Open-ended photoacoustic spectroscopy cell for thin-layer chromatography and other applications

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DISCUSSION

Oxygen is most frequently measured with an oxygen electrode (23). This electrode is similar to our CL device. In both, oxygen diffuses across a membrane and then reacts to yield a measurable signal. Both devices measure oxygen partial pressure rather than concentration. In both devices, response varies considerably with temperature, requiring a correction factor for accurate measurements. Both devices have similar response times.

There are also some significant differences between an oxygen electrode and the CL oxygen probe. The CL oxygen probe as described here is more sensitive than the typical oxygen probe but is more subject to changing response due to reagent consumption. If the CL oxygen probe is operated with a less permeable membrane and/or a smaller membrane area, its response will be less sensitive but longer lived. For example, if the rate of oxygen consumption is decreased by a factor of 100, the CL oxygen probe should operate for 2-3 months without requiring new reagent. It would still have a detection limit comparable to most oxygen electrodes. Further improvements may be realized by substituting pure TMAE for the 10% EIA solution presently used in the oxygen probe. TMAE is a nonviscous liquid at room temperature while EIA is a solid.

The oxygen electrode is subject to interference from oxidizing gases such as chlorine which can diffuse through the membrane and be reduced at the cathode. It is also subject to interference from reducing gases such as H_2S which poison electrode response causing inaccurate measurements. These gases are not expected to interfere with the CL oxygen probe since they do not react with tetraaminoethylenes to yield CL.

The CL oxygen probe requires that ambient light be excluded. This is its most serious limitation relative to the oxygen electrode. Conceivably, this problem could be eliminated if an opaque membrane material could be found. The CL oxygen probe cannot be turned off like the electrode. It should be stored in an oxygen-free environment when not in use to prolong its lifetime.

We believe that the CL oxygen probe may prove to be a superior alternative to the oxygen electrode for many applications. However, considerable further work is required to verify that the CL probe can operate for extended times without replacing reagent as well as to show that the CL probe is free of interferences affecting the oxygen electrode. We plan to pursue this work by using a less permeable membrane material and TMAE as the CL reagent.

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Open-Ended Photoacoustic Spectroscopy Cell for Thin-Layer Chromatography and Other Applications

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The design and applications of an open-ended photoacoustic spectroscopy cell are described. This cell allows rapid nondestructive analysis of a variety of solid, liquid and gel samples. The quantitative analysis of samples on thin-layer chromatography (TLC) plates (e.g., rose bengal dye samples and developed palmitic acid chromatograms) is described. Spectra of spots on TLC plates containing 0.4 μg of rose bengal were obtained with a signal/noise ratio of 3.3.

Photoacoustic spectroscopy (PAS) has been used to study the optical and thermal properties of various opaque as well as transmitting samples of condensed phases (*1-3*). Several theoretical treatments (*4-6*) and a digital model (*7*) of the PAS effect have been published.

In the conventional PAS experiment the sample is sealed in a tightly closed, fixed volume, cell. The sample is then irradiated through the cell window with chopped monochromatic light. The radiant energy absorbed by the sample is converted by radiationless decay pathways to heat. This heat flows to the surrounding gas and generates a pressure pulse that is detected with a microphone connected to the sample chamber. The output from the microphone is fed to a lock-in-amplifier referenced at the frequency of the chopped in-

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cident light. The PAS absorption spectrum is then generated by plotting the lock-in-amplifier output vs. the wavelength of the incident light.

The necessity of a tightly closed cell puts some constraint on the nature of the sample examined, which must be of a form that fits into the PAS cell sample holder. For example, the analysis of samples on thin-layer chromatography (TLC) plates by PAS was first discussed in the literature in 1975 (*8*). More recently, Kirkbright and co-workers (*9*) have demonstrated the quantitative analysis of fluorescein samples on TLC plates. In these experiments the developed analyte spots were scraped from the thin-layer plates and placed in a sealed PAS cell for analysis. The authors noted difficulty in preventing the loss of analyte during the transfer from the plate to the sample cell and in preventing dilution of the sample of interest by inclusion of excess silica gel. Moreover, it is well-known that the PAS response is strongly dependent on the form of the sample. Thus the particle size in a powder sample or its degree of packing will affect the PAS signal.

We describe here a new PAS sample cell that allows the routine quantitative analysis of samples on precoated hard TLC plates without removal of the sample from the plate. Further, the cell reduces the restrictions on larger sample sizes while still maintaining a fixed, small internal volume (*10*). The cell need not be stationary with respect to the optical path of the PAS spectrometer and can be moved from point to point on a sample surface.

EXPERIMENTAL SECTION

Instrumentation. The PAS instrument was a single beam type and has been described elsewhere (*1*). The light source was a 2500-W high-pressure xenon lamp (Hanovia, Newark, NJ, Model 975C298). The collimated light from the lamp was focused through an $f/1$ quartz lens on the entrance slits of a $^1/_4$-m $f/3.6$ monochromator (Jarrell-Ash, Waltham, MA, Model 82560). The mechanical light chopper (Princeton Applied Research, Princeton, NJ, Model 192) provided a useful chopping range from 25 to 2000 Hz. The throughput from the lamp–monochromator combination averaged 1.1 mW over the region 400–800 nm. The output from the 1-in. ceramic microphone (General Radio Type 1560–9065, −61.6 dB re 1 V/μbar) was fed via shielded cable 91 cm in length to the differential inputs of a lock-in amplifier (Princeton Applied Research, Model 5204). The signal from the lock-in was then digitized and stored in an electronic recorder (Bascom-Turner, Model 8110). Normalization of the sample PAS spectra to the power spectrum of the lamp was accomplished by dividing the sample spectrum by the PAS spectrum of carbon black powder in the cell. Both sample and carbon black reference spectra were stored in the electronic recorder, and the division was performed by using the preprogrammed functions in the recorder memory.

Materials. Precoated hard-layer TLC plates (Catalog No. 47521) were obtained from Analtech, Newark, DE. Nafion membrane material was obtained from E. I. du Pont de Nemours and Co., Wilmington, DE. The Nafion was cleaned prior to use by boiling for 30 min in 1:1 nitric acid–water. This was followed by boiling for an additional 30 min in distilled water. After this treatment, the membrane material was clear and retained none of its original amber color. Rose bengal dye was technical grade and manufactured by Eastman Kodak, Co., Rochester, NY.

Chromatography Techniques. TLC plates were spotted with aqueous solutions of rose bengal dye with a microsyringe. The plates were allowed to air-dry in the dark, to prevent any possibility of photooxidation of the dye. Analysis of a particular sample spot was accomplished by placing the illuminated area of the PAS cell sample chamber over the analyte spot. Exact positioning of the cell was done by viewing the illuminated area of the plate and the sample spot through the glass backing of the TLC plate.

Palmitic acid was spotted near one end of the TLC plate with a microsyringe. The chromatograms were developed with a solvent system of 70% petroleum ether, 29% anhydrous ether, and 1% acetic acid. After development, the TLC plates were removed from the solvent tank and allowed to air-dry for 1 h. The plates were then sprayed with 3 M sulfuric acid and charred for 25 min at 160–170 °F. The developed chromatograms showed ill-defined elliptically shaped spots whose color varied from light brown at the edges to dark brown at the center, where the palmitic acid concentration was highest.

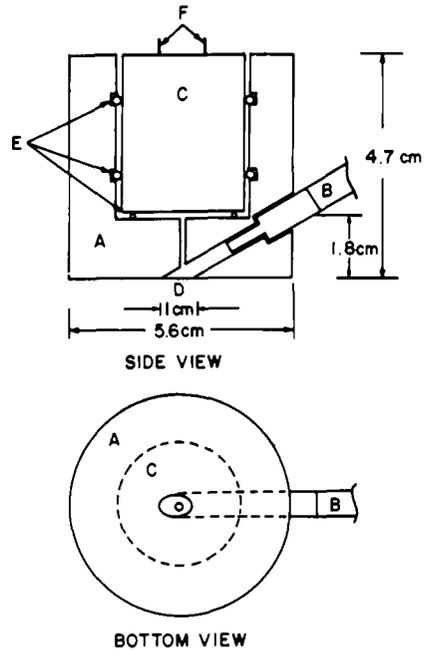

Figure 1. Open ended PAS cell; center section: (A) aluminum cell body; (B) fiber optic probe; (C) microphone; (D) illuminated area of sample chamber; (E) O-rings; (F) microphone leads.

Photoacoustic Cell. The cell, shown in Figure 1, was machined from aluminum with the bottom face highly polished. The light enters the cell through a flexible fiber optic probe as previously used by Cahen et al. in PAS (*11*); the other end of the 51 cm long probe is mounted at the exit slit of the monochromator. The fiber optic probe is sealed to the cell body with silicone rubber sealant (Dow Corning, Midland, MI). The microphone is O-ring sealed and electrically isolated from the aluminum cell. The total internal cell volume, including sample chamber, microphone, and connecting channel, is 1.13 cm^3. The illuminated area of the sample in the plane of the lower cell face is 0.35 cm^2. The total cell weight is 330 g.

The spectra of powdered samples were obtained by placing a small amount (1–2 mg) of sample on a flat surface, typically glass, and setting the sample chamber of the cell over the sample. The spectra of solution samples were obtained by placing the sample in a channel milled into the face of a flat surface, usually a Plexiglass sheet.

RESULTS AND DISCUSSION

The usual PAS cell is tightly sealed to prevent gas leakage which would lead to a decrease in the pressure in the cell and degradation of signal. In the open-ended PAS cell, the highly polished bottom face of the cell forms a partial seal by virtue of its intimate contact with the flat surface of the sample. The weight of the cell (330 g) is sufficient to keep the cell in close contact with the sample surface and to reduce the cell sensitivity to mechanical vibration that might be transmitted through the fiber optic probe. To isolate the cell from mechanical vibrations arising from blowers, scan motors, etc., that are located on the laboratory bench, we placed the cell and sample on a flat aluminum plate that is vibrationally isolated from the bench by a small, partially inflated inner tube. The inflation pressure which produced the greatest mechanical isolation is easily found by trial and error and depends to some extent on the frequency of the vibrational interferences. The inherent acoustical isolation of the cell is such that normal laboratory activity generated no interference. However, sharp acoustical interference was noticed during

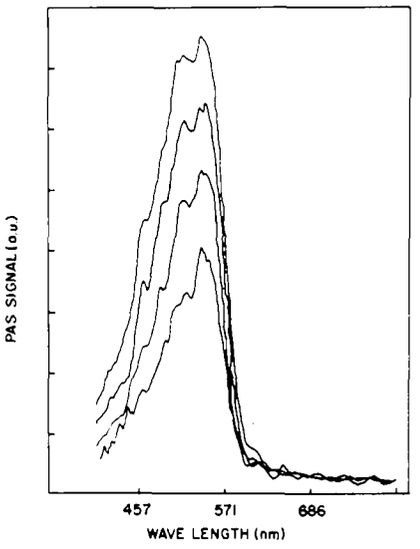

Figure 2. Mass of spots on TLC plate. Mass of dye in spots from top to bottom, respectively, is 12.6, 6.30, 3.15, and 1.58 μg.

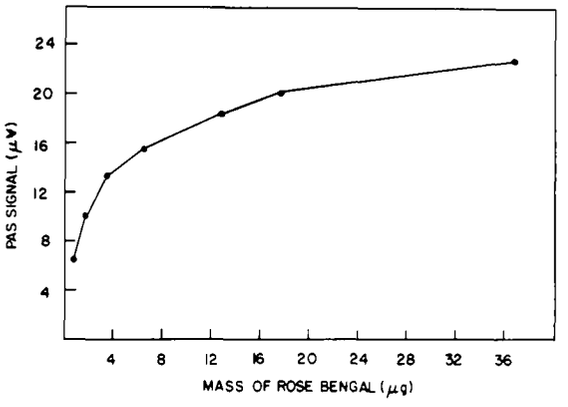

Figure 3. Calibration curve showing PAS signal (μV) vs. mass of rose bengal dye in the TLC sample spot (μg).

events such as door slams or sudden jolts to the laboratory bench. Further, it was necessary to move the lamp power supply, which is forced-air cooled by an integral 35 cm diameter fan and generated a considerable amount of noise, out of the room containing the spectrometer.

Aamodt and Murphy (*10*) showed that the PAS signal was inversely proportional to cell volume as long as the length of the gas volume was greater than the thermal diffusion length in the gas at the given chopping frequency. Thus, the internal cell volume was minimized, yielding the configuration shown in Figure 1. It may be possible to increase the cell sensitivity further by bringing the end of the fiber optic probe closer to the sample surface. Since this would necessitate lengthening the connecting channel between the sample chamber and the microphone, the optimum cell geometry would have to be determined.

Thin-Layer Chromatography Samples. Figure 2 shows the PAS spectra of different amounts of rose bengal dye samples on TLC plates. The magnitude of the PAS signal at a wavelength of 541 nm, corrected for the background signal from the blank silica gel, can be used to determine the amount of dye in the sample spot. The standard curve for the determination of the mass of dye in the analyte spot was obtained by spotting the TLC plates with 5-μL volumes of aqueous solutions containing known concentrations of rose bengal (0.16–7.33 μg/μL). The calibration curve is shown in Figure 3. The smallest mass of dye analyzed was 0.4 μg, which yielded a signal-to-noise ratio of 3.3 for a single scan with a lock-in-amplifier time constant of 3.0 s. Clearly signal averaging techniques could be used to improve the sensitivity further.

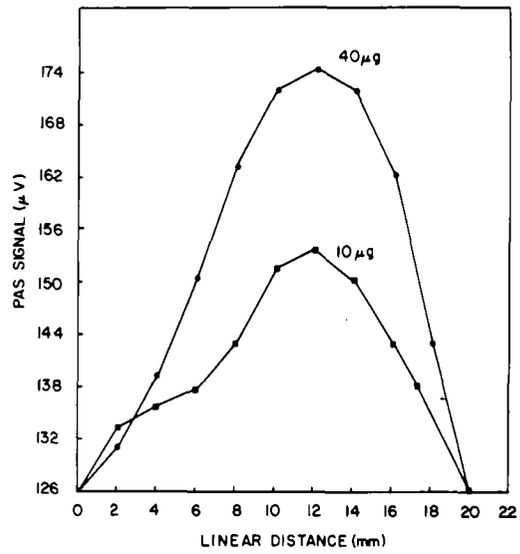

Figure 4. Analysis of palmitic acid chromatogram, PAS signal (μV) vs. linear distance along the TLC plate (mm) for (●) 40 and (■) 10 μg of palmitic acid, white light (xenon lamp) excitation.

The spectra of Figure 2 are typical of the rose bengal structure (*12*). The calibration curve shown in Figure 3 was obtained by using spots of constant area but with varying masses of dye. The relative standard deviation of the measurements was 1.1%. The absorption coefficient of the smallest sample measured corresponded to a $\beta = 4\ cm^{-1}$. The nonlinearity of the calibration curve may result from a number of factors (e.g., deviation from Beer's law) but probably can be attributed to the thermal saturation effect present in PAS. The extent of the nonlinearity depends on the relationship between the optical properties of the absorbing sample and the thermal properties (thermal diffusivity) of the silica gel substrate (*4*). In principle this effect should provide an optical method for determination of the thermal properties of the nonabsorbing substrate through the experimental determination of the critical value of the sample system (*7*).

A graph of the PAS signal magnitude vs. linear distance along the plate for palmitic acid chromatograms is shown in Figure 4. The spectra of palmitic acid samples were obtained with polychromatic white light. The higher incident light intensity led to a corresponding increase in the PAS signal. As noted, the developed chromatograms of palmitic acid produced spots that were diffuse and larger in area than the illuminated area in the sample chamber of the PAS cell. A micrometer drive from the specimen stage of a microscope was used to scan the PAS cell over the area of the analyte spot to analyze these samples. The polished lower face of the cell allowed the cell to move easily across the surface of the TLC plate without disturbing the integrity of the silica gel. The cell was initially positioned at a point before the leading edge of the spot, where no developed analyte could be observed. The signal level at this point was taken as the background signal level. The cell was then positioned a fixed distance, e.g., 1 mm, and the PAS signal level noted. This was repeated until the entire area of the analyte spot had been examined. The mass of palmitic acid present was proportional to the area under the curve obtained by plotting PAS signal magnitude vs. linear distance along the plate. A linear calibration graph ($r^2 = 0.996$) was obtained over the range 10–80 μg of palmitic acid.

Other Samples. A variety of other types of samples were examined to gauge the scope of applications of the cell. Among these were single-crystal semiconducting cadmium sulfide,

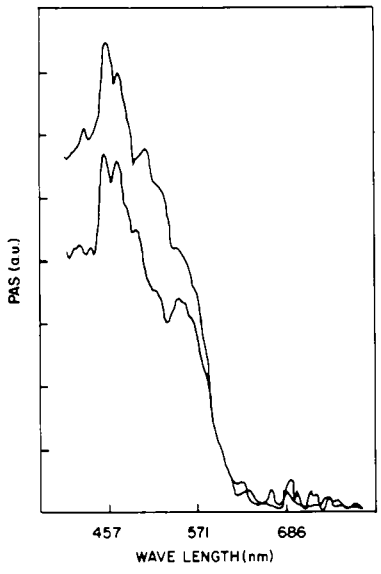

Figure 5. PAS of cosmetics: Cadillac Red lipstick on cotton (upper trace); Dragon Red nail polish on glass (lower trace).

holmium oxide powder, and cytochrome *c* crystals (*13*). In all cases the spectra taken with the open-ended photoacoustic cell were identical with transmission optical absorption spectra for these same samples.

A smear of Revlon Cadillac Red lipstick on white cotton and Dragon Red nail polish spotted on a glass plate are shown in Figure 5. These cosmetic samples indicate the ease of application of the PAS technique to this type of sample in the environment where they may be encountered, e.g., a lipstick smear on white cotton.

Recent experiments in our laboratory have been concerned with the absorption of various ions into ion-exchange membranes, such as the perfluorinated cation exchange material Nafion (*14*). A variety of materials can be taken up by this membrane, and characterization by PAS (or spectrophotometric techniques) is possible. For example, Figure 6 is the PAS spectrum of $Ru(bpy)_3^{2+}$ (bpy = bipyridine) absorbed in a Nafion membrane. The membrane is a 10 mL thick disk of area 0.283 cm^2. The membrane had been allowed to stand overnight in 2 mL of an aqueous solution containing 5 mM $Ru^{II}(bpy)_3$. After approximately 12 h, the solution was clear and the Nafion showed the intense red-orange color typical of $Ru^{II}(bpy)_3$. The PAS technique may provide a useful tool for probing events within the membrane and determining concentrations of absorbed species. A PAS spectrum of an aqueous rose bengal solution (concentration = 7.33 $\mu g/mL$) was obtained by placing 0.15 mL in a sample cup milled into a Plexiglass plate (*13*). The sample cup had an area of 0.8 cm^2 and a depth of 0.1 cm. The spectrum obtained was typical of rose bengal dye.

CONCLUSIONS

The open-ended PAS cell provides a convenient and accurate method for the nondestructive analysis of TLC plates and other flat, large area samples. Although the flexible fiber optic probe limits spectral investigations to the region above 385 nm because of its transmittance characteristics, it does allow the cell to be moved to the sample and easily positioned

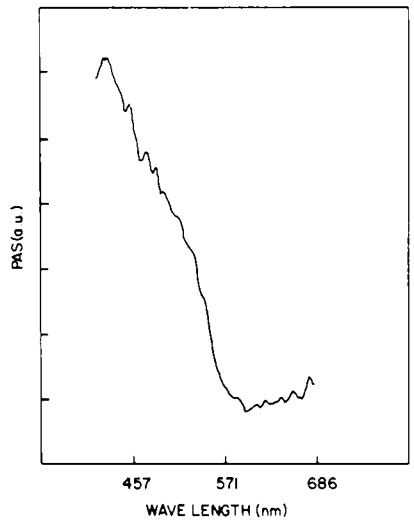

Figure 6. PAS of $Ru(bpy)_3^{2+}$ in Nafion membrane.

at different places on the sample surface. The sensitivity and lower limits of detection could be improved by replacing the ceramic microphone used here with a condenser microphone of similar size. Since the open-ended PAS cell is not fixed in position, care must be taken to isolate the cell from mechanical vibration. The condenser microphone has a 17-dB advantage when compared to a ceramic microphone of the same size with respect to reduced sensitivity to mechanical vibration (*15*). Refinements in the sample-detector chamber geometry should lead to further improvements in cell sensitivity.

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