endurable under high vacuum was first constructed to be incorporated into the synchrotron radiation beam line as the light source. The performance of the present system was confirmed to be satisfactory in the high vacuum off-line experiment by monitoring the CD spectra of ammonium *d*-camphor-10-sulfonate aqueous solutions.

Circular dichroism (CD) spectroscopy is powerful for analyzing the structure of optically active materials such as biopolymers. However, no commercial-type CD spectrophotometer is capable of measuring the CD in the vacuum ultraviolet (VUV) region below 190 nm because of some technical difficulties involved in the light source, optical device, and sample cell. The extension of CD measurements into the VUV region can provide more detailed and new information on the structure of biopolymers based on the higher energy transition of chromophores such as hydroxyl and acetal groups. A great deal of efforts has been made to construct the vacuum ultraviolet circular dichroism (VUVCD) spectrophotometer.[1–4] A breakthrough for the instrumentation occurred in the 1980's by using synchrotron radiation (SR) as an intense light source.[5–8] However, the short wavelength limit is so far about 170 nm in aqueous solution and such a VUVCD spectrophotometer does not come into wide use, because it requires the vacuum-proof special devices and a big facility to generate SR.

We aim at constructing a VUVCD spectrophotometer to measure the CD spectra of biomaterials in aqueous solutions in the 310–140 nm wavelength region under high vacuum, using a small-scale SR source (HiSOR) at Hiroshima Synchrotron Radiation Center. This paper reports the construction of the optical system and the sample cell to be incorporated into the SR beam line. The satisfactory performance of these units was proved by the off-line CD measurements of ammonium *d*-camphor-10-sulfonate.

Figure 1 shows the block diagram of VUVCD spectrophotometer. All optical devices and the sample cell are set up in two separate vacuum chambers, i.e., polarization modulation chamber and sample chamber, in order to avoid the absorption of light by air and water vapor. The sample chamber is separated by gate valve (GV) from the polarization modulation chamber, so that the sample can be exchanged, while the modulation chamber is kept under high vacuum. Both chambers can be degassed to 2×10^{-8} Torr with a Varian turbo molecular pomp V-70LP.

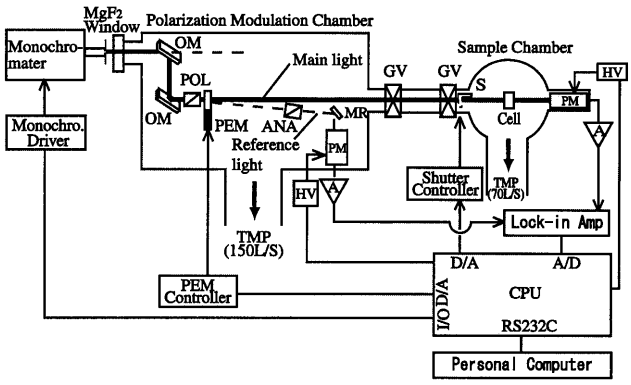

Figure 1. A block diagram of VUVCD spectrophotometer. Abbreviations indicate the following optical devices: OM, offset mirror; POL, polarizer; PEM, photoelastic modulator; ANA, analyzer; MR, mirror; PM, photomultiplier; S, shutter; HV, high voltage supply; A, preamplifier; GV, gate valve.

In the off-line experiments, in place of SR, an Osram X130-150W/4 xenon lamp was used as a light source in combination with a d.c. power supply (1.5 A). A JASCO J700 spectropolarimeter was used as the monochromator. The incident light passing through the MgF_2 window is led to the center of polarization modulation chamber by two offset mirrors (OM). The incident light can be removed from the light pass by rotating the first mirror, whenever it is needed for other experiments. The light reflected by the second offset mirror is separated into two orthogonal linearly polarized light beams by a Karl Lambrecht magnesium fluoride Rochon prism (POL). The separation angle between two light beams is 5.1° at 200 nm and 5.4° at 140 nm. Both linearly polarized light beams are modulated to circularly polarized light at 50 kHz by a JASCO LiF photo-elastic modulator (PEM). The main light beam in the center of the chamber is led to the sample cell and the CD signal is detected using a Hamamatsu R6836 photomultiplier tube (PM), which is covered by a MgF_2 window. The a.c. signals accumulated by preamplifier (A) are rectified, amplified by the dual lock-in amplifier (JASCO), and finally recorded on a personal computer. Another light beam is used as the reference signal to synchronize the polarization modulation. The offset signal caused by the strain birefringence of MgF_2 window on the PM can be minimized by rotating the PM with a differential pumping rotational feed-through apparatus (RMTG-450, MDC Co.). In order to protect the sample from the damage by VUV light irradiation, we attached the shutter which opens automatically only during the accumulation of signals.

In order to control PEM accurately and to stabilize the lock-in amplifier under high vacuum, we used the optical servo-control system.[9] In VUV region, driving voltage for PEM should be lower than linearly extrapolated one because of refractive index dispersion in PEM. Therefore, it is difficult to obtain optimal driving voltages by electric method in the region. This system realizes achromatic modulation and compensation for the thermal drift of PEM by use of double beam configuration.

The block diagram of a sample cell is shown in Figure 2. The cell consists of a stainless steel container with a cylindrical screw and two MgF_2 windows of 20-mm diameter and 1-mm thickness (Ohyo Koken, Co.). The *c*-axis cut MgF_2 disc was used to eliminate the birefringence of the windows. The optical path length can be adjusted within an accuracy of 1 μm with a doughnut-shaped aluminum spacer. The sample solution in the cell is sealed with six rubber O-rings that can be pressed uniformly by the cylindrical stainless screw.

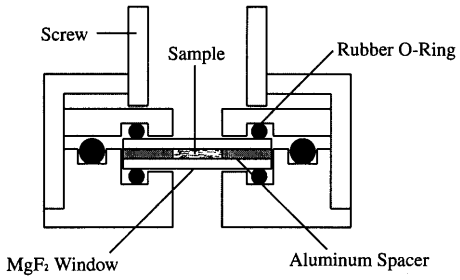

Figure 2. A block diagram of sample cell.

The performance of the present VUVCD spectrophotometer constructed was tested by monitoring the CD spectra of ammonium *d*-camphor-10-sulfonate (ACS), purchased from Katayama Chemical Co. The lamp house and the monochromator were purged with nitrogen gas at a flow rate of 10 L/min. The polarization modulation chamber and the sample chamber were kept under vacuum better than 1×10^{-5} Torr during the CD measurements. Figure 3 shows the CD spectra of 100 mM ACS aqueous solutions at 25 °C, which were measured with the MgF_2 sample cell of 20-μm path length. The spectrum obtained by the presently constructed VUVCD apparatus is completely superimposed upon the spectra observed by a commercial JASCO J720 spectropolarimeter. The characteristic peaks were observed at 291 and 192 nm with the intensity ratio of 1:2 as expected for the normal operation of the instrument.[10] No change was found in the spectrum after the sample cell was held under vacuum for 10 h, indicating no leakage of the sample solution. The CD spectrum was reproducible within 5% when the spacer and solution were exchanged. The inset of Figure 3 shows plots of the ellipticity at 291 nm as a function of ACS concentration. The experimentally observed ellipticity is in good agreement with the calibration line calculated with the molar ellipticity of 7910 deg·cm^2·dmol^{-1} at 291 nm[10] in the 0–400 mM range of ACS concentration. These results indicate that the MgF_2 windows are free from the strain birefringence and that the path length remains constant under high vacuum.

In this preliminary report, we showed a successful construction of the optical system and the sample cell, which can

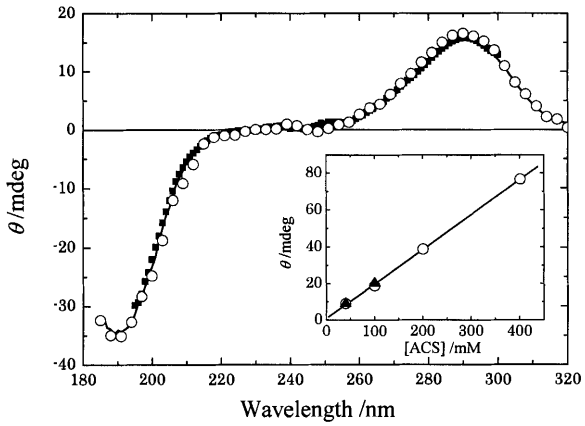

Figure 3. CD spectra of 100 mM ACS aqueous solutions at 25 °C. Open circle indicates the spectrum measured under vacuum using the optical device and the MgF_2 sample cell. Solid line and filled square show the spectra measured by a JASCO J720 spectropolarimeter before and after the sample cell was kept under vacuum for 10 h, respectively. The inset shows the ACS concentration dependence of θ_{291}. Open circle and filled triangle indicate the θ_{291} and the one-half of absolute value of θ_{192}, respectively. Solid line is calculated with the molar ellipticity, 7910 deg·cm^2·dmol^{-1} at 291 nm.[10]

be operated normally under high vacuum as well as at the atmospheric condition. They should also be utilized for the CD measurements in the VUV region below 190 nm, when the intense light source such as SR and the vacuum-proof monochromator are available. Recently, we have connected these CD units with a 1-m normal incident grating monochromator on the beam line BL15 of Hiroshima Synchrotron Radiation Center (HiSOR). The preliminary on-line experiment has confirmed that the VUVCD spectrum of ACS can be measured in aqueous solutions in the VUV region down to 140 nm.[11]

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