Stirred Flow Reactor Incorporating a Spectrophotometer Cell And pH Electrodes

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The stirred flow reactor and auxiliary apparatus have been designed and constructed specially for kinetic studies with enzymes.

The Reactor Cell

Cell A–F (fig. 1) has a capacity of 3.6 ml. It is mounted with a reference cell G on an aluminum platform (not shown). The platform fits on the slide carriage of the Beckman DU–2 spectrophotometer, and cells A and G are interchangeable in the light path.

Reactor cell A is made from a 20-mm. glass tube ground precisely 0.500-inch deep. Two 1/8-inch optical grade quartz plates are cemented to each side. At the base of the cell on one side is a pivot bearing which accommodates a screw-type Teflon stirrer activated by motor H. This is a 24–29 volt direct current motor with speeds up to 8,000 rpm. It is 1.25 inches in diameter and 2.75 inches long.

Vigorous stirring by the screw stirrer on a 1/16-inch shaft mixes solutions from inlets D as they enter the reactor. Both shaft and stirrer are made from Kel-F. The upper bearing for stirrer E at the top of the cell is made from a section of 2-mm. capillary glass tubing. This also serves the cell as a solution outlet.

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The 1-mm. capillary tubes C, D, and F enter the cell without tapering. The capillary tubes were cut and ring sealed into the reactor without distortion of their flat end. This allows a precise determination of the effective volume of the cell because there are no regions of indeterminant mixing.

Tube E has been similarly sealed into place. A rubber cup fitted around tube E acts as a temporary waste reservoir. A suction line (not shown) removes the waste solution from this rubber cup. An outlet, tube F, permits emptying and cleaning the cell. The pH is measured by using the salt bridge and calomel electrode C and the glass electrode with O-ring seal set into a specially ground tapered opening B.

To increase the width of the compartment of the DU spectrophotometer, a 1-inch spacer and two additional specially fabricated thermospacers have been incorporated. Thermospacers available commercially did not permit flow of sufficient water to maintain adequate temperature control.

The piston outlet at K is connected to the inlets D by 3/32-inch Teflon tubing. The glass tubes K and D were chosen so that leakproof, press-fit, Teflon-inside-glass connections can be made and broken repeatedly.

A constant air temperature plywood black box (not shown) with fan, temperature regulator, heater, and appropriate inlets and outlets

covers the reactor cell A shown in figure 1. In order to maintain constant temperature of the entire lengths of the flow transport lines connecting D (fig. 1) with K (fig. 2), a 2-inch long, 2-inch diameter aluminum tube extends from the bottom of the air box and dips into the constant temperature water bath. The Teflon tubes which run through the aluminum tube are thus kept at constant temperature at all points. The constant temperature water bath contains the pistons.

The Precision Piston

Piston J is made of Kel-F and lubricated with Halocarbon grease. It has an O-ring seal with an outside diameter of 3/8 of an inch.

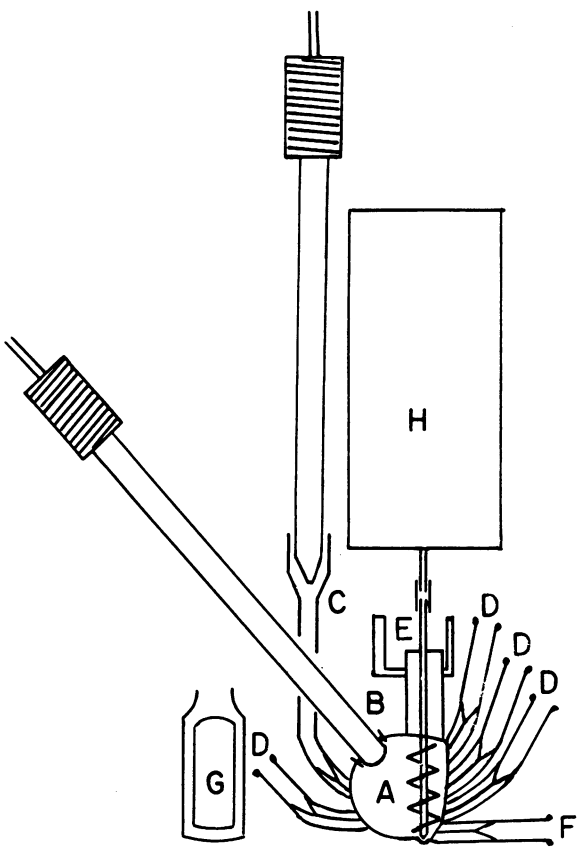

Figure 1. Stirred flow reactor cell
A. 3.6-ml. cell with quartz window plates on each side and screw stirrer B. Glass electrode with O-ring seal C. micro salt bridge and calomel electrode D. Solution inlets E. Stirrer bearing with cup for outflowing solution F. Outlet for cleaning cell G. Reference cell H. Miniature motor

Dimensions must be accurate to permit free movement of the piston.

Polyethylene bags, which serve as noncontaminable containers for the enzyme and substrate solutions, are molded to fit precisely inside the 24/40 joint K and loosely into L. To detect leakage from the bags, a platinum wire is sealed into the bottom of J and dilute salt water is poured in J and around L. One lead of an 100,000-ohm meter is inserted into the constant temperature water bath and the other lead in any outlet above K. Any deflection of the meter indicates leakage. Cylinders J and L must be completely filled with liquid so that the solution will flow smoothly.

Speed and Flow Control Mechanism

The mechanism for attaining both constant and intermittently variable speeds is shown in figure 3. Its 1/8-hp. synchronous reversible type motor M rotates at precisely 1,800 rpm. and is equipped with a dual switching mechanism so that it cannot be reversed without first stopping the motor. Gearbox N with outlets for speeds of 180, 18, 1.8, and 0.18 rpm. is shown. Intermediate speeds are attainable using the change gearbox P. Speeds are varied by combining four 20-pitch gears in pairs so that the sum of the teeth in each pair is 100. The gears are chosen so that the logarithm of output speeds may be varied in units of about 0.25.

Precision drive screw Q is ground at 40 threads per inch. Four drive shafts (only two are shown) attached to the drive block Q have speeds corresponding to the gear settings in N and P and allow up to four separate flows from J_3. A shear plate to prevent possible damage to the drive is shown with change gearbox P.

Apparatus Q-U permits a continuously variable control of flow. Screw QT set inside the bar QT is precisely and equally divided into two sections of which one half has a right-hand thread and the other a left-hand thread. Furthermore, the nuts V and V′ and the end threads on QT start at the same rotational and lengthwise positions so V and V′ are always equidistant from the center or from the pivot points Q and T.

Horizontal drive plates R are fixed precisely

perpendicular on a square shaft set into a square sliding bearing, thus preventing them from rotating. Drive shaft S, which could be placed at different vertical settings by opening set screw S, activates pistons J_1 and J_2. Knob U permits continuous control in positioning V and V′. The pivot point at T is fixed, but a sliding pivot point is necessary at Q. The groove is necessarily precisely perpendicular to the main drive screw so that the pivot height does not change due to the horizontal positioning of the pivot.

The combined flows from J_1 and J_2 exactly equal the flow from any one unit at J_3; thus, as V approaches T, correspondingly V′ approaches Q. Upon placing pure water in J_2 and a solution of 2Y molar in J_1, the combined outflow from J_1 plus J_2 can be made to vary continuously between 0 and 1Y molar at the same total flow rate as from any one of the J_3 units. Two of these units, parallel to each other, are provided. The main use is to vary the pH through alteration of the concentration of acid or base solution flowing into the reactor without changing the total flow rate. Therefore, a series of runs at given concentrations and different pH's is possible without refilling the tubes.

A Typical Rate Determination

Upon filling the polyethylene bags with the solutions of enzyme, substrate, base, or acid, and whatever other solutions may be required, the J tubes are positioned in the constant temperature water bath within the support framework (not shown) so designed for them. The Teflon tubes from L are connected to D and the reactor is positioned in the constant temperature box. Knob U is turned to the desired position for pH adjustment, and the drive plates R are positioned against the drive pins at V and V′. After starting the pistons and allowing cell A to fill, the stirring is begun. The pH is measured and the spectrophotometric reading is recorded after a volume of liquid equal to 3–4 fillings of the cell has been wasted.

Calibration curves of optical densities against various concentrations of the product or reactant to be analyzed are determined in the appropriate solvent. It may be necessary to correct for the presence of other substances,

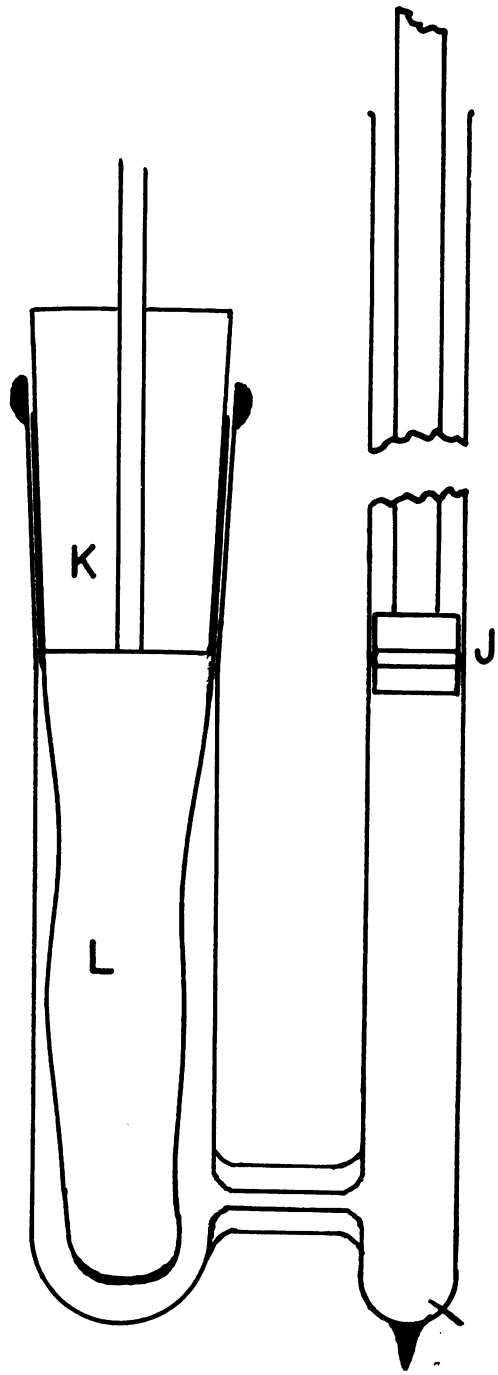

Figure 2. Details of the piston syringe
K. 24/40 standard taper joint and glass or Teflon plug with fitted polyethylene bag at L J. Kel-F piston and O-ring inside a ⅜-inch precision bore tube

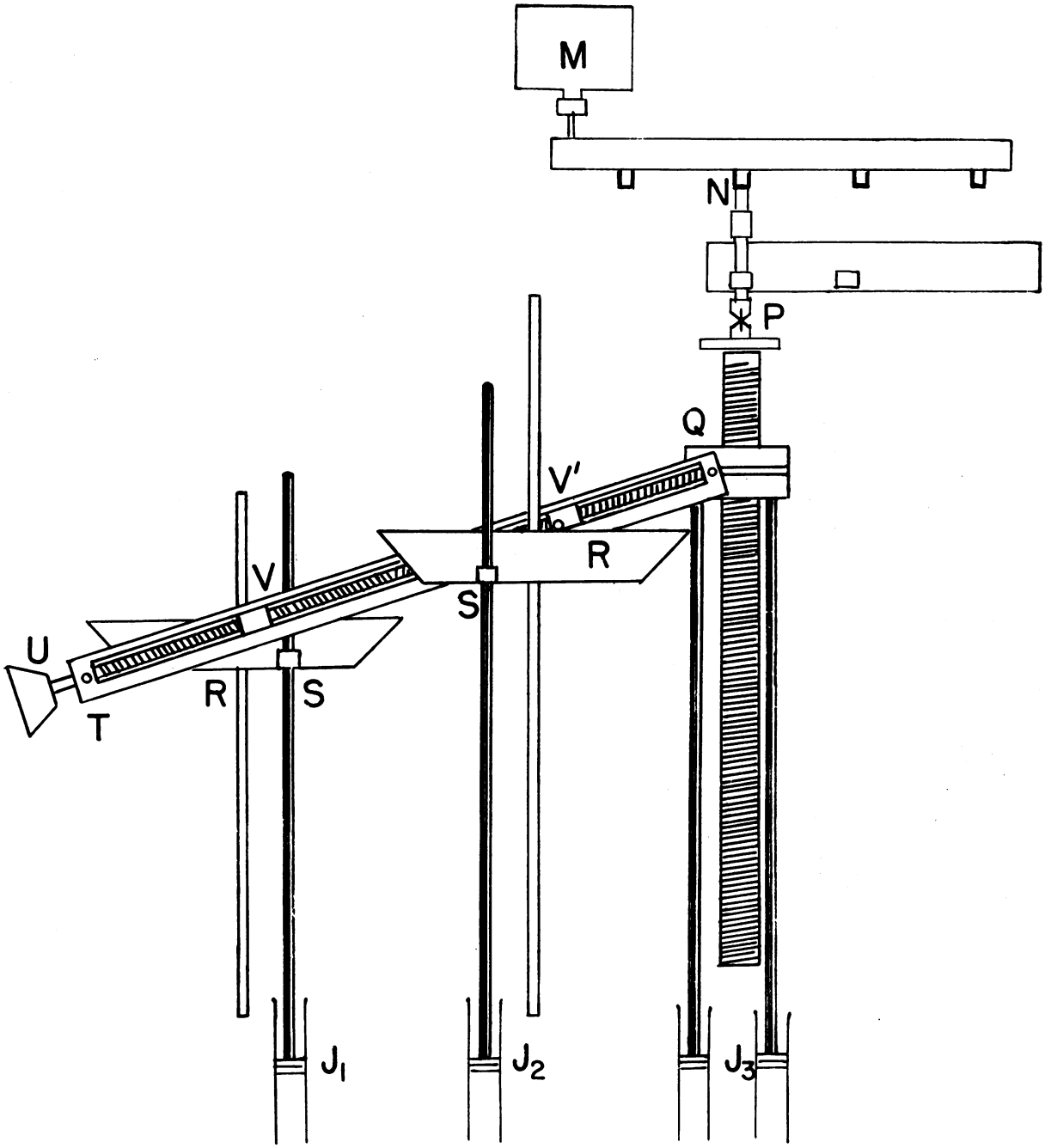

Figure 3. Speed and flow control apparatus M. ⅛-hp. synchronous motor N. Multiple outlet gearbox P. Change gearbox with shear plate coupling Q. Precision screw with drive block and sliding pivot in the bar QT R. Perpendicular square shafts support the horizonal plate driven by pivots at V S. Drive shaft for pistons at J T. Fixed pivot U. Knob to turn screw which controls positions of V V. Two nuts—one having a right-hand thread and the other a left-hand thread so that the two nuts move in opposite directions

particularly if their concentrations vary with the completion of the reaction.

Because the concentration of the enzyme and substrate are kept constant throughout the investigations, the relative rates obtained with each variation may be plotted against pH.

To repeat the run at another pH the cell is emptied through F, a new setting of U is made, and the flow of solution is renewed.

Cineradiographic Circle Unit

The cineradiographic circle unit permits simultaneous recording of three parameters of mandibular movements for better understanding of the working role of the temporomandibular joint. The unit consists of two 7-foot disks standing on edge and fastened parallel to each other by 4-foot interconnecting shelves. The patient's chair is located between the disks that rotate on floor-mounted rubber bearings. This rotating unit supports and maintains the X-ray tube and the image intensifier-recording camera in proper alinement to each other on two opposing interconnecting shelves. Thus the cineradiographic equipment rotates around the patient who is seated in the center of the double circle system.

This feature provides a 120° choice of X-ray angulations that can be used without tilting the patient's head as is necessary in some current techniques that introduce a deviation from normal intermaxillary relationships.

Biplane data can also be recorded; as the primary motion picture camera records the output of the image intensifier, a secondary cinecamera records the facial movements of the patient as viewed through the open center section of the front circle support.

To record exact orofacial movements in a study, a 16 mm. Auricon camera directly in front of the patient is synchronized to the Milliken high-speed cineradiographic camera. Sound film records programed instructions by investigators and the corresponding response of the patient. Electromyographic recordings are synchronized to frontal and cineradiographic cameras by a shutter pulse signal generated within the high-speed camera and transmitted to the multichannel oscillographic recorder. Therefore each frame of the motion picture films from both circle unit cameras can be positively related to each other and to the time base on the electromyographic tracings.

This system allows the patient to sit in a natural, comfortable position during the entire data recording session. This aids in the reduction of "operator-induced error," a part of the hithertofore difficult task of positioning the patient for temporomandibular joint studies. The new system also reduces the patient-induced error introduced into data recording when the patient attempts to hold the head in a tilted position for the duration of the study.—HARRISON M. BERRY, Jr., D.D.S., M.Sc., and F. ALLAN HOFMANN, *University of Pennsylvania School of Dental Medicine, Philadelphia.* *This invention was developed under Public Health Service grant No. DE–00240.*