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

Noriyuki Ojima,[†,††] Kenichi Sakai,[†††] Koichi Matsuo,[†††] Takashi Matsui,[†] Tomoyuki Fukazawa,[††††]
Hirofumi Namatame,[†] Masaki Taniguchi,[†] and Kunihiko Gekko*[†,†††]
[†]*Hiroshima Synchrotron Radiation Center, Hiroshima University, Higashi-Hiroshima 739-8526*
[††]*Institute of Pharmaceutical Sciences, Faculty of Medicine, Hiroshima University, Hiroshima 734-8551*
[†††]*Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Higashi-Hiroshima 739-8526*
[††††]*Spectroscopic Instruments Division, JASCO Corporation, Tokyo 192-8537*

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With the aim of measuring the circular dichroism (CD) spectra of biomaterials in aqueous solutions in the vacuum ultraviolet region between 310 and 140 nm, an optical system withstanding high vacuum was incorporated into the beam line 15 of Hiroshima Synchrotron Radiation Center (HiSOR). The performance of the constructed system was confirmed to be satisfactory in the high-vacuum on-line experiments by monitoring the CD spectra of ammonium *d*-camphor-10-sulfonate, D- and L-alanines in water.

Circular dichroism (CD) spectroscopy is powerful for analyzing the structure of optically active materials such as biopolymers. However, no commercial CD spectrophotometer is capable of measuring the CD in the vacuum ultraviolet (VUV) region below 190 nm because of technical difficulties involved in the light source, optical device, and sample cell. CD measurements extended to 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 hydroxy and acetal groups. In the 1980's, a breakthrough in CD spectroscopy instrumentation was realized by using synchrotron radiation (SR) as an intense light source.[1–4] Since then, however, the short wavelength limit has only improved to approximately 170 nm in aqueous solution and as a result the VUVCD spectrophotometer has not become widely used. According to the recent reports of Wallace,[5,6] two new facilities having the short wavelength limit of 160 nm are expected to come on-line within a year; one at the Synchrotron Radiation Source (Daresbury, UK) and another at the Aarhus Storage Ring (Aarhus, Denmark).

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 (0.7 GeV) at Hiroshima Synchrotron Radiation Center (HiSOR). We have constructed the optical devices and the sample cell endurable under high vacuum, which were proved to be satisfactory by the off-line CD measurements.[7] This optical system was incorporated into the beam line 15 at HiSOR. This paper reports the good performance of the constructed VUVCD spectrophotometer in the high vacuum on-line experiments by monitoring the CD spectra of ammonium *d*-camphor-10-sulfonate, D- and L-alanines in water.

Figure 1 shows schematically the layout of the beam line 15 at HiSOR. The beam line is composed of four mirrors (M0, M1, M2, and M3) and a McPherson's Model 225M2 1-m normal incidence monochromator (NIM) which consists of an entrance slit S1, two interchangeable concave gratings coated with Pt (1200 lines/mm) and MgF_2/Al (1200 lines/mm), and an exit slit S2. The distance between the grating and two slits is 1000 mm and the angle between the incoming and outgoing beams is 15°. The grating rotates along the bisector of the beam angle, keeping the monochromated beam focused onto S2. The synchrotron radiation beam from the bending magnet is first reflected by a cylindrical mirror M0 (quasi-toroidal mirror), and then focused onto S1, by the combination of a toroidal mirror M1 and a spherical mirror M2. In order to minimize the aberration on S1, the beam reflected by M1 is horizontally focused in front of M2. The available photon energy region at the NIM beam line is from 4 to 40 eV, corresponding to 310 to 31 nm. The resolution of the monochromator is 0.2 Å. Two focal points are available; a 3 mm ϕ point focus and a 0.2 × 4 mm line focus at 2000 and 2500 mm from a toroidal mirror M3, respectively.

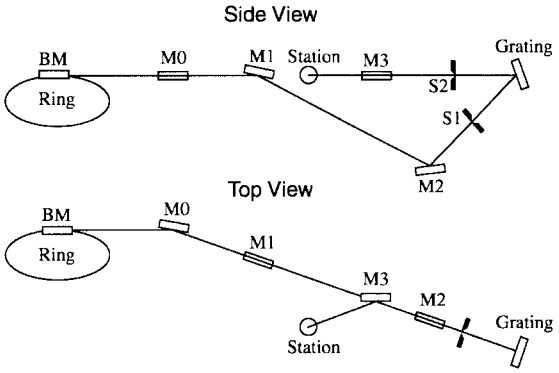

Figure 1. Optical layout of the beam line BL15 at HiSOR. Abbreviations BM, M, and S represent the bending magnet, mirror, and slit, respectively. The distances: BM–M0, 3200 mm; M0–M1, 2600 mm; M1–M2, 4840 mm; M2–S1, 630 mm; S2–M3, 1500 mm; M3 Station, 2000 or 2500 mm.

The VUVCD spectrophotometer was set at the end station of the beam line. Details of the optical devices and the sample cell are shown in a previous paper.[7] The spectrophotometer consists of two separate vacuum chambers, i.e., polarization modulation chamber and sample chamber. The sample chamber is separated by a gate valve from the polarization modulation chamber, so that a sample solution can be exchanged, while the modulation chamber is kept under high vacuum. Both chambers can be degassed to 2×10^{-6} Torr with a Varian turbo molecular pomp V-70LP.

The synchrotron radiation light monochromated by NIM is led through a MgF_2 window to the polarization modulation chamber and separated into two orthogonal linearly-polarized light beams by a Karl Lambrecht MgF_2 Rochon prism (POL). Both linearly-polarized light beams are modulated to circularly-polarized light beams at 50 kHz by a JASCO LiF photo-elastic modulator (PEM). In order to control PEM accurately and to stabilize the lock-in amplifier under high vacuum, the optical servo-control system was used.[7,8] This system realizes achromatic modulation and compensation for the thermal drift of PEM by use of the double beam configuration. The main light beam in the center of the chamber is led to the sample cell and the CD signal is detected with a Hamamatsu R6836 photomultiplier tube (PM). The other light beam is used as the reference signal to synchronize the polarization modulation.

The performance of the VUVCD spectrophotometer was tested with a standard material, ammonium *d*-camphor-10-sulfonate (ACS), purchased from Katayama Chemical Co. Figure 2 shows the VUVCD spectrum for a 100 mM ACS aqueous solution at 25 °C, which was measured with a MgF_2 sample cell of 20-mm diameter and 20-μm path length. The base line signal for water is perfectly straight within an accuracy of ±2 mdeg, indicating no birefringence of the cell windows. The spectrum for ACS obtained by the presently constructed VUVCD spectrophotometer is completely superimposed upon that obtained by a commercial JASCO J720 spectropolarimeter in the wavelength region down to 185 nm. The characteristic peaks are observed at 291 and 192 nm with an intensity ratio of 1:2 as expected for the normal operation of the instrument. The CD spectrum was reproducible within 5% when the spacer and solution were exchanged.

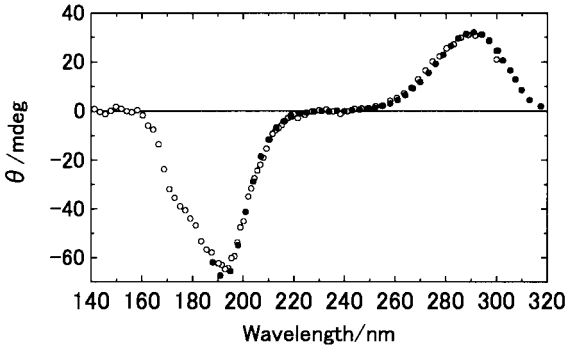

Figure 2. VUVCD spectra of 100 mM ACS aqueous solutions at 25 °C. Solid line shows the base line for water. Open and solid circles indicate the spectra measured with the same sample solution using a HiSOR–VUVCD and a JASCO J720 spectropolarimeter, respectively. The spectra are recorded with a 20-μm path length MgF_2 cell, 1.0 mm slit, a 16-s time constant, 4 nm/min scan speed, and 4–16 accumulations.

Figure 3 shows the VUVCD spectra of D- and L-alanines. Evidently, both amino acids show the symmetrically inverted spectra of positive and negative ellipticities, consistent with the theoretical requirement for optical isomers. The symmetrical spectra were also observed for D- and L-isomers of other amino acids (to be reported). In any case, the spectra obtained by the present VUVCD apparatus were superimposed upon those that were observed by a commercial JASCO J720 spectropolarimeter in the wavelength region down to 200 nm.

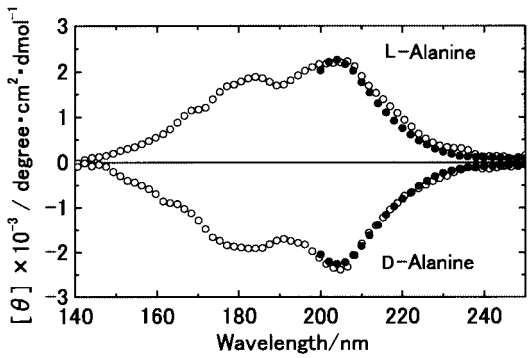

Figure 3. VUVCD spectra of 10 % aqueous solutions (pH 6.2) of D- and L-alanines at 25 °C. Open and solid circles indicate the spectra measured by a HiSOR–VUVCD and a JASCO J720 spectropolarimeter, respectively. These spectra were recorded under the same conditions as used in Figure 2.

As clearly shown in this paper, the constructed optical system and the sample cell can normally operate under high vacuum as well as under the atmospheric condition. For the first time, we have succeeded in measuring the CD spectra of ACS and two optical isomers of alanine in water in the VUV region down to 140 nm by using synchrotron radiation as a light source. The result is difficult to attain by any commercially available instrument. A remaining problem is the improvement of signal-to-noise ratios of the spectra. The DC signal and noise level are expected to greatly improve, since the power of the light source at HiSOR will be increased from 100 mA to 200 mA within a few months. A temperature-control unit for the sample cell which incorporates a Peltier thermoelectric element is currently under development. This VUVCD spectrophotometer should be sufficiently powerful for assignment of the structure and conformational change of proteins, nucleic acids, and, in particular, polysaccharides which show CD peaks only in the VUV region.

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