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REPORT NO. 209

**SPECIAL CUVETTES FOR SPECTROPHOTOMETRIC
MEASUREMENTS OF MICRO-ORGANISMS AND LIVING TISSUES***

by

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ABSTRACT

SPECIAL CUVETTES FOR SPECTROPHOTOMETRIC MEASUREMENTS OF MICRO-ORGANISMS AND LIVING TISSUES

OBJECT

To construct cuvettes for spectrophotometry in which the biological conditions can be modified during the measurement.

RESULTS AND CONCLUSIONS

The cuvettes gave perfect service in combination with a modified Beckman DR spectrophotometer and with a General Electric recording spectrophotometer.

RECOMMENDATIONS

None.

Submitted 19 August 1955 by:
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SPECIAL CUVETTES FOR SPECTROPHOTOMETRIC MEASUREMENTS OF MICRO-ORGANISMS AND LIVING TISSUES

I. INTRODUCTION

In the course of the study of the spectral reflectance of human and animal skin over a wide spectral range (1-5) the integrating sphere (6) and its combination with various spectrophotometers, originally not equipped with it, (1, 7, 8) has been thoroughly investigated. Jacquez has demonstrated that this improved method of measuring spectral reflectance is versatile and applicable also to spectrophotometric absorption measurements on turbid and even opaque systems. He has obtained absorption spectra of living tissues and micro-organisms which compare favorably with those obtained with more elaborate and sensitive techniques (9). It is expected that the improved method will be useful in other fields, such as the study of the kinetics of respiratory enzymes, photosynthesis, and photoreactivation.

In Jacquez's experiments two special cuvettes, one for suspensions of micro-organisms, and a slightly different one for tissue samples, backed by a block of MgCO_3 during the reflectance measurement were used (9). They permitted changing the biological conditions of the systems during measurement without removing the sample from the cuvette. To facilitate further applications of the improved method, the cuvettes are described here in detail.

II. DESIGN, FUNCTION AND ASSEMBLY

The basic design for the two cuvettes is as follows. Two plates of fused quartz, each of which was 1/16 in. thick and 10 cm in diameter, were held together by 4 small screw clamps placed along the periphery. In one of the cuvettes used, a Plexiglass spacer ring with a 5.4 cm inner diameter was sealed tightly between the two quartz plates by applying a thin layer of stopcock grease or petrolatum on each face of the ring; this cuvette was used to measure the reflectance of suspensions. In the other cuvette, a Plexiglass spacer ring with a 7 cm inner diameter was sealed between the plates; this cuvette was used to measure the reflectance of tissue samples. Spacers of different thicknesses (one-fourth to one-eighth in.) can be used to vary the length of the light path or to accommodate tissue samples of different thickness.

The difference between the two types of cuvettes is in the design of the spacers. The spacer used for the suspension cell, shown in

Figure 1A, has four radial bores holding hypodermic needles (15 gauge, both ends cut off). The needles are sealed in with chloroform. They provide openings for admitting the sample into the cell, for adding chemicals and for the inlet and outlet of gases. The bubbling space at the gas outlet prevents the liquid from being forced out. The suspension cuvette is assembled first and then filled with the system to be measured.

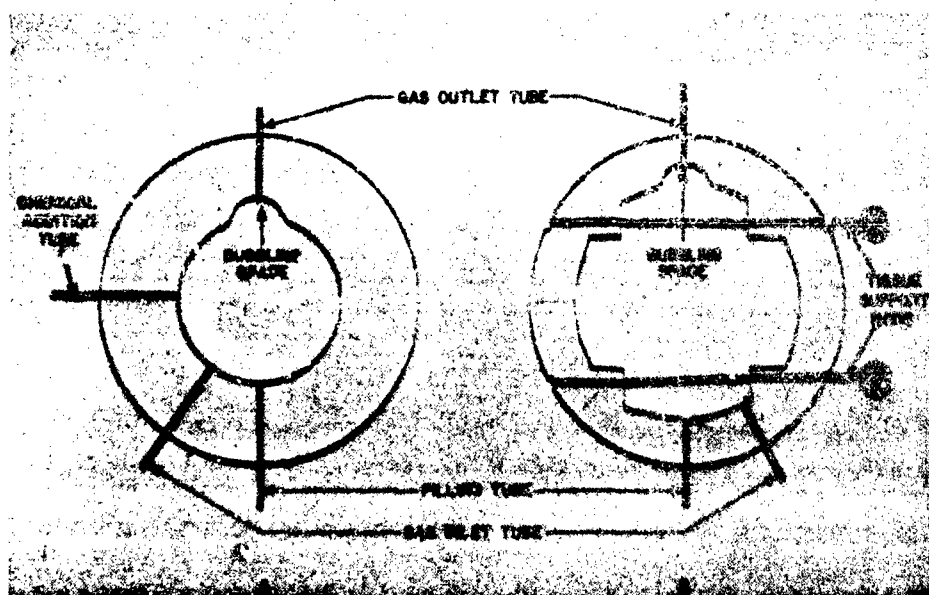


FIG. 1 - THE SPACERS FOR THE SUSPENSION (A) AND TISSUE (B) CUVETTES.

The spacer for the tissue cuvette, with a slightly different shape as shown in Figure 1B, has only three radial bores with sealed-in hypodermic needles. In addition, two parallel nickel-silver rods, 1/16 in. thick, are sealed into the spacer.

Two opposite edges of the tissue sample (usually rectangular) can be fastened to the rods with at least four 11 mm Michelle clips on each edge holding the sample flat. The rods and clips serve as electrodes for electrical stimulation of the sample (9). For most tissue samples a one-fourth in. spacer is convenient. This cuvette must be assembled after the tissue sample is mounted. It is shown, containing a strip of abdominal rat muscle, in Figure 2.

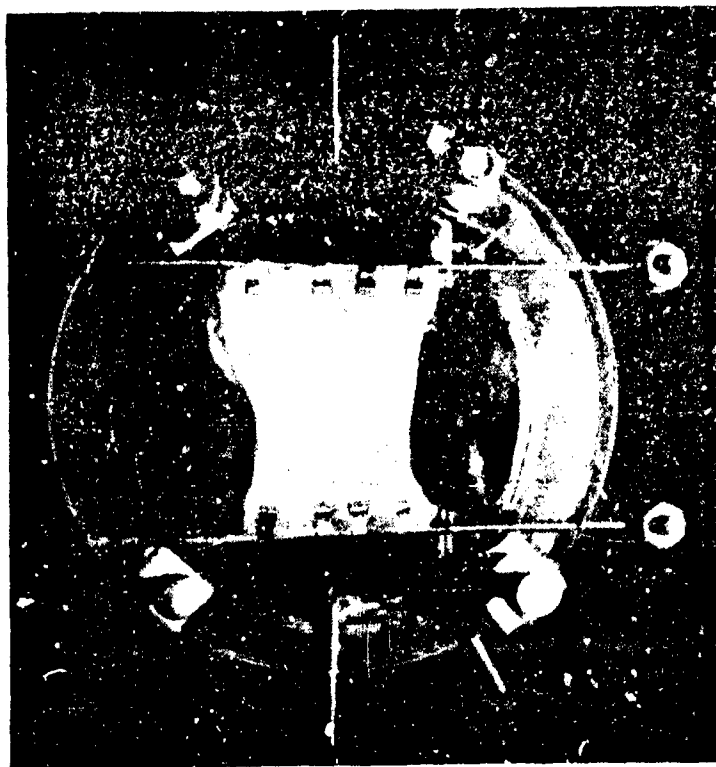


FIG. 2 - THE ASSEMBLED TISSUE CUVETTE

III. DISCUSSION AND CONCLUSIONS

The two cuvettes gave perfect service in combination with the modified (8) Beckman DR spectrophotometer. They were also used in combination with the GE recording spectrophotometer, in this case without the screw clamps because the spring-activated covers for the openings of the integrating sphere supplied enough pressure for holding the cell parts tight.

IV. RECOMMENDATIONS

None.

V. BIBLIOGRAPHY

1. Jacquez, J. A., J. T. Randall, M. Goodman, W. McKeehan, J. M. Dimitroff and H. F. Kuppenheim. The Spectral Reflectance of Human Skin in the Region 235-1000 m μ . AMRL Report No. 159, Fort Knox, Kentucky, 1 December 1954.
2. Jacquez, J. A., J. Huss, W. McKeehan, J. M. Dimitroff and H. F. Kuppenheim. The Spectral Reflectance of Human Skin in the Region 0.7-2.6 μ . AMRL Report No. 189, Fort Knox, Kentucky, 18 April 1955.
3. Jacquez, J. A., H. F. Kuppenheim, J. M. Dimitroff, W. McKeehan and J. Huss. The Spectral Reflectance of Human Skin in the Region 235-700 m μ . AMRL Report No. 193, Fort Knox, Kentucky, 20 April 1955.
4. Dimitroff, J. M., J. A. Jacquez, and H. F. Kuppenheim. Spectral Reflectance of the Skin of Rats and Rabbits in the Region 420-1000 m μ . AMRL Report No. 177, Fort Knox, Kentucky, 2 March 1955.
5. Dimitroff, J. M., H. F. Kuppenheim, P. M. Melotti, J. Huss, I. C. Graham, W. McKeehan and D. W. Swanson. Spectral Reflectance of the Skin of Rats, Rabbits and Hairless Mice in the Regions 243-700 m μ and .707-2.660 μ . AMRL Report No. 203, Fort Knox, Kentucky, 19 July 1955.
6. Jacquez, J. A., and H. F. Kuppenheim. The Theory of the Integrating Sphere. AMRL Report No. 150, Fort Knox, Kentucky, 26 November 1954.

7. Jacques, J. A., W. McKeehan, J. Huss, J. M. Dimitroff and H. F. Kuppenheim. An Integrating Sphere for Measuring Diffuse Reflectance in the Near Infrared. AMRL Report No. 188, Fort Knox, Kentucky, 14 April 1955.
8. Jacques, J. A., W. McKeehan, J. Huss, J. M. Dimitroff and H. F. Kuppenheim. An Integrating Sphere for the Measurement of Reflectance with the Beckman Model DR Recording Spectrophotometer. AMRL Report No. 192, Fort Knox, Kentucky, 19 April 1955.
9. Jacques, J. A., with the technical assistance of C. W. McKeehan, J. D. Huss, I. C. Graham, J. M. Melotti, Dimitroff and D. W. Swanson. The Use of the Integrating Sphere in Spectrophotometric Studies of Living Tissues and Micro-Organisms. AMRL Report No. 194, Fort Knox, Kentucky, 15 May 1955.

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