Thermal Lens Technique: A New Method of Absorption Spectroscopy

Abstract. *Both a single beam and a dual beam (with synchronous detection) thermal lens technique have been employed in the measurement of "colorless" organic compounds in the range 15,700 to 17,400 cm^{-1}. Combination overtones of C–H stretching vibrations in benzene have been identified and agree with previous results obtained by conventional spectroscopy with a long optical path. Extinction coefficients as low as 1 × 10^{-6} liter mole^{-1} cm^{-1} have been accurately determined. The sensitivity of the technique has been further demonstrated by measuring the* $S_0 \rightarrow T_1$ *absorption of anthracene; the spectrum compares favorably with results obtained by conventional techniques.*

We report the application of the thermal lens technique to absorption spectroscopy. The method is based on the temperature rise in an illuminated liquid induced by the absorption of small amounts of energy from a laser beam passing through the sample. The localized temperature change brings about a transverse gradient in the index of refraction, which can be probed optically as a "thermal lens." In recent years such thermal lensing has been well characterized (*1, 2*), and it has been shown that under optimal conditions the optical properties of the lens can be simply related to the absorption coefficient of the molecule.

Hu and Whinnery (*3*), Whinnery (*4*), and others (*5*) have applied this method to absorptivity measurements of a number of compounds at a fixed frequency—the 632.8-nm line of the He-Ne laser. Their results show systematic variations of the absorptivity within a particular homologous series. However, measurements at a single wavelength are not sufficient to properly characterize the absorption. This problem seemed ideally suited for an argon ion pumped tunable dye laser system. We have used a tunable laser–based thermo-optical spectrometer to investigate weak absorptions in organic compounds in the liquid phase. We report here some preliminary results that demonstrate the usefulness and extremely high sensitivity of the technique.

The thermal lens effect was first reported and discussed in detail by Gordon *et al.* (*1*), whose analysis forms the basis of the present work. A liquid sample is placed in the beam of the laser. The liquid is heated by the absorbed power and, in the absence of heat conduction. a transverse temperature profile is established which matches the intensity profile of the laser. If the mode of this beam is TEM_{00}, the intensity profile is Gaussian. Sufficiently close to the center of the beam, the laser intensity profile is parabolic and, even after heat conduction takes place, so is the temperature profile. Since most liquids have a positive coefficient of thermal expansion, the temperature coefficient of the index of refraction, dn/dT, is negative, and the thermal lens is divergent. For the steady-state limit, where the heat deposited by the laser is balanced by the outward conduction, the focal length of the thermal lens in centimeters is

$$f = \frac{\pi J k w^2}{P \alpha l (dn/dT)} \qquad (1)$$

where J is Joule's constant (4.18 joule/cal), k the thermal conductivity (cal cm^{-1} sec^{-1} ^{0}K^{-1}), w the beam waist (cm), P the incident laser power (watts), α the absorptivity (cm^{-1}), and l the sample length (cm). By measuring the strength of this steady-state thermal lens, Gordon *et al.* (*1*) calculated the absorptivity of the liquid.

Refinements in the measurement technique were developed by Hu and Whinnery (*3*). Our apparatus, derived from their work, is shown schematically in Fig. 1a. An 8-watt argon ion laser pumps a continuous wave rhodamine 6G dye laser. Approximately 25 mw (~ 1 percent of the total output power) is delivered to the sample cell, which is placed one "confocal length" (*6*) past the focus of a long focal length lens. Hu and Whinnery demonstrated that this configuration allows the thermal lens to exhibit the maximum defocusing effect on the laser beam. The laser beam expands onto a target containing a 500-μm pinhole at its center. The time development of the thermal lens is followed by sampling the beam intensity with the pinhole-photomultiplier configuration. Intensity measurements at the instant the shutter opens, I_0, and when the steady-state condition is achieved, I_s, are used in calculating the absorptivity according to

$$\alpha = -\left(\frac{I_0 - I_s}{I_s}\right) \frac{J \lambda k}{P l (dn/dT)} \qquad (2)$$

where λ is the wavelength of the incident light (cm) and all other symbols are as before.

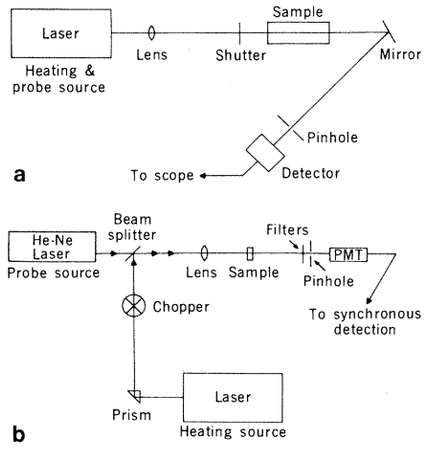

Fig. 1. Experimental setup: (a) single beam apparatus and (b) dual beam apparatus.

The characteristics of the thermal lens depend not only on the laser mode but also on the power absorbed in the liquid and the exposure time, both of which should be minimized to avoid convection and distortion effects on the lens (*7*). One must also ensure that the laser maintains a TEM_{00} mode.

We have used the single beam technique to accurately measure absorptivities (*8*). However, this is a d-c method, which is not only time-consuming but also inherently less sensitive than methods which take advantage of synchronous detection. Therefore, we have also designed and constructed a dual beam synchronous instrument (*9*), which lends itself to continuous recording (*10*).

Figure 1b shows a schematic of the dual beam experiment. The chopped heating beam forms a continuously pulsating thermal lens in the sample. The He-Ne probe beam passes through the sample and senses the lens. The heating beam is blocked from the detector by a cutoff and a narrow bandpass filter. The modulated probe beam is then synchronously detected. This signal is corrected for the heating beam power by a separate measurement.

Figure 2 shows the absorption of neat benzene over the tuning range of the dye laser determined by each of the two methods discussed above. A 6.8-cm cell was used for the single beam experiment and a 1.0-cm cell for the dual beam experiment. The absorptivity at the He-Ne laser line (15,800 cm^{-1}) agrees well with previously reported values (*4*). The dramatic failure of perdeuterobenzene to reveal any lens effect, in contrast to ordinary benzene, rules out electronic absorption as the cause of this absorption. It does, however, strongly implicate higher order (overtone and combination) vibrational transitions involving hydrogen motion. In fact, thermal lensing studies of various substituted benzenes show a dependence of the intensity of this peak on the number of ring hydrogens (*11*). The peak location agrees very well with the fifth overtone of the C–H stretching vibration reported in 1928 by Ellis (*12*), who used conventional spectroscopy with cells

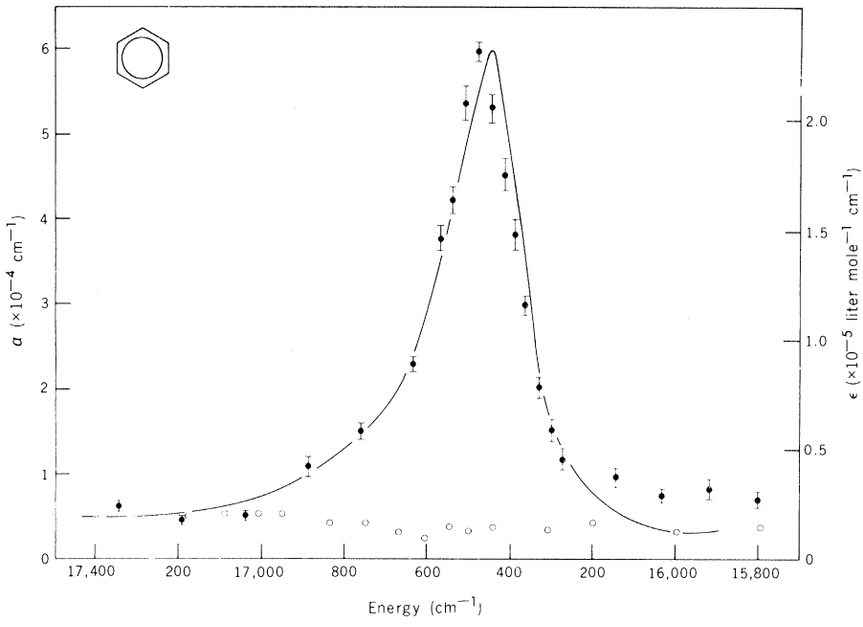

Fig. 2. Absorption of neat benzene. (•) Single beam spectrum; (curve) dual beam spectrum normalized to single beam spectrum at peak; (○) single beam spectrum of deuterated benzene.

up to 7 m long. The thermal lens method provides an accurate determination not only of peak location but also of absorption strength without complications found in conventional low absorbance measurements.

A reasonable extension of this thermal lens technique is the determination of singlet-triplet absorption spectra. Figure 3 shows the singlet-triplet absorption spectrum of a $10^{-2}M$ solution of anthracene in ethyl iodide measured in a 10-cm cell. The arrows refer to a shoulder, valley, shoulder, and peak (left to right) seen for the same system by conventional spectroscopy using a 20-cm cell (*13*). A higher order C–H vibrational transition of anthracene is expected at 16,500 cm^{-1} in the region of the first singlet-triplet transition, and it is possible that this causes some of the activity observed both in this and the earlier work (*13*). Isotopic substitution of anthracene would distinguish between the two types of transitions.

We can extrapolate the ultimate sensitivity of the thermal lens technique from our measurements on the overtone absorption spectrum of benzene. Using only 1 percent of the available laser power and 1-cm cells, we measured molar extinction as low as 2×10^{-6} liter $mole^{-1}$ cm^{-1}. With full laser power, 5-cm cells, and better-stabilized detection electronics, we visualize an ultimate sensitivity of less than 10^{-9} liter $mole^{-1}$ cm^{-1}. With detection limits so sensitive, purification procedures will be severely strained.

The sensitivity of the thermal lens technique compares favorably with that of other techniques for measuring small absorptivities. Stone (*14*) described an interferometric technique that senses the change in optical path length accompanying a temperature rise in the solution induced by absorption from a high-intensity arc lamp. The low intensities and therefore poor spectral resolution achievable with an incoherent source limit this method. Other workers (*15*) have explored the optoacoustic technique, first devised by Bell (*16*) in 1880, which senses pressure changes induced by absorption from a modulated light source. This method is directly applicable to measurements of weak absorption in gases and can be used on solids and liquids enclosed in a pressurized optoacoustic cell. Pinnow and Rich (*17*) have described a calorimetric method for measuring optical absorption in bulk solids. By placing their calorimeter inside a laser cavity, they were able to measure absorptivities of optical fibers as low as 6×10^{-6} cm^{-1}, comparable to the sensitivity in this work.

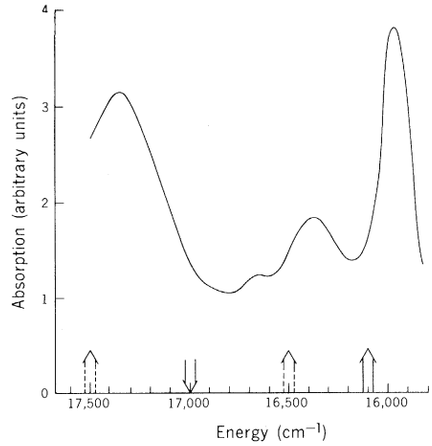

Fig. 3. Singlet-triplet absorption of anthracene in ethyl iodide, corrected for solvent absorption. Arrows are discussed in the text.

The thermal lens technique is probably best suited for liquids since the index of refraction is most sensitive to temperature in the liquid phase. It should be noted that thermal lens methods do not sense the fraction of the absorbed energy that is dissipated radiatively. For quantitative work such pathways must be considered; and, in fact, quantum yields for luminescence might be determined if the correct absorptivity is already known (*3*).

The experimental possibilities of the thermal lens technique are promising. In addition to vibrational overtones and spin-forbidden electronic transitions, it may be possible to detect multiphoton absorption and transient species [excited states or phototransients (*18*)], and to make quality determinations of low absorption materials (*19*).

M. E. Long*
Robert L. Swofford
A. C. Albrecht
Department of Chemistry,
Cornell University,
Ithaca, New York 14853

References and Notes

1. J. P. Gordon, R. C. C. Leite, R. S. Moore, S. P. S. Porto, J. R. Whinnery, *J. Appl. Phys.* **36**, 3 (1965).
2. R. I. Carman and P. I. Kelley, *Appl Phys. Lett.* **12**, 241 (1968); F. W. Dabby, R. W. Boyko, C. V. Shan, J. R. Whinnery, *IEEE J. Quantum Electron.* **QE-5**, 516 (1969); D. C. Smith, *ibid.*, p. 600; J. N. Hayes, *Appl. Opt.* **11**, 455 (1972); P. Calmettes and C. Laj, *J. Phys. (Paris)* **33**, 125 (1972); H. Kleiman and R. W. O'Neil, *Appl. Phys. Lett.* **23**, 43 (1973); R. G. Buser and R. S. Rhode, *Appl. Opt.* **14**, 50 (1975).
3. C. Hu and J. R. Whinnery, *Appl. Opt.* **12**, 72 (1973).
4. J. R. Whinnery, *Acc. Chem. Res.* **7**, 225 (1974).
5. R. C. C. Leite, R. S. Moore, J. R. Whinnery, *Appl. Phys. Lett.* **5**, 141 (1964); D. Solimini, *Appl. Opt.* **5**, 1931 (1966); *J. Appl. Phys.* **37**, 3314 (1966); Y. Kohanzadeh and D. H. Auston, *IEEE J. Quantum Electron.* **QE-6**, 475 (1970).
6. A. E. Siegman, *An Introduction to Lasers and Masers* (McGraw-Hill, New York, 1971), chap. 8.
7. J. R. Whinnery, D. T. Miller, F. Dabby, *IEEE J. Quantum Electron.* **QE-3**, 382 (1967); S. A. Akhmanov, D. P. Krindach, A. V. Migulin, A. P. Sukhorukov, R. V. Khoklov, *ibid.* **QE-4**, 568 (1968); F. W. Dabby, T. K. Gustafson, J. R. Whinnery, Y. Kohanzadeh, P. L. Kelley, *Appl. Phys. Lett.* **16**, 362 (1970); W. G. Wagner and J. H. Marburger, *Opt. Commun.* **3**, 19 (1971); C. M. Vest, *Phys. Fluids* **17**, 1945 (1974); R. D. Boyd and C. M. Vest, *Appl. Phys. Lett.* **26**, 287 (1975).
8. R. L. Swofford and A. C. Albrecht, paper presented at the 30th Molecular Spectroscopy Symposium, Columbus, Ohio, 16 to 20 June 1975.
9. M. E. Long and A. C. Albrecht, paper presented at the 30th Molecular Spectroscopy Symposium, Columbus, Ohio, 16 to 20 June 1975.
10. A proposal for such an instrument, but for sensitive infrared spectroscopy, has been made independently by G. W. Flynn, in *Chemical and Biochemical Applications of Lasers*, C. B. Moore, Ed. (Academic Press, New York, 1974), p. 163.
11. R. L. Swofford, M. E. Long, A. C. Albrecht, in preparation.
12. J. W. Ellis, *Phys. Rev.* **32**, 906 (1928); *ibid.* **33**, 27 (1929); *Trans. Faraday Soc.* **25**, 888 (1929).
13. S. P. McGlynn, T. Azumi, M. Kasha, *J. Chem. Phys.* **40**, 507 (1964).
14. J. Stone, *J. Opt. Soc. Am.* **62**, 327 (1972); *Appl. Opt.* **12**, 1828 (1973).
15. L. B. Kreuzer, N. D. Kenyon, C. K. N. Patel, *Science* **177**, 347 (1972); W. R. Harshbarger and M.

B. Robin, *Acc. Chem. Res.* **6**, 329 (1973); A. Rosencwaig, *Anal. Chem.* **47**, 592A (1975).
16. A. G. Bell, *Proc. Am. Assoc. Advan. Sci.* **29**, 115 (1880); *Philos. Mag.* **11**, 510 (1881).
17. D. A. Pinnow and T. C. Rich, *Appl. Opt.* **12**, 984 (1973).
18. K. Darie, *Opt. Commun.* **4**, 238 (1971).
19. V. P. Egorov, *Sov. J. Quantum Electron.* **4**, 1184 (1975); A. V. Nurmikko, *Appl. Phys. Lett.* **26**, 175 (1975); D. C. Burham, *Appl. Opt.* **7**, 1727 (1970).
20. We are grateful for support from the National Science Foundation and the Materials Science Center of Cornell University. One of us (M.E.L.) thanks H. Mahr for many helpful discussions.
* Present address: Research Laboratories, Eastman Kodak Co., Rochester, New York 14650.

10 September 1975; revised 12 November 1975

Metaphase Chromosome Anomaly: Association with Drug Resistance and Cell-Specific Products

Abstract. *Large, homogeneously staining chromosome regions which lack the longitudinal differentiation ordinarily revealed by cytogenetic "banding" methods have been found in antifolate-resistant Chinese hamster cells and also in human neuroblastoma cells established in vitro. The drug-resistant cells are characterized by excessive production of the target enzyme, dihydrofolate reductase, while the human neuroblastoma cells have phenotypes of normal neuronal cells. The homogeneously staining region appears to represent a novel metaphase chromosome anomaly which may have functional significance in cells with specialized properties.*

Chromosome staining methods for revealing differential banding patterns along the length of metaphase chromosomes are a useful means of identifying structurally rearranged "marker" chromosomes (*1*). With that objective, we utilized a modified trypsin-Giemsa staining technique (*2*) for investigation of two groups of cell lines having strikingly long marker chromosomes as seen after conventional aceto-orcein staining. The first group comprised a series of amethopterin (methotrexate) and methasquin (a quinazoline antifolate) resistant Chinese hamster sublines (*3*) with very long, seemingly translocated segments on chromosomes 2 and 4 in particular. The second group consisted of continuously cultured human neuroblastoma cells which also possessed long, clearly abnormal marker chromosomes (*4*). Our initial and unanticipated finding was that the long chromosome segments in drug-resistant Chinese hamster cells did not "band," that is, did not manifest the relatively short, sequential regions of differential staining that characterize normal Chinese hamster chromosomes (*5*). We were thus enabled to recognize a similar anomaly occurring in two out of the four neuroblastoma lines which we analyzed. We now present some cytological features of the large, homogeneously staining region (HSR) present on specific chromosomes of antifolate-resistant Chinese hamster cells and on several chromosomes of human neuroblastoma cells.

Chinese hamster cells exposed to high concentrations of either amethopterin or methasquin developed high levels of resistance and cross-resistance to the antifolates

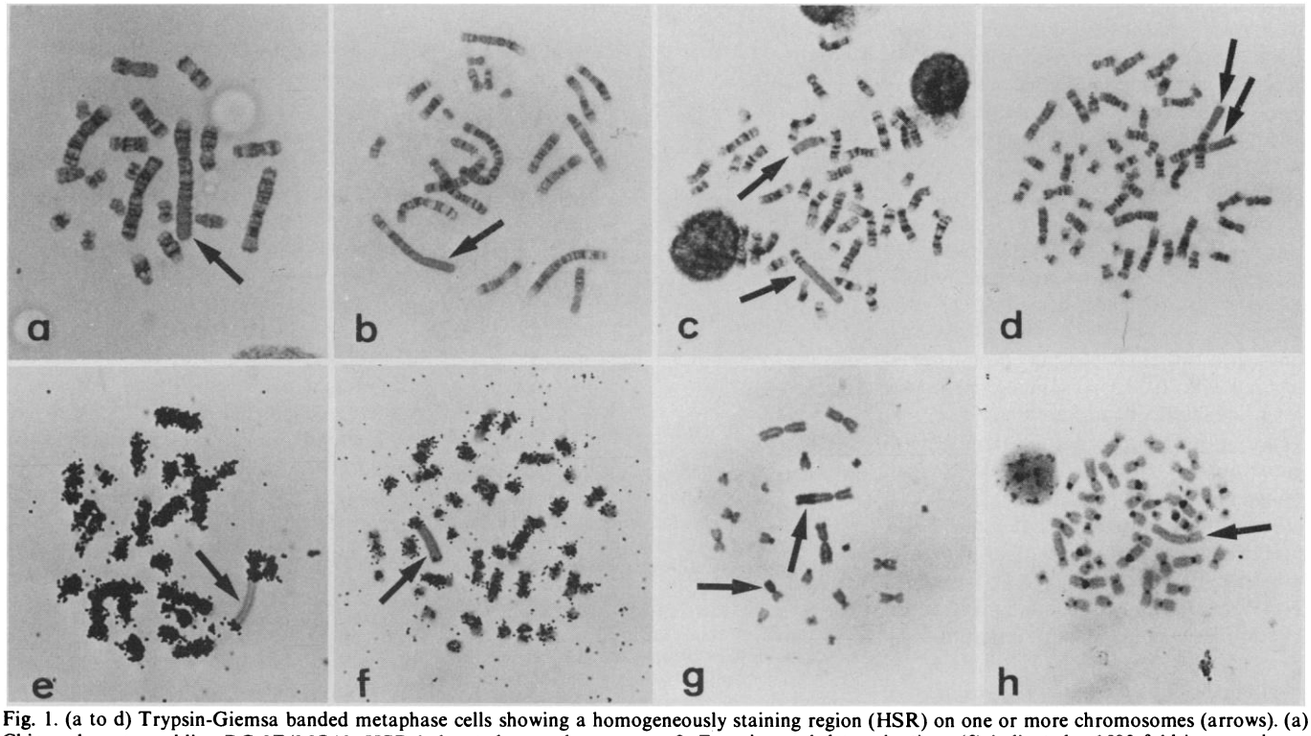

Fig. 1. (a to d) Trypsin-Giemsa banded metaphase cells showing a homogeneously staining region (HSR) on one or more chromosomes (arrows). (a) Chinese hamster subline DC-3F/MQ19. HSR is located on a chromosome 2. Experimental determinations (*3*) indicated a 1583-fold increase in resistance to methasquin and a 151-fold increase in specific activity of dihydrofolate reductase as compared to drug-sensitive parent cells. (b) Subline DC-3F/A3 cell showing unidentified marker chromosome with an HSR. The Chinese hamster line developed a 108,400-fold increase in resistance to amethopterin and a 170-fold increase in dihydrofolate reductase activity after exposure to drug. (c) Human neuroblastoma line SK-N-BE(2). In this cell, HSR's are located on a chromosome 10 and a chromosome 19. (d) A metaphase cell of the IMR-32 neuroblastoma line with overlapping HSR-bearing No. 1 chromosomes. (e) Heavily labeled DC-3F/A3 cell exposed to tritiated thymidine during third quarter of S phase. Except for distal end of arm, there are no silver grains over long segment (arrow) corresponding to HSR. (f) Radioautogram of a metaphase cell of human neuroblastoma line SK-N-BE(2). The No. 10 chromosome (arrow) with an HSR was identified by G-banding prior to application of photographic emulsion. (g) C-banded cell of DC-3F/MQ19 subline. Region corresponding to HSR of chromosome 2 (center arrow) and long arm (arrow) of an X chromosome show positive staining. The entirely heterochromatic X chromosome is absent. (h) C-banded metaphase cell of SK-N-BE(2) neuroblastoma line. Abnormally long chromosome (arrow) is presumptive HSR-bearing chromosome 6. Centromeric regions are strongly C-band positive, while segment or segments corresponding to an HSR in G- or Q-banded cells did not stain by the C-banding technique (× 756).