Journal of Chromatography, 262 (1983) 321–327
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,840

Note

Gas chromatography–circular dichroism system for detection of optically active substances*

J.S. GAFFNEY and E.T. PREMUZIC*
Department of Energy and Applied Science, Brookhaven National Laboratory, Upton, NY 11973 (U.S.A.)
T. ORLANDO and S. ELLIS
Department of Chemistry, Southampton College, Southampton, NY 11968 (U.S.A.)
and
P. SNYDER
Department of Chemistry, Florida Atlantic University, Boca Raton, FL 33431 (U.S.A.)
(Received March 8th, 1983)

The use of specific molecular property detectors in chromatographic applications has given rise to a number of highly selective and sensitive new analytical tools. These include ozone chemiluminescent specific detection of olefins and reduced sulfur compounds[1–3], photothermal laser spectroscopic applications in gas chromatography (GC)[4], and the detection of chiral substances by circular dichroism (CD) detection in liquid chromatography[5,6]. This work describes the application of CD detection to the specific monitoring of optically active molecules in GC. CD is a measure of the difference in the absorption by an optically active substance of the left- and right-handed circularly polirized light at a given wavelength[7]. When a mixture containing volatile optically active and inactive substances (such as a crude extract of natural products) is gas chromatographed, only the eluents which are optically active will yield a CD signal. This signal specificity, as well as the strong wavelenght dependence of the CD detector, gives rise to the inherent selectivity of a GC–CD analytical system. This paper describes the construction of a first generation detector and its application to the selective analysis of optically active molecules.

EXPERIMENTAL

The major components of the system are presented in Fig. 1. The light source (S) (Oriel high-pressure 200 W Hg–Xe arc lamp) is powered by a d.c. supply and has been found to be most stable when supplied with 20 V at a current of 7.5 A. Light emerging from the source is focused directly into the monochromator (Beckman DU-2). The beam emerging from the monochromator is focused onto a spherical focusing and collimating mirror (M) which has a focal length of 23.0 cm and a diameter of 10.8 cm. The mirror beam collimation was found to reduce the baseline noise level

* This research was performed under the auspices of the United States Department of Energy under Contract No. DE-AC02-76CH00016.

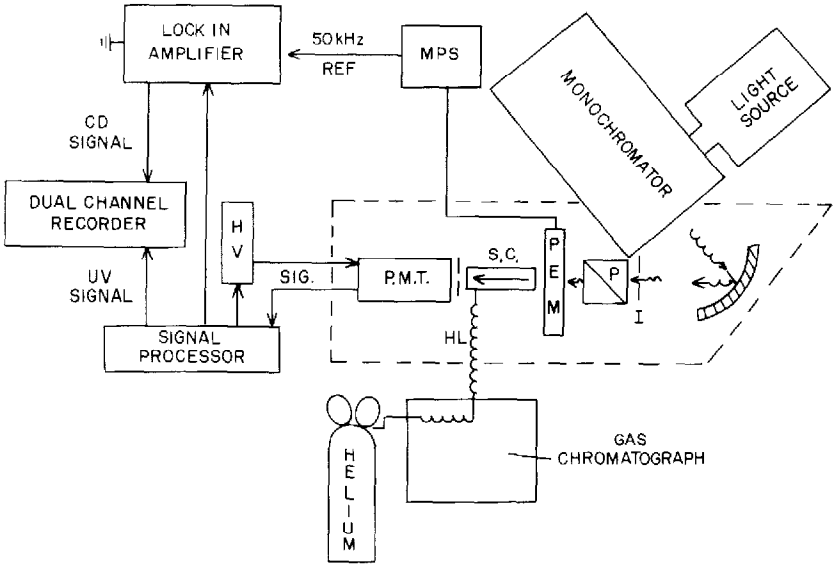


Fig. 1. Schematic of experimental system for GC – CD detection. I = Double iris; P = Wollaston prism; PEM = photoelastic modulator; SC = gas sample cell; PMT = photomultiplier; MPS = PEM modulator power supply; HV = high-voltage power supply; HL = heated transfer line. Dotted line indicates light-tight optical enclosure.

over the entire instrumental spectral range (200–650 nm). The collimated beam passes through an iris (I), which allows a 2 mm circular beam of light to impact on a 40° MgF_2 Wollaston polarizer, P, (24 mm aperture, 1.4° angular seperation). The linearly polarized light then travels through a CaF_2 photoelastic modulator (PEM; Hinds Int. PEM-CF4), which acts as a quarter-wave retarder and alternately produces right and left circularly polarized light at a 50-kHz frequency. The PEM power supply (MPS) simultaneously supplies a 50-kHz reference to the lock-in amplifier (Princeton Applied Research 186A). The modulation voltage amplitude to the crystal was calibrated and found to be linear over the wavelength range used. For GC measurements, the modulation voltage amplitude was manually set at the desired monitoring wavelength. The circularly polarized light from the PEM was passed through an aluminium gas detection cell (SC) of 6 cm path length (see Fig. 2). This cell has a 1.38 ml dead volume and is fitted with 0.125 cm diameter, 2 mm thick LiF windows. The windows are mounted by a screw clamp–O-ring compression fitting which allows a gas-tight fit with minimal window stress. Torque on the windows was found to give rise to appreciable birefringence, thus requiring this type of a window mount. The gas cell was interfaced to an Aerograph gas chromatograph using a heated capillary gas transfer line. Resistive heating (tape and cartridges) was used to effectively heat the gas cell to 175°C and the transfer line (HL) to 200°C, and the temperatures were monitored with the use of thermocouples.

Upon exiting the gas cell, the modulated light beam strikes the photomultiplier tube (PMT; Gencom 9635QB). The CD signal arises from the differential absorptions of either left or right-handed circularly polarized light by the eluent which is optically active yielding an a.c. signal (modulation frequency at 50 kHz). This signal is superimposed on a much larger d.c. signal due to the total light striking the PMT.

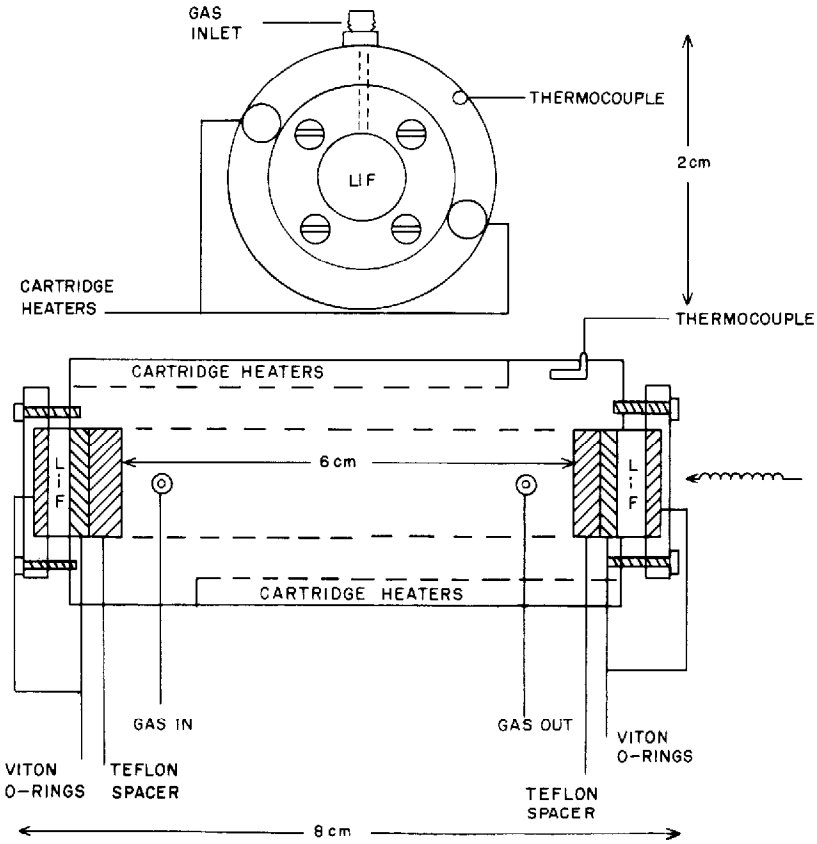


Fig. 2. Gas detection cell for GC–CD system.

To extract the small CD a.c. signal, the d.c. current of the PMT is controlled and maintained by an automatic gain control feedback network[8]. This circuitry is housed in the signal processor unit (SP)[8], which automatically amplifies and normalizes the 50-kHz a.c. signal. This is accomplished by a feedback to the remote controlled photomultiplier voltage supply (HV; Bertran 303), which compensates for light source d.c. variations due to sample absorption and lamp variability. The signal processor d.c. controller voltage level is used to monitor the total UV–VIS absorption.

The CD signal has been shown to be proportional to the ratio of the a.c. and d.c. voltages ($V_{a.c.}$ and $V_{d.c.}$, respectively)[7,9,10] according to:

$$\mathrm{CD} = k\left(\frac{V_{a.c.}}{V_{d.c.}}\right)\left(\frac{1}{cd}\right)$$

where c is the concentration, d the pathlenght and k an instrumental constant. Since $V_{d.c.}$ is kept constant by the feedback network, with appropriate calibration, the measurement of $V_{a.c.}$ directly yields CD. CD signals observed were monitored using the lock-in amplifier operated on 2- or 5-mV sensitivity scales with 3- or 10-sec time constants and a two-channel strip-chart recorder (Omniscribe).

The CD system was calibrated by comparison of spectra taken on this equipment with previous literature data for *d*-camphor and cyanocobalamin solutions, using a standard 2-mm quartz cell. The observed solution spectra were found to be in excellent agreement with the reported studies[6,11].

GC conditions were as follows: 10 ft. × 1/8 in. 10% OV-101 column (100–120 mesh); column temperature, 150°C; injector temperature, 175°C; transfer line temperature, 175°C; gas cell, 150°C; and gas flow-rate, 24 ml helium/min.

Chemical samples were of analytical grade commercial purity (> 95%) as follows: (+)-3-methylcyclopentanone and *l*-verbenone (Aldrich), *l*-fenchone and *d*-dihydrocarvone (PCR Research Chemicals), and *d*-camphor (Fluka).

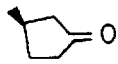
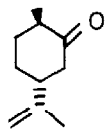
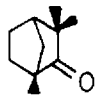
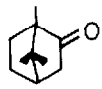
RESULTS AND DISCUSSION

The GC–CD analytical system has been applied to the analysis of five optically active ketones: (+)-3-methylcyclopentanone (MCP), *d*-dihydrocarvone (DHC), *l*-fenchone (F), *d*-camphor (C), and *l*-verbenone (VB) (see Table I).

A GC–CD analysis of a mixture of these five compounds at two wavelengths (300 and 340 nm) is presented in Fig. 3. Resolution of the five compounds is indicated for the 300 nm data. Notice that the red shifted $n - \pi^*$ transition in VB allows the selective monitoring of this compound at 340 nm. Calibration curves have been found to be linear with 10% reproducibility from injection to injection. Typical calibration

TABLE I

CURRENT DETECTION LIMITS FOR GC–CD SYSTEM FOR FIVE OPTICALLY ACTIVE KETONES

Compound	*Structure*	$[a]_D$	*Wave-length (nm)*	ϵ*	$\Delta\epsilon$	*GC–CD detection limit (μg)*
(+)-3-Methylcyclopentanone		+ 148°	300	20.4	+ 2.5	7
d-Dihydrocarvone		+ 18°	300	21.3	+ 0.70	40
l-Fenchone		− 49.5°	300	11.9	− 0.54	35
d-Camphor		+ 45°	300	18.9	+ 1.6	20
l-Verbenone		− 193°	340	32.3	− 1.0	10

* UV Extinction coefficient (ϵ = molar absorptivity) determined with Beckman DU-8.

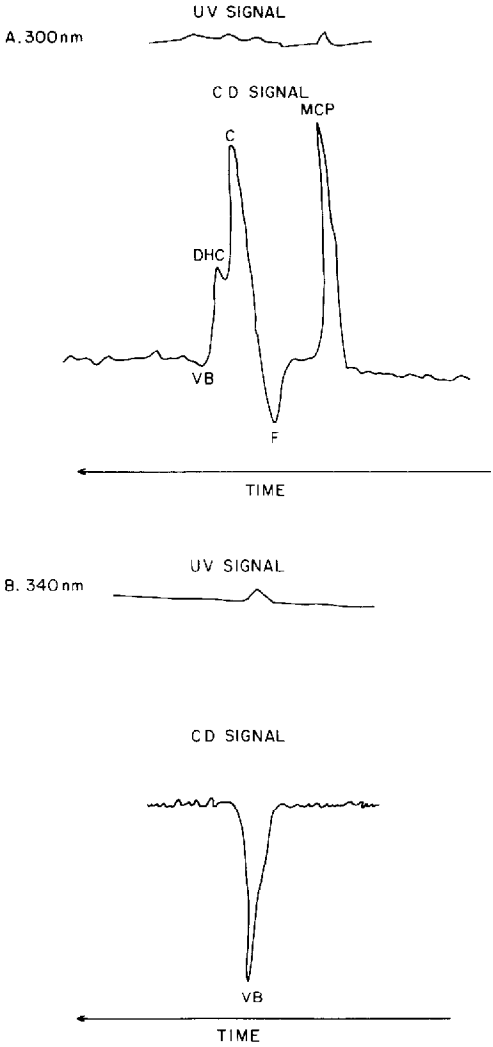


Fig. 3. GC–CD analysis of a mixture of five optically active ketones at two wavelengths (A, 300 and B, 340 nm). MCP = 284 μg (+)-3-methylcyclopentanone; DHC = 290 μg *d*-dihydrocarvone; F = 284 μg *l*-fenchone, C = 500 μg *d*-camphor; VB = 290 μg *l*-verbenone (10-μl injection in heptane).

curves for *d*-camphor and *l*-verbenone are given in Fig. 4. From these calibrations detection limits were calculated for a case with signal-to-noise ratio = 2.

Current detection limits for the ketones studied are given in Table I. These values are all in the tens of μg range, for compounds with $\Delta\epsilon$ coefficients in the +2 to −2 range for these weak $n - \pi^*$ transitions. For compounds with stronger $\Delta\epsilon$ values (+20 to −20), this sytem should be able to detect compounds in the 0.5 to 1 μg range. The present detection limits are comparable to those reported for high-performance liquid chromatograph–CD detection[5,6] and those using optical rotatory dispersion (ORD)[13]. The inherent specificity of CD over ORD, however, offers some advantage

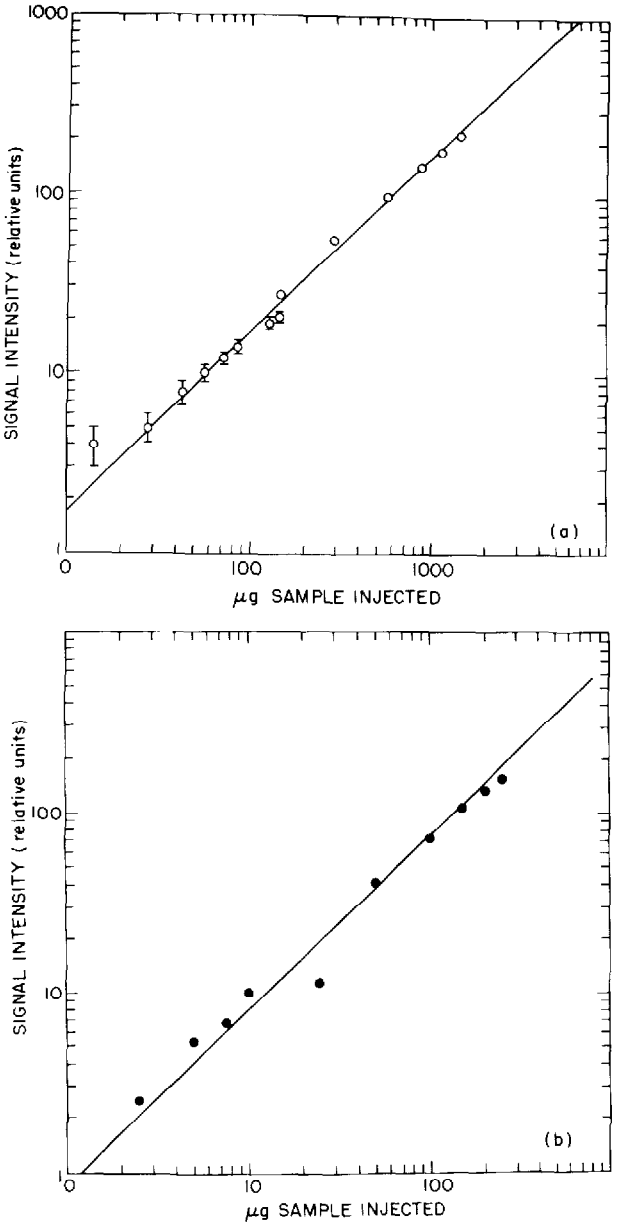


Fig. 4. Calibration curves for (+)-3-methylcyclopentanone (a) and *d*-camphor (b) on the GC–CD system. Lines are unit slope.

over ORD detection. Relative to ORD (which monitors the overall changes in the optical rotation over a very wide wavelength range), CD is associated with the narrow wavelength of the chromophore associated with an asymmetric optically active center. Therefore, groups of optically active substances with a common chromopheric system can be selectively monitored at given wavelengths by CD (*e.g.*, 290–300 nm for car-

bonyl). Further work is underway to use synchrotron light[13] as a means of monitoring optically active eluents (GC–CD) in the vacuum ultraviolet (VUV) (120–200 nm) region.

Optically active molecules in a shorter wavelength region should further increase the detection capability of the device. Sample cell improvements (*i.e.*, lower dead volume and longer pathlength) are underway to improve detection and allow gas capillary column work with this detector.

CONCLUSIONS

From the presented data, it has been demonstrated that the detection of CD signals from a gas chromatographic effluent is feasible. Current detection limits indicate that this instrument is capable of analysis in the μg region for moderately weak absorbers. The strong dependence of the CD signal with wavelength, makes it a potentially useful tool for fingerprinting of natural product mixtures as a function of optically active chromophoric systems (as indicated here for ketones and conjugated ketones). It is expected that the extension of the wavelength capability into the VUV using synchrotron radiation will further increase the detection sensitivities and thus allow many types of natural products to be characteristically fingerprinted by this technique. Applications in organic geochemistry and the petroleum, syn-fuels and biomedical industries are anticipated.

ACKNOWLEDGEMENTS

The work was supported by the Division of Chemical Sciences, United States Department of Energy, Washington, DC, U.S.A. under Contract No. DE-AC02-76CH00016. One of us (P.S.) wishes to acknowledge the support of the NSF CHE 8108534, The Research Corporation; and Division of Sponsored Research at Florida Atlantic University. We wish to thank Drs. G. Senum and H.B. Lin of Brookhaven National Laboratory for helpful discussions.

REFERENCES

1 W. Bruening and F.J.M. Concha, *J. Chromatogr.*, 142 (1977) 191–201.
2 W. Bruening and F.J.M. Concha, *J. Chromatogr.*, 112 (1975) 253.
3 T.J. Kelly, J.S. Gaffney, M.F. Philips and R.L. Tanner, *Anal. Chem.*, 55 (1983) 135–138; and references cited therein.
4 H.B. Lin, J.S. Gaffney and A.J. Campillo, *J. Chromatogr.*, 206 (1981) 205–214.
5 A.F. Drake, J.M. Gould and S.F. Mason, *J. Chromatogr.*, 202 (1980) 239–245.
6 S.A. Westwood, D.E. Games and L. Sheen, *J. Chromatogr.*, 204 (1981) 103–107.
7 L. Velluz, M. Legrand and M. Grosjean, *Optical Circular Dichroism. Principals, Measurements and Applications*, Academic Press, New York, 1965.
8 P.A. Snyder and E.M. Rowe, *Proceedings of the 6th International Conference on Vacuum Ultraviolet Radiation*, University of Virginia Publication, 3 (1980) 33–35.
9 W.C. Johnson, *Rev. Sci. Instrum.*, 42 (1971) 1283–1286.
10 O. Schnepp, in C. Sandorfy, P.J. Ausloos and M.B. Robin (Editors), *Chemical Spectroscopy and Photochemistry in the Vacuum Ultra Violet Region*, NATO Advanced Study Institute, Sec. C8 Reidel, Dordrecht, 1974, pp. 211–222.
11 O. Schnepp, S. Allen and E.F. Pearson, *Rev. Sci. Instrum.* 41 (1970) 1136-1141.
12 E.J. Yeung, L.E. Steenkoek, S.D. Woodruff and J.C. Kuo, *Anal. Chem*, 52 (1980) 1399.
13 P.A. Snyder and E.M. Rowe, *Nuclear Instr. Methods*, 172 (1980) 345–349.