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A Thermostat Cell-holder for the Beckman Spectrophotometer

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The Beckman spectrophotometer is very convenient for following the progress of many enzyme reactions by measuring the change of absorption spectrum which accompanies the reaction. For this purpose it is necessary that the temperature of the cell should be kept accurately constant in the spectrophotometer in spite of the heat from the neighbouring lamp-house. Moreover, for studies on enzyme kinetics it is important to be able to work at different temperatures covering a wide range from 0° upwards.

Several temperature-controlled cell-holders have been devised for this purpose, e.g. that of Bell & Stryker (1947), consisting usually of some form of water-jacket through which water is circulated from an external thermostat. In all of these, however, so far as the writer is aware, the circulating water does not come into contact with the cells themselves, but the cells are placed in a well in the holder in such a way that they are surrounded by a layer of air. This tends to insulate the cells thermally from the surrounding jacket and greatly prolongs the time necessary for the cell contents to reach the desired temperature. Bell & Stryker, for instance, estimate that an equilibration period of 10 min. is necessary. This is a serious drawback for enzyme work.

This paper describes an easily constructed holder in which the cells are actually immersed directly in the circulating water, as a result of which the rate of thermal equilibration has been increased about tenfold. This device has been in constant use for the past two years for work on enzymes by a number of workers in this department, especially for studies on fumarase kinetics by Massey (1953), and it has proved so useful that it has been thought worth while to publish details of its construction.

It consists essentially of a Perspex box surrounding two standard 10 mm. quartz cells (Figs. 1, 2); watertight joints between the cells and the box are made by means of compressed rubber gaskets. The holder is suspended by rigid brackets from a brass plate which forms the lid of the spectrophotometer cell chamber and which can be slid so as to bring either cell in turn into the path of the light beam. The lid carries on its under-surface another brass plate, the ends of which act as stops; these are cut so that when either end is brought up against the wall of the chamber the light beam passes through the centre of the corresponding cell. Two rubber tubes for the circulating water are brought down through closely fitting holes in the lid to the cell-holder. The holder is hinged to the supporting brackets so that when it is removed from the chamber the lid can be swung down to the side to allow free access to the cells for filling. A small stand fitting the bottom of the holder loosely is useful to support it when it is removed from the instrument.

The problem of providing windows in the water jacket which do not absorb in the ultraviolet has been avoided by having nothing in the path of the light beam but the quartz cells themselves and their contents. This has been achieved by providing the window apertures in the front and back of the holder with sliding frames carrying rubber gaskets on their inner ends. These are so arranged that when the frames are pushed home the gaskets press on to the cells to make a watertight joint, and at the same time the pressure causes them to expand sideways and press against the sides of the apertures, so sealing them. The frames have projecting flanges within the gaskets which direct the pressure outward and prevent them from bulging into the light path.

Where the cells pass through the top of the holder, watertight joints are similarly made by compression of rubber gaskets contained in the apertures in plate *B* by screwing down plate *A*; this causes them to press both on to the top of the box and on to the cells, so making a complete seal.

The cells can be withdrawn for cleaning purposes simply by releasing the pressure on the gaskets, though this has not been found necessary in practice.

Since the holder is 3 cm. in thickness, it is necessary to replace the cell chamber of the spectrophotometer by another made up from three pieces of $\frac{3}{8} \times 1\frac{3}{16}$ in. brass bar, fastened together with screws and soldered at the corners, and drilled to fit the locating pins and bolts of the Beckman spectrophotometer. It is convenient to have an inlet at the bottom for a stream of dry air to prevent the cells from steaming up when working at 0°. It will be necessary to replace the four bolts holding the photocell compartment by longer ones.

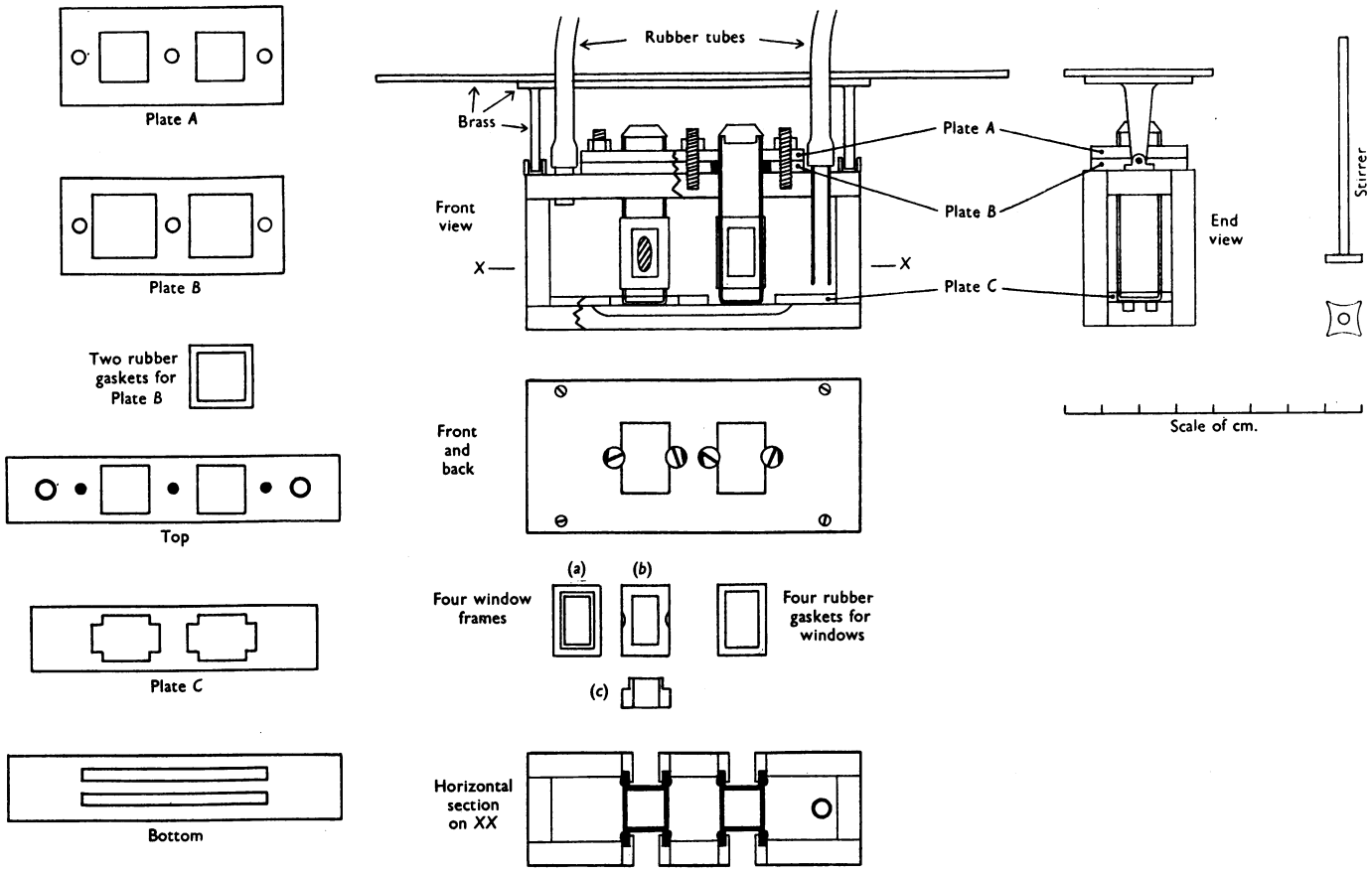

Fig. 1. Working drawings of cell-holder and its parts (half actual size). *Front view* (shown partly in section): the shaded area shows the position of the widest part of the light beam; with the Beckman instrument used this was obtained with a distance of 6 cm. between the lower surface of the top brass lid and the upper surface of the bottom plate of the holder, but this should be checked with the instrument for which the holder is intended; the dimensions of the lid are 17 cm. × 4 cm. × $\frac{1}{16}$ in. The dimensions of the other parts are as follows. *Front and back plates*: 9 cm. × 41 mm. × $\frac{1}{4}$ in., with two rectangular holes 19 mm. × 13 mm. with centres 13 mm. from centre lines and lower sides 10·5 mm. from bottom edge of plate. *Four window frames* ((*a*) back view, (*b*) front view, (*c*) horizontal section) 19 mm. × 13 mm. × $\frac{5}{16}$ in., pierced with holes 13 mm. × 7 mm. and with a 2 mm. × 3 mm. rebate cut round one face. *Four rubber gaskets* 19 mm. × 13 mm. with holes 15 mm. × 9 mm., cut from a $\frac{3}{16}$ in. sheet of soft rubber with smooth surface. *Plate A* 6 cm. × 26 mm. × $\frac{1}{8}$ in., with two holes 13 mm. square and three 4 BA clearance holes. (The centres of the square holes in plates *A–C* and the top are all at 13 mm. from the centre line, and the 4 BA holes in plates *A* and *B* and the top are spaced 25 mm. apart.) *Plate B* 6 cm. × 26 mm. × $\frac{1}{8}$ in., with two holes 17 mm. square and three 4 BA clearance holes. *Two rubber gaskets* 17 mm. square, with holes 13 mm. square. cut from $\frac{3}{16}$ in. sheet. *Top Plate* 9 cm. × 17·3 mm. × $\frac{1}{4}$ in., with two holes 13 mm. square and two holes to fit the Perspex tubes, and with three 4 BA brass studs tapped in. *Plate C* 77·3 mm. × 17·3 mm. × $\frac{3}{32}$ in., with two holes 13 mm. square with extensions as shown. *Bottom plate* 9 cm. × 17·3 mm. × $\frac{1}{4}$ in., with two grooves about $\frac{1}{8}$ in. × $\frac{1}{8}$ in. milled in upper surface. *Two end plates* (not shown) 28·3 mm. × 17·3 mm. × $\frac{1}{4}$ in.

CONSTRUCTIONAL DETAILS

The parts of the Perspex box are preferably squared-up accurately to size in the milling machine. Two slots are milled in the upper surface of the bottom piece as shown to permit some of the water to pass below the cells. The window frames should be an easy sliding fit in the apertures and are held in position by small brass screws with flat heads 6 mm. in diameter. The tapped holes for these must not pass right through the front and back pieces. The heads are recessed into these pieces and into the window frames in such a way that when the screws are home the heads are flush with the outside of the holder and the frames are also held flush with the front and back surfaces. After fitting, the frames are removed, the screws replaced and driven home, and then unscrewed for half a turn. The part of the heads which overlaps the window apertures is then removed with a file. This enables the window frames to be released later for removing the cells by a half turn of the screws.

The top is drilled and tapped for three 4 BA brass studs as shown, care being taken that the holes do not penetrate through to the under side. It is also drilled to fit the two pieces of Perspex tube accurately and these are fixed in position with Perspex cement. Plate *C*, which locates the bottoms of the cells, is stuck on to the bottom piece with Perspex cement.

The holder is then assembled by coating the joining surfaces with Perspex cement and immediately fastening firmly together with screws, so as to obtain watertight joints throughout.

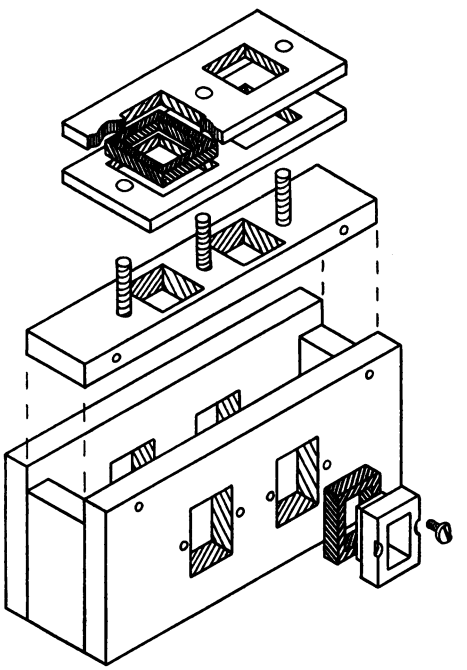

Fig. 2. Isometric drawing of cell-holder showing details of assembly.

Plate *B* with its two rubber gaskets is placed in position on the brass studs, followed by Plate *A*. 4 BA nuts are placed on the studs, but not tightened. The quartz cells are then placed in position and the nuts tightened so that they are held firmly. The gaskets should be cut so that when the nuts are screwed down the cells are quite firmly held, but without excessive pressure on their faces. If necessary, the pressure may be adjusted by placing thin washers on the studs between Plates *A* and *B*.

Finally the window frames, with their rubber gaskets, are pressed in from both sides simultaneously and secured by their screws. The whole jacket should then be watertight.

The water jacket is attached by hinges to the suspending brackets fixed to the lid. The photocell assembly is then removed from the spectrophotometer and the light turned on. The cell compartment is held in position and the cell-holder placed in it. It is then easy to verify that the ends of the brass plate on the under side of the lid have been cut correctly so that the light beam is accurately centred in the window frames when the lid is slid as far as it will go in either direction.

Some difficulty was met with in cutting the rubber gaskets until the following method was devised. A wafer-type safety razor blade is broken off to a length about $\frac{1}{2}$ mm. longer than the cut desired. The breaks are made at an angle of about 80° so that the cutting edge is the widest part. The piece of blade is held firmly in a small hand vice. The sheet of soft rubber is placed on a flat wooden surface, the edge of the blade is carefully placed in the desired position and the blade is pressed straight down through the sheet without any sawing motion. As the blade is a little too long, the cuts cross slightly at the ends and give square corners. None of the pieces of rubber should be removed from the sheet until all the cuts have been made, otherwise there will be some distortion of the rubber during cutting. For the same reason a margin should be left unused at the edge of the sheet.

Water is circulated from an external thermostat of about 3 l. capacity by means of a no. 10 Stuart centrifugal pump run with a speed-controlling resistance in series. A rate of about 300 ml./min. is quite adequate.

A small Perspex stirrer as shown is useful for quickly mixing the cell contents. It is shaped so that it cannot come into contact with the optical faces of the cells.

SUMMARY

A description is given of a cell-holder for the Beckman spectrophotometer in which the temperature of the cells is held constant by water circulating in contact with them. This gives rapid equilibration and accurate control.

The cell-holder was made for me by Mr H. Overhill in this department. I am indebted to him for the care which he took in its construction.

REFERENCES

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