Crossed-beam apparatus for simultaneous spectrophotometric observation and microwave exposure of biochemical samples*

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A spectrophotometer has been adapted for use as a simultaneous observation–exposure system for irradiation of biochemical samples by 1.7–2.6 GHz electromagnetic radiation. A waveguide applicator is mounted vertically in the sample compartment of a Cary 15 spectrophotometer such that the sample can be placed simultaneously in the light path and in a slot in the applicator. A standard 1-cm path square cell contains the sample which is irradiated through the open top of the cuvette. Temperature of the sample and an unirradiated reference are monitored continuously without measurable interference from the microwave field. Temperature can be controlled within a few tenths of a degree between 15° and 40°C, and an absorbed dose rate can be calculated using thermal measurement techniques. Microwave generator output and reflected power are monitored at all times. The frequency can be changed easily and standing waves minimized by adjusting a waveguide tuning section. The microwave energy within the basic frequency range can be applied as continuous wave or modulated. The quality of the absorption spectra is not measurably impaired, and the flexibility of the spectrophotometric technique remains intact.

INTRODUCTION

As the potential for exposing large segments of the population to microwave radiation has increased in recent years, so has concern for effects other than those thermally induced which may be present at low radiation levels.[1–3] Until recently, most investigators exposed their test system to radiation and analyzed the system for effects after exposure. However, the most likely time to find an effect due to direct interaction of microwave radiation with the test system may be during the irradiation. Several workers have designed ingenious exposure facilities and experimental systems in an attempt to take advantage of this point with varying degrees of success[4–7]; however, in such cases, information concerning absorbed energy often is not sufficient to make meaningful comparisons with other experiments.

Spectrophotometric measurements have been extremely fruitful in providing information about structural details of molecules as well as mechanism of action of many chemical and biochemical processes. This paper describes the adaptation of a double beam spectrophotometer such that a sample may be exposed to 1.7–2.6 GHz radiation while coincident spectroscopic measurements are being made, thus combining the wide adaptability of the spectrophotometric technique with the concept of simultaneous irradiation. The double beam capability of the spectrophotometer allows *direct* comparison of an irradiated sample to an identical unirradiated sample. Furthermore, absorbed dose rate measurements can be made. This instrumentation can be extremely useful for investigating the effects of microwave radiation on a wide variety of *in vitro* systems.

DESCRIPTION OF THE APPARATUS

A Cary 15 spectrophotometer provides the capability for spectral measurement between 200 and 800 nm and is the basis for the apparatus, schematically represented in Fig. 1. The microwave energy is generated by an AIL 125 source. The output of the generator passes through a Narda coaxial directional coupler, reverse mounted so that the reflected energy can be monitored. The output is then fed via a coaxial cable to a coax-to-waveguide adapter (Raytheon, cat. no. 7099-5002G1), then through a Microlab/FXR 310 series tuner (cat. no. R130A), and finally to an applicator section (Raytheon, cat. no. 7097-1001G1) which deposits the microwave energy in the sample. Reflected power is measured using a calibrated thermoelectric-type power meter attached to the side port of the directional coupler. Forward power is monitored by connecting another calibrated thermoelectric power meter to the "sample rf" port of the generator, which in this case is a measured 30.0±0.2 dB down from the main output port. An oscilloscope is connected to the detected output ("modulation monitor") of the generator in order to monitor the generator waveform and modulation envelope.

Microwave energy is propagated in the basic TE_{10} mode using RG-104/U waveguide (internal dimensions 10.92 ×5.46 cm). As the microwave radiation propagates along the waveguide assembly, the electric field vector is parallel to the narrow dimension, and the magnetic field vector is parallel to the wide dimension of the guide. The applicator tapers in the narrow or "*b*" dimension to about 1 cm inside (see Fig. 2). An axial slot 1.43×7.30 cm cut in the applicator is large enough to accommodate a standard 1-cm path

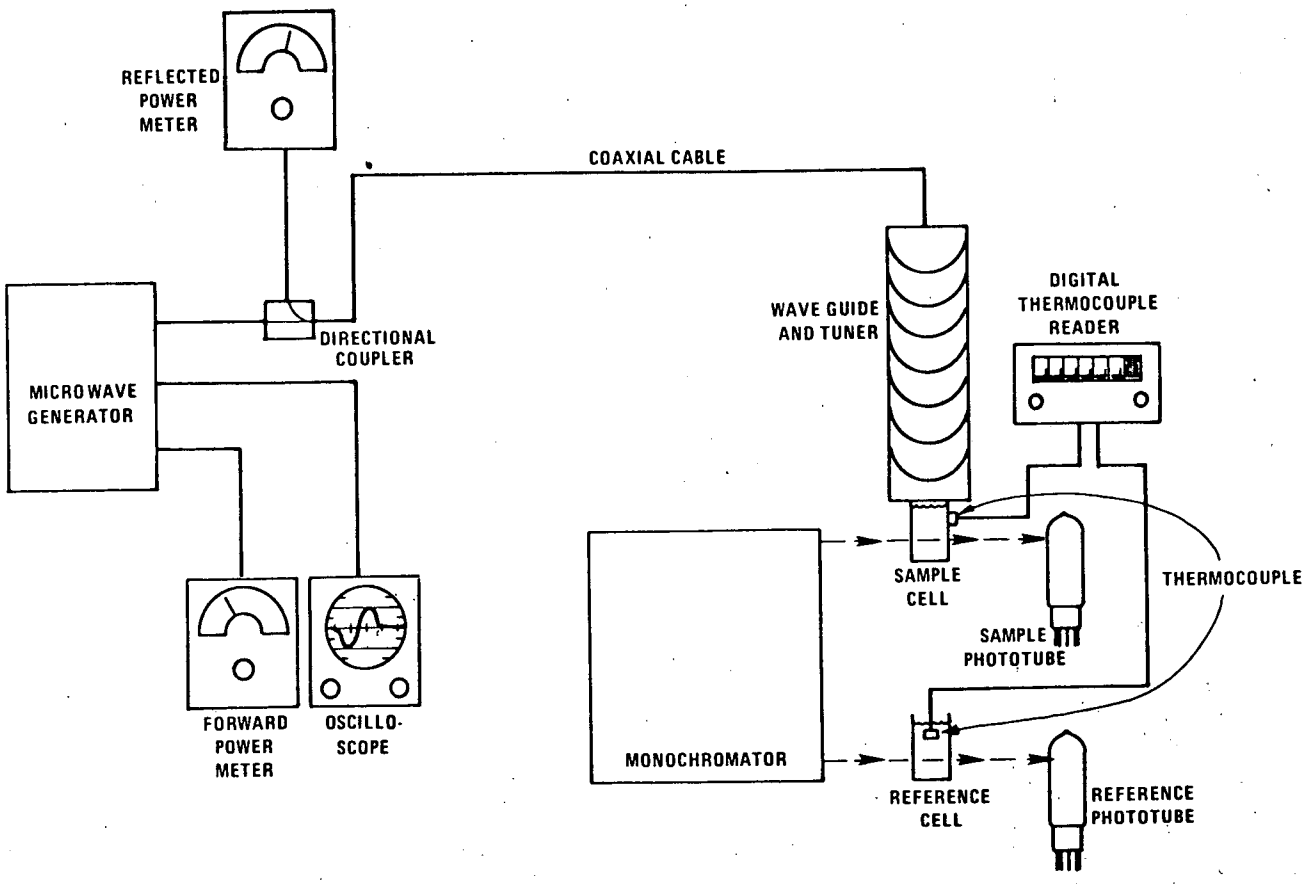

FIG. 1. Schematic of crossed-beam apparatus. The spectrophotometer is represented by a monochromator and phototubes only for simplicity.

spectrophotometer cell (outside dimensions 1.25×1.25×4.8 cm high).

The waveguide assembly is suspended over the sample (upper) compartment of the Cary 15 in such a way that the applicator is positioned in the sample compartment. The axial slot is aligned so that the light beam passes unobstructed through the slot (and cell, if present) and then to the phototube detector. The sample cell is held in position by a custom made Plexiglas holder. Figure 2 illustrates how the cell can be aligned in the light path of the spectrophotometer and simultaneously in the slot of the waveguide termination. The holder is constructed to slide in the base plate along the direction of the light path in order to facilitate inserting and removing the sample cell.

Except for the region occupied by the sample cell, the applicator is terminated by a short (i.e., the waveguide is simply closed at the end). In order to avoid excessive mismatch conditions, foam absorbing material possessing a high loss factor and permittivity close to unity has been placed on each side of the slot in the applicator. This helps to absorb the unwanted energy; use of the stub tuner allows a very close match to be made between the load and the generator. The tuning procedure will be described later.

The microwave energy enters the sample cell from the top and passes into the sample which is about 3 cm in depth. This arrangement cannot be expected to yield a uniform distribution of microwave energy absorption throughout the sample, and indeed a theoretical calculation confirms this point. This calculation is discussed in a later section.

Since the energy distribution in the sample is not uniform, it is very important to be able to measure the thermal properties of the portion of the sample that the spectrophotometer is observing. To this end, a microthermocouple (Omega Engineering) has been installed through the frosted side of a spectrophotometer cell. The thermocouple is positioned approximately 1 mm above the light path through the cell, allowing continuous measurement of the temperature of the sample just above that being observed spectrally. The solution whose temperature is measured, therefore, is as close to that which is being observed spectrally as is possible without interfering with the light beam. This procedure tends to overestimate slightly the temperature of the solution being observed spectrally. The thermocouple readings are monitored continuously by a digital thermocouple thermometer (Doric DS-350). A constant heat sink for the sample cell is maintained by circulating water from a constant temperature bath through the sample compartment walls. Temperature of the sample can be controlled under irradiation over the range 15°–40°C. A temperature rise from equilibrium of 20°C or more can be attained in the irradiated sample.

The reference cell, which is not irradiated, is maintained at the desired temperature by a second constant temperature bath circulating water through a jacketed cell holder. The temperature of the reference solution is also monitored continuously as above, except that the thermocouple is placed in the solution through the open top of the cell. In this manner the temperatures of both the irradiated and unir-

radiated solutions can be matched and maintained within a few tenths of a degree during the experiment.

MICROWAVE SYSTEM CALIBRATION AND USE

Frequency

Each time a frequency setting is made or checked, the output of the generator is monitored either using a frequency counter or a wave meter (PRD LS-518) coupled to a voltmeter. A frequency can be set to three significant figures and read to four.

VSWR

Voltage-standing-wave ratio (VSWR) measurements are made after each frequency change or when otherwise necessary. The VSWR is minimized with the sample in place by adjusting the stub tuner in the waveguide portion of the system. Adjustments are made until VSWR ≤ 1.15 (reflected power $\leq 0.5\%$). The measurement is carried out using either the slotted-line technique or by minimizing reflected power at constant forward power. The latter technique has been found in practice to be much quicker and easier.

Radiation leakage

Leakage of microwave radiation was checked at all points in the system using a Narda model 8100 survey meter. No detectable leaks were found except at the slot in the applicator, where leakage was less than 20 $\mu W/cm^2$ with the sample cell in position.

Thermocouple interaction with microwave field

The microthermocouple is always present in the sample during irradiation. Therefore, the problem of interaction between the probe and the microwave field must be considered. In this case we have sought to minimize the physical presence of the probe. Each thermocouple wire is 0.013 cm in diameter, and the junction is 0.028 cm in diameter. Furthermore, only about 1 cm of the probe is under the waveguide applicator. Half of this is in the solution, about 0.7 cm below the surface (the cell is always filled with 3.00 ml of solution), and the other half is outside the cell but protected from the radiation by the absorbing material adjacent to it. In practice, there is no pickup that is detected as an apparent change in temperature when the radiation is turned on or off. If the thermocouple is merely placed over the top of the cell into the sample, however, an apparent instantaneous

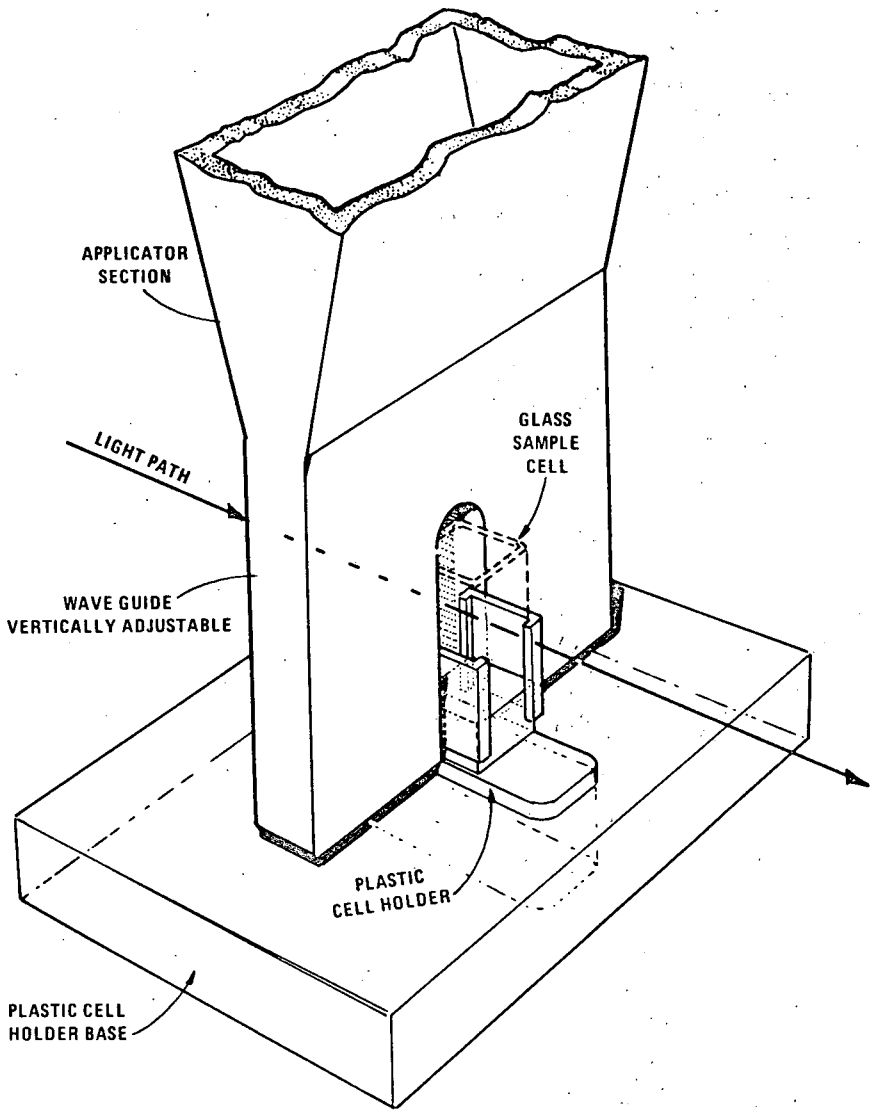

FIG. 2. Applicator and sample cell. The sample cell, held by the plastic cell holder, is illustrated partially inserted under the microwave applicator.

TABLE I. Temperature distribution in 3.0 ml 0.02 *M* KCl irradiated for 30 min. Temperature at equilibrium before irradiation, 20.7°C.

Distance below sample surface (cm)	Temperature (°C)
0.4	29.4
0.9	29.4
1.4	29.2
1.9	28.9
2.4	28.2
2.9	26.9

temperature change is detected by turning the generator on or off. This apparent change is about 0.2°C per watt of generator output.

The second manifestation of probe interaction is direct heating of the probe by the radiation, which subsequently transfers heat to the sample. Again, the small size of the probe compared to the sample, plus the fact that the heat capacity of water is much higher than that of metals, would make the likelihood of any significant heating of the sample appear remote. Temperature measurements were performed, however, with and without a thermocouple present in a sample during irradiation. No differences were detected within the reproducibility of the experiment (0.2°C). Thus, we concluded that probe interaction with the microwave field did not significantly affect our measurements.

Temperature distribution in sample

As noted previously the temperature distribution in the sample cannot be expected to be uniform in this experiment. The strong attenuation of the radiation of this frequency range by an aqueous sample should lead to significantly less energy being deposited at the bottom of the sample than at the top. In addition, cooling of the sample may be more efficient in the lower part of the cuvette simply because the bottom provides additional area for the transfer of heat. In order to obtain a temperature profile, measurements as a function of sample depth were taken at half-centimeter increments using a microthermocouple probe. These readings were made after the 0.02 *M* KCl sample had been irradiated for 30 min at 2450 MHz and 1.0 W output power.

The results, summarized in Table I, show that the temperature is fairly uniform in the top half of the sample but drops rapidly in the bottom third of the sample. The permanently installed thermocouple is located 0.7 cm from the sample surface, and the light path is located between 0.8 and 1.8 cm from the surface. Because the temperature rise at 0.9 cm is 8.7°C and at 1.9 cm 8.2°C, there is less than 6% variation of temperature over the portion of sample being observed spectrophotometrically. Thus, the spectrophotometer is monitoring a portion of the sample that is irradiated at a relatively uniform rate of energy deposition, and the thermocouple mounted in the cuvette is giving an accurate representation of that rate.

THEORETICAL ANALYSIS OF WAVEGUIDE SYSTEM

By means of a conventional transmission line analysis of the loaded waveguide system, approximate solutions can be obtained for the distribution of energy deposition along the cell and for the total power absorbed by the sample cell. A complete solution of this problem requires a knowledge of both the characteristic impedance and the complex propagation factor for the three different regions of the waveguide transmission line (see Fig. 3). Region 2 represents the test cell and is assumed to be composed of 0.02 molar KCl solution whose dielectric parameters at 2.45 GHz are given by $\epsilon_r = 76$ for relative permittivity and $\sigma = 1$ mho/m for conductivity. Two simplifying approximations may be made for this region, based on the finite but sufficiently low conductivity of the dielectric: the propagation factor can be assumed to be the same as that obtained for the case of a lossless dielectric, and the characteristic impedance may be assumed to be purely real. Expressions can then be developed for the propagating electric and magnetic fields at the center of the waveguide ($x = a/2$) in the three different regions, assuming that the fields in all three regions conform to the basic TE_{10} mode distribution. By matching tangential components of E and H fields at the two boundaries between the various regions, it is possible to solve for the unknown amplitude constants in region 2, thereby obtaining an expression for the normalized electric field in the cell $|E_{2y}/E_0|^2$ as a function of distance "z" along the cell. Using this expression and the values obtained earlier for the various propagation characteristics of the waveguide system, computations are then made of the normalized power distribution in the cell (see Fig. 4).

It is apparent that only about 18% of the *incident* power is transmitted into the cell. Note how the fields in the cell are rapidly attenuated by the lossy dielectric; the energy remaining at the base of the cell is only 4% of that which is incident. If the small sinusoidal component created by standing waves in the system is neglected and an average exponential decay curve is drawn, as shown in Fig. 4, then the percentage of incident power actually absorbed by the sample is approximately 16%.

These theoretical curves represent instantaneous power deposition in the aqueous sample. The measured values of temperature in Table I represent a steady state situation where thermal mixing of neighboring layers is taking place. However, it is interesting to note that the measurement and the calculations both show a relatively constant area of energy deposition between 0.4 and 1.4 cm below the surface.

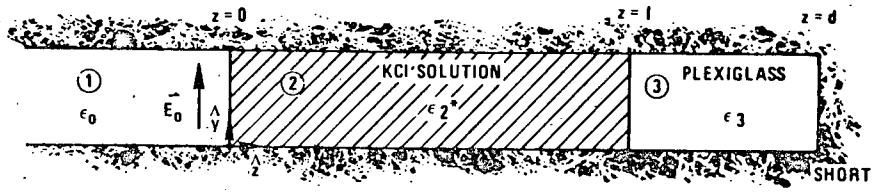

FIG. 3. Transmission line model for cell and base. The model represents a cross section taken at the center of the wide side of the tapered waveguide, $x = a/2$.

MEASUREMENT OF DOSE ABSORBED BY SAMPLE

Because the exposure of the sample is essentially a waveguide exposure, measurement of the absorbed dose rate is the most convenient method of quantifying dose for this system. It is also the most useful measure for comparing *in vitro* exposures with one another or with animal exposures. This measurement may be accomplished by assuming that all the microwave energy absorbed is converted to heat within a short period of time. The thermal energy deposited in the sample can be calculated from the cooling rate constant of the sample in the exposure system and the temperature change induced in the sample by the radiation. From these data the absorbed dose rate may be calculated in milliwatts per gram or other convenient units. Details of this method will be presented elsewhere. Absorbed dose rates up to 200 mW/g can be conveniently obtained from this system.

Using this method the dose rate delivered to the sample (at the thermocouple location) by a generator output power of 1 W has been measured to be 60 mW/g. From the previous section the power absorbed in the 3-ml sample has been calculated to be 16% of the incident power or 160 mW for 1 W of generator output. For an aqueous sample the absorbed dose rate averaged throughout the sample would be about 53 mW/g. Allowing for the nonuniform deposition of power in the sample, these values are in excellent agreement.

SPECTROPHOTOMETER TESTS

Because the spectrophotometer was not designed for the application described in this paper, some checks must be made in order to determine that the absorption spectra do not contain artifacts which are due to the presence of the microwave equipment.

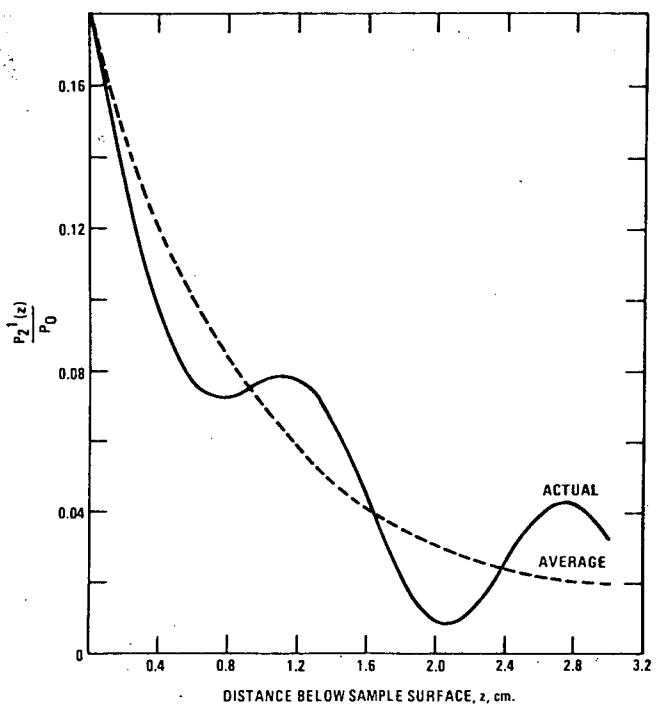

FIG. 4. Normalized power transfer along axis of cell. The ratio of instantaneous power distribution in the sample to incident power is represented as a function of distance below the surface of the sample.

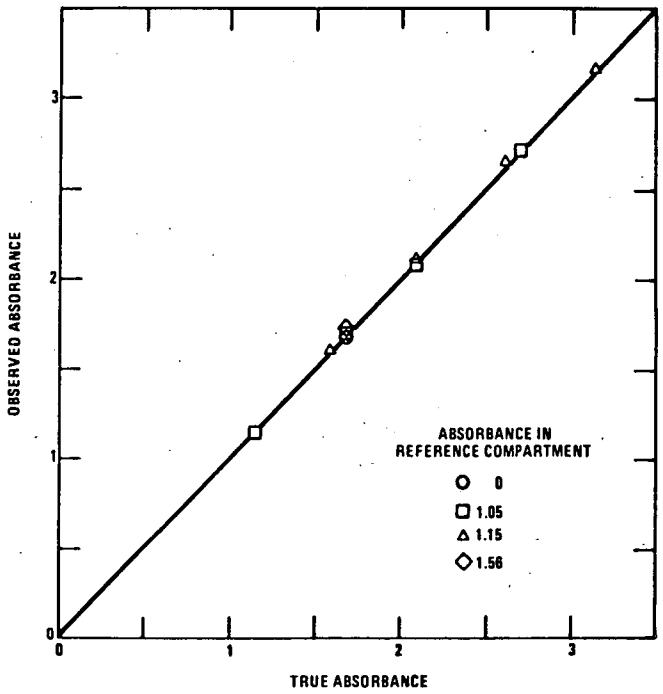

FIG. 5. Stray light measurement. The observed absorbance plotted vs true absorbance shows no negative deviation from Beer's law (line) to $A=3.1$.

Fidelity of absorption spectra

A series of absorption spectra were measured to ensure that the quality of the spectrophotometric measurements has not been affected. The spectra were run from 300 to 240 nm using a protein solution having a maximum absorbance of 1.0 at 280 nm. Aliquots of the same solution were placed in the reference and sample compartments simultaneously, and the spectra were recorded on the expanded scale (0.10 absorbance units full scale). This procedure maximized the probability of identifying a deviation from the normal result.

Spectra were run under the following conditions: (1) empty cells in both compartments and no microwave equipment installed (to establish a basis for comparison); (2) protein solution in each compartment, no microwave equipment; (3) as in (2) but using the plastic cell holder; (4) as in (3) but with the waveguide components in place; and (5) as in (4) but using the cell with the thermocouple implanted. No differences were detected between the results of any of these measurements.

Stray light

Because the sample chamber cannot be closed in the normal manner when the waveguide apparatus is in place, a black rubberized cloth has been used to keep outside light from entering the sample compartment and ultimately reaching the phototube. To establish the effectiveness of this procedure, measurements to detect the presence of stray light were made.

Neutral density filters were placed in the sample and reference compartments singly and in combination so that

measurements could be made up to an absorbance of about 3.1 at 280 nm. The data are plotted in Fig. 5. The line represents Beer's law, and the effect of stray light would be a negative deviation from this line. The data show no such deviation up to a true absorbance of 3.0, indicating that the stray light in the sample compartment is less than 0.01%,[8,9] or within a factor of 10 of the specification for the unmodified instrument at 280 nm.

*This report has been reviewed by the Office of Research and Development, EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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