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CIRCULAR DICHROISM DETECTION OF OPTICALLY ACTIVE COMPOUNDS IN GAS CHROMATOGRAPHY USING VACUUM ULTRAVIOLET SYNCHROTRON RADIATION

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SUMMARY

Circular dichroism has been employed as a detection technique in gas chromatography for specific monitoring of optically active compounds which absorb in the vacuum ultraviolet region. The synchrotron radiation from U9A beamline of National Synchrotron Light Source, Brookhaven National Laboratory, was used as the light source. The detection limit established using this system is at the nanogram level for a selected group of hydrocarbons.

INTRODUCTION

In development of specific molecular property detectors for chromatographic applications, detection of optically active compounds has been the subject of some recent studies[1–4]. In early studies by two independent groups, detection systems were developed for monitoring optical activity of the eluent from a liquid chromatograph[1,2]. In both these studies, commercial circular dichroism (CD) spectrophotometers were modified to use in combination with liquid chromatographs. These two groups demonstrated the versatility of coupling high-performance liquid chromatography with CD detection (HPLC–CD) in separation of synthetic racemic mixtures and natural product extracts. In another development of HPLC–CD, high sensitivity and selectivity were achieved by combining microbore liquid chromatography with a laser-based CD detector[4].

Application of CD detection in gas chromatography (GC–CD) for specific monitoring of optically active compounds was described in an earlier work from this laboratory[3]. This GC–CD system consisted of a homemade CD spectrometer with Hg-Xe arc lamp as the light source and a specially designed heated gas cell. All of the above mentioned systems of HPLC–CD and GC–CD can be used for separation and detection of optically active compounds absorbing in the visible (VIS) and ul-

traviolet (UV) regions. There are large number of compounds, such as hydrocarbons, which do not absorb in the VIS and UV range but absorb strongly in the vacuum ultraviolet (VUV). Therefore, in the present work we have extended GC–CD technique to the VUV region (GC–VUVCD) using synchrotron radiation as the light source. In fact, the present instrument can be used in the wavelength region covering 600–135 nm.

THEORY OF MEASUREMENT

CD spectroscopy is a special kind of absorption spectroscopy which uses circularly polarized light. In CD spectroscopy, the measured quantity, $\Delta\varepsilon$, is the difference in absorption of left and right circularly polarized light by the compound at any given wavelength; *i.e.*

$$\Delta\varepsilon = \varepsilon_L - \varepsilon_R \tag{1}$$

where ε_L and ε_R are the molar extinction coefficients for left and right circularly polarized light, respectively, and ε is defined by Beer's Law as

$$\text{optical density or absorbance } (A) = \log_{10}(I_0/I) = \varepsilon l c \tag{2}$$

In eqn. 2, I_0 and I are intensities of the light incident on the sample and emerging from the sample, respectively, l is the path length of the cell in cm, c is the concentration of the sample in mol l^{-1}. It is clear from eqn. 1 that $\Delta\varepsilon$ can be either positive or negative depending on the relative magnitudes of ε_L and ε_R. Using Beer's Law, the difference in absorption of left and right circularly polarized light can be written as

$$A_L - A_R = (\varepsilon_L - \varepsilon_R) l c = \Delta\varepsilon l c \tag{3}$$

A frequently used method for the generation of circularly polarized light is to use a quarter wave plate. A quarter wave plate is placed such that its fast axis makes a 45° angle with the plate of the linearly polarized light, which in automatic instruments is modulated at a frequency of 50 kHz. The circularly polarized light is passed through a sample and is received by a detector such as a photomultiplier. In the absence of any optically active substance in the light path, the detector produces a d.c. signal proportional to the intensity of light at a given wavelength. When an optically active sample is placed in the light path, the detector produces a small a.c. signal superimposed on a large d.c. signal. It has been shown[5] to a good approximation, that the intensity of the a.c. signal is a measure of the circular dichroism of the sample according to the following relationship,

$$A_L - A_R = C(I_{a.c.}/I_{d.c.}) \tag{4}$$

where C is a constant characteristic of the instrument. Eqns. 3 and 4 give the relationship

$$(I_{a.c.}/I_{d.c.}) = (1/C) \Delta\varepsilon l c \tag{5}$$

Constant C can be determined by calibration with a compound of known $\Delta\varepsilon$.

EXPERIMENTAL

Instrumentation for VUVCD spectroscopy

A complete description of the VUVCD instrument which was built by us on U9A beamline of National Synchrotron Light Source (NSLS), Brookhaven National Laboratory, has been given in an earlier report[6]. It is similar in design to the instrument constructed at the Synchrotron Radiation Center, University of Wisconsin, Madison, WI, U.S.A.[7,8].

The basic layout of U9A beamline on the VUV ring at NSLS is shown in Fig. 1[9]. The radiation input from the ring is divided into two portions directed to U9A and U9B beamlines by two sets of gold-coated mirrors (one spherical and one plane mirror in each set) placed in a mirror box. U9A portion of the beam passes through a LiF window before entering the monochromator. A cylindrical mirror placed before the entrance slit and a toroidal mirror placed after the exit slit allow the beam to be focused into the center of the outer sample chamber (or vacuum chamber), which is a six-way stainless-steel cross. Other optical components, sample cells, detectors (such as photomultipliers), etc., can be connected to the vacuum chamber using 8 in. conflat flanges.

A schematic representation of the CD experimental setup is shown in Fig. 2. The VUV synchrotron radiation is passed through a 0.5-m Seya-Namioka monochromator ($f/24$) equipped with a 1200-grooves-per-mm grating. Overall dispersion of the monochromator is 16.6 Å per mm. The light beam coming from the synchrotron ring is highly linearly polarized ($>95\%$) along the plane of the electron orbit[10]. The linearly polarized light is converted into circularly polarized light using a CaF_2 quarter wave retarder[11] (Hinds International Model PEM80) moduled at a frequency of 50 kHz. The CaF_2 quarter wave plate is mounted on a conflat flange so that it
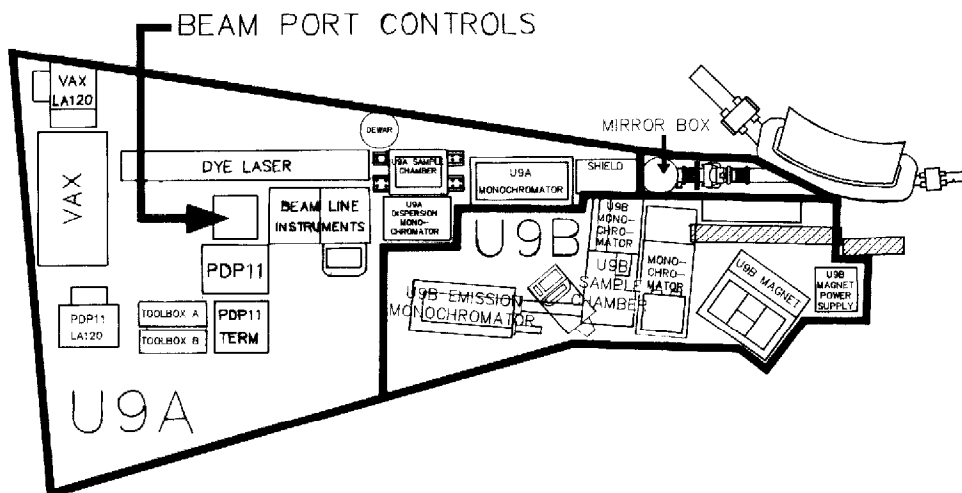

Fig. 1. A schematic representation of U9 beamlines of NSLS[6]. (Provided to us by Dr. J. M. Preses, who is in charge of U9A beamline of NSLS.)
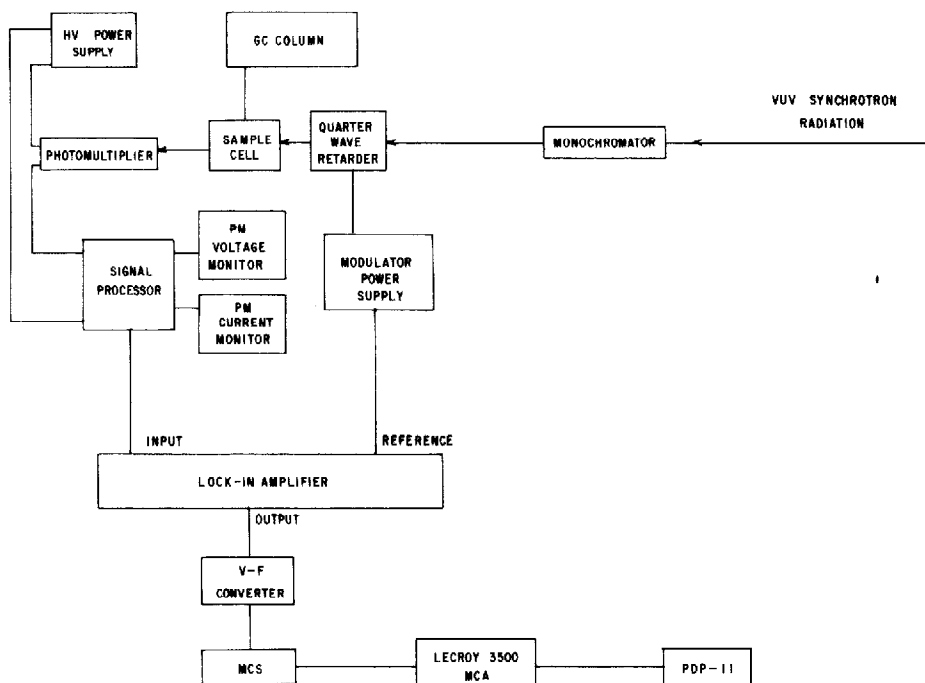
Fig. 2. Schematic representation of the CD experimental setup on U9A beamline.

can be connected to the U9A beamline vacuum chamber. The instrument has been designed to accommodate both vapor and liquid phase samples in different cell assemblies. In the present experiment, a temperature controlled gas cell (13 cm × 1.3 cm I.D.) fitted with two LiF windows (1.9 cm diameter × 2 mm thickness plates from Harshaw Chemical Company) is used. One end of the cell is mounted on an 8 in. conflat flange so that the flange can be connected to the main chamber. The other end of the cell is directly connected to the photomultiplier housing.

The light beam emerging from the sample cell is detected by an EMI 9635-QB photomultiplier tube (PMT). A sodium salicylate coating was used on the PMT window for the scans between 160 and 135 nm. The signal from the photomultipler and the high-voltage power supply (Bertan Model 303) to the photomultiplier are connected to a signal processer unit built at Brookhaven National Laboratory for this purpose, which maintains a constant phototube current throughout a scan. The circuit for the signal processer is a slightly modified version of the original circuit used by Snyder[7]. The main function of this unit is to serve as an automatic gain control. The unit has been designed for automatic normalization of the a.c. signal (at 50 kHz modulation frequency) by controlled changes in PMT gain. The controlled changes in PMT gain are required to compensate for the variations of dc signal due to variations in light intensity with wavelength and time. The voltage and current meters, connected to the signal processor unit, monitor the high-voltage input to phototube and the output signal from the phototube. The voltage monitor gives a measurement which is related to the sample absorbance. The output signal from the

signal processor (DC controller) unit is fed into a lock-in-amplifier (PAR Model 186A) through a differential preamplifier (PAR Model 117). A phase reference output from the photoelastic modulator power supply is used as the external reference for the lock-in-amplifier. The signal is processed through a voltage-to-frequency (V–F) converter and multichannel scaling module (LeCroy 3521A) and the data are stored in a LeCroy 3500 multichannel analyzer. The entire data collection system is run by a PDP 11/73 computer. Data are transferred to a VAX 11/730 computer for the purpose of plotting spectra while PDP 11/73 computer acquires data.

The performance of the VUVCD instrument described above and the reliability of the results were tested by reproducing previously reported vapor phase spectra of (+)-3-methylcyclopentanone[12–15] and (−)-α-pinene[8,15–18]. Further, (+)- and (−)-α-pinene clearly showed a mirror image relationship as expected.

Chromatographic conditions

A 1/8 in. × 10 ft. stainless-steel column packed with 5% Carbowax 20M on Supelcoport 100/120 (Supelco, stock No. 1-1793) was directly connected to the gas cell described in the previous section. The other end of the column was connected to an injection port which was connected to a pressure regulator for nitrogen (99.99%) carrier gas. The injection port, GC column, and the gas cell were maintained at 60 ± 1°C. The gas cell was completely purged with nitrogen and kept at atmospheric pressure. Since nitrogen does not absorb at wavelengths longer than 150 nm, it could be used at 186 nm. To use this apparatus at shorter wavelengths, a different gas such as helium would have to be used.

Standard samples

The chemicals used and their purities are as follows: (+)-3-methylcylopentanone (99%) from Aldrich, (−)-α-pinene (>99.5%), (+)-α-pinene (>99%), and (+)-limonene (>99%), all from Fluka. Carefully measured quantities of (−)-α-pinene and (+)-limonene were dissolved in perfluorohexane (99%, Aldrich) to prepare dilute solutions. Perfluorohexane was selected for this purpose because it has very low absorption coefficients and no CD signals in the wavelength regions of interest, and it has a high volatility.

RESULTS AND DISCUSSION

Two terpene compounds, (−)-α-pinene and (+)-limonene, were selected to demonstrate GC–VUVCD analytical system. The two compounds have high vapor pressures and hence allow the convenience of low column temperature operations. Two chromatograms taken with the monochromator setting of 186 nm (bandwidth of the light beam 1.6 nm) are shown in Fig. 3. At 186 nm, (−)-α-pinene has a positive CD signal[8,16–18] and (+)-limonene has a negative CD signal[19]. The wavelength of 186 nm was specifically chosen to illustrate one advantage of this analytical system, i.e., the identification of the stereoisomers using positive or negative nature of the chromatographic peak. For example, with 186 nm light, (+)-limonene gives a negative signal while (−)-limonene gives a positive signal.

The calibration curve for (+)-limonene is shown in Fig. 4. The measurement corresponding to a 10-μl injection showed an absorption saturation (open circle in
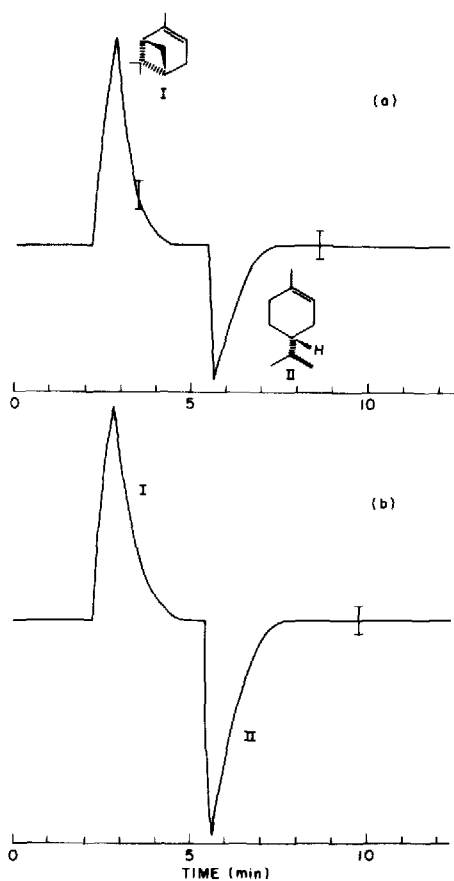
Fig. 3. GC–VUVCD detection of ($-$)-α-pinene(I) and ($+$)-limone(II) using 186 nm radiation. (a) 43 ng of I and 42 ng of II, (b) 43 ng of I and 67 ng of II. These scans were obtained with 1-s time constant. The noise level is indicated by the vertical bars.

Fig. 4). The concentration at which the absorption saturation occurs depends on the excitation wavelength and the intensity of light. The calibration measurements shown in Fig. 4 were obtained with beam currents of 100–500 nA, because in this region synchrotron beam at NSLS has a lifetime of more than 2 h. The synchrotron radiation intensity monitored on U9A beamline (by measuring the photoion yield from *cis*-butene-2 at 123.6 nm) is usually in the order of 10^{11} photons per second per Ångstrom of bandwidth per ampere of beam current. The signal-to-noise ratio (S/N) of a CD signal is proportional to the square root of the intensity according to the following relationship[5,20]

$$S/N = -2.303 \, \Delta\varepsilon lc I^{1/2} = -2.303 \, \Delta\varepsilon lc I_0^{1/2} \cdot 10^{-\varepsilon lc/2} \tag{6}$$

It has been shown that the S/N ratio is optimum at an absorbance of 0.86[5,20]. Since the synchrotron beam intensity decreases with time, it is difficult to determine the detection limits of GC–VUVCD system precisely. Based on the available results, the
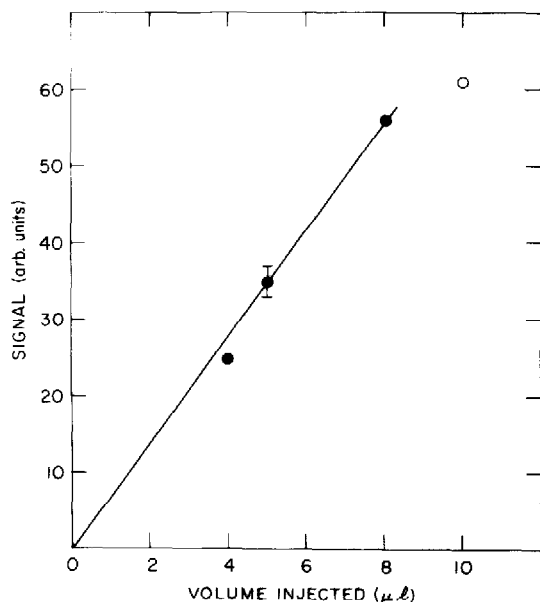
Fig. 4. Calibration curve for (+)-limonene obtained using 1% solution in perfluorohexane. A 10-μl sample showed an absorption saturation, and the open circle indicates the level at which saturation occurred (see text).

detection limits were calculated for a case with S/N = 2, and these estimated detection limits are given in Table I. However, it must be mentioned here that it should be possible to determine even smaller quantities than those listed in Table I with high intensities of synchrotron beam. This aspect was not tested in this work, because at the present time, the synchrotron beam of NSLS decays rapidly from about 800 mA to about 500 mA. Also, S/N ratio is a function of $\Delta\varepsilon$, therefore, the detection limits can be different with different wavelengths.

CONCLUSION

The detection of a GC effluent as a CD signal in the VUV region has been demonstrated using synchrotron radiation as the light source. The detection limit

TABLE I

CHROMATOGRAPHIC DATA FROM GC–VUVCD SYSTEM

Species	Retention time* (min)	Estimated detection limit** (ng)
(−)-α-Pinene	2.2	11
(+)-Limonene	5.4	17

* Column operated at 60°C.
** Measurements made using 186 nm radiation.

established using GC–VUVCD system is at the nanogram level for monoterpenes selected for this work. The detection limits established for some selected ketones using UV light in the previously published work from this laboratory were at microgram levels. Since synchrotron radiation is tunable and highly intense, the analytical system described here will be very useful for fingerprinting natural product mixtures such as oil fractions. A disadvantage of the GC–VUVCD system is the change of intensity of the synchrotron beam with time which requires a number of chromatographic calibrations such as the one shown in Fig. 4 at few different beam currents.

ACKNOWLEDGEMENTS

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