

Transient Response of the Glass Electrode to pH Step Variations

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A dc amplifier with electrometer input stage is described making it possible to display pH changes on the oscilloscope screen for physiological applications. Sensitivity is 0.003 pH unit per cm beam deflection; noise 50 μ v; drift 300 μ v/hr (\approx 0.005 pH unit). A simple hydraulic mechanism is used to produce very fast pH steps under the electrode. The speed of the electrode response is shown to tend towards a finite limit when the speed of the pH change increases. The form of the transient in the fastest records is close to an exponential curve $e^{-\alpha t}$, with $1/\alpha \approx 30$ msec. The response is independent of the membrane thickness (5 to 10 μ). Some evidence exists to show that the response of the electrode to pH steps is analogous to the response of a low-pass RC-filter to a corresponding voltage step, where R and C are, respectively, the resistance and the capacity of the glass membrane. R and C values determined from the analysis of the electrode response to step voltages in series give $RC \approx 30$ msec in agreement with the experimental results.

INTRODUCTION

AS shown by Dubuisson,¹⁻⁷ pH changes which take place during muscle activity can be detected by means of a glass electrode with its flat active membrane in close contact with the muscle surface through a thin film of Ringer solution. A twin-tube balanced electrometer stage (Dubuisson and Debot)⁸ and a galvanometer were used for photographic recording. The time required for an instantaneous pH change to be recorded as a full galvanometer deflection was found experimentally to be 4 sec, corresponding to the galvanometer period. This rather high delay does not bring too much distortion in the recordings of the pH variations which occur during the contraction of a smooth muscle (Dubuisson),⁵ or during slow stretching of striated muscle (Dubuisson),⁶ but makes it impossible to study the pH changes during a single twitch of the striated muscle of a frog.

As pointed out at the time, besides the time lag due to the recording instrument, one has to know the delays introduced by the intrinsic properties of the electrode and by the diffusion processes which characterize the CO₂ exchanges between the muscle fibers and the film of Ringer under the glass membrane.

A theoretical estimation of the delay brought by diffusion has been given by Hill.⁹

As a first attempt to improve the technique, we have made the delay due to the recording device (electrode excluded) negligible, by feeding the electrometer stage output to a dc high gain amplifier in order to display the pH changes on the oscilloscope screen. This has

been possible thanks to the very great help received from A. V. Hill and his collaborators.

The next step was to measure the delay introduced by the electrode by recording its transient response to an instantaneous pH change.

I. EXPERIMENTAL TRANSIENTS CORRESPONDING TO VERY FAST pH STEP VARIATIONS

Technique

A. Electrometer, DC Amplifier, Oscilloscope

The electrometer circuit is shown in Fig. 1. It is essentially similar to that described by Dubuisson and Debot,⁸ except that the voltage gain is increased from $\times 0.3$ to $\times 0.7$ by using higher plate loads and a higher plate supply voltage. The circuit was initially designed for large-sized Gecovalve ET1 electrometer triodes, but as the Gecovalve ET3 sub-miniature triodes proved less microphonic, they were preferred.

The grid current is sufficiently small for potential measurements with glass electrodes of 100 megohms internal resistance: identical galvanometer deflections are obtained for a given dc input

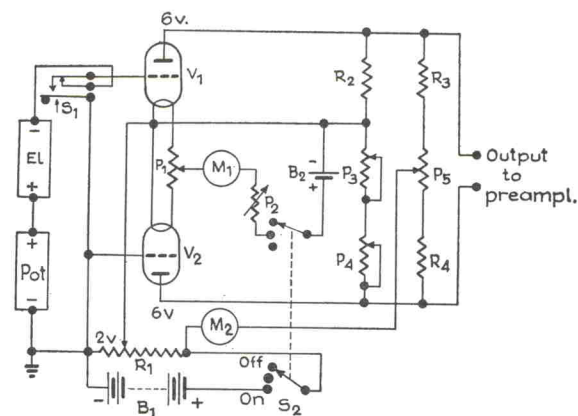


FIG. 1. Circuit diagram of valve electrometer for pH measurements. $R_1 = 12K$ with tap. $R_2 = 250K$. $R_3, R_4 = 35K$. $P_1 = 5\omega$ single wire pot. $P_2 = 25\omega$ single wire pot. $P_3 = 50K$ helical pot. $P_4 = 5 \times 45K$ on S.p.-6 pos.-rotary sw. $P_5 = 10K$ helical pot. EL = electrode assembly (see page 870). Pot. = precision potentiometer. (All resistors are precision wire wound.) S_1 = make-before-break high insulated switch. S_2 = D.p.-3 pos.-rotary sw. $M_1 = 0-50$ mA. $M_2 = 0-500\mu$ A. $B_1 = 12$ -v battery. $B_2 = 2$ -v. battery. V_1, V_2 = Gecovalve ET3 sub-miniature electrometer triode.

¹ M. Dubuisson, Proc. Soc. Exptl. Biol. Med. **35**, 609 (1937).
² M. Dubuisson, Proc. Physiol. Soc. Phila. **90**, 1 (1937).
³ M. Dubuisson, Pflügers Arch. ges Physiol. **239**, 314 (1937).
⁴ M. Dubuisson and W. Schulz, Pflügers Arch. ges Physiol. **239**, 776 (1938).
⁵ M. Dubuisson, J. Physiol. (London) **94**, 461 (1939).
⁶ M. Dubuisson, Arch. intern. physiol. **50**, 203 (1940).
⁷ M. Dubuisson, Proc. Roy. Soc. (London) series B, **137**, 63 (1950).
⁸ M. Dubuisson and A. Debot, Arch. intern. physiol. **50**, 54 (1940).
⁹ A. V. Hill, Proc. Roy. Soc. (London) series B, **135**, 446 (1948).

potential whether fed to the grid through a 100-megohm resistor or directly.

As this instrument is intended to record continuous pH changes to within 0.01 pH unit and less, great care has to be taken to eliminate the zero drift. It can be shown experimentally that with carefully matched tubes, there is always a setting of potentiometer P_1 (heater current balance), for which one is able to find a setting of potentiometer P_5 (plate load balance) which suppresses the zero drift between plates, or reduces it to a very small value. Once this equilibrium is reached, a slight change to the right or to the left in the setting of potentiometer P_5 starts the drift in one or the other direction. The equilibration is carried out with a sensitive galvanometer with suitable shunt, plugged in between plates. To shift the zero P_2 and P_4 are used. At minimum drift point, provided the total filament current is always adjusted to the same value and the filament supply battery is maintained in the flat portion of its discharge curve, the H.T. battery can be used down $\frac{1}{4}$ of its discharge curve without drift trouble. The minimum drift reached this way is always continuous. Spurious drifts and jumps indicate defective batteries, poor connections, insufficient insulation (input grid circuit, grid switch, electrode assembly, moisture, or dirt on the electrometer valves), sudden temperature changes, etc. The usual precautions against such sources of disturbance have been taken.

The major trouble when the output of the ET1 and ET3 electrometer valves is amplified and displayed on the oscilloscope screen, the amplification factor being as high as $\times 190\,000$, is their very marked microphony. To eliminate vibrations and sound pick-up, the valves have to be mounted in an electrically shielded sound-proof cabinet, on a spring suspension of the type designed by Hill¹⁰ for vibrationless galvanometer mountings, and similar to the system described by Frankenhaeuser and Mac Donald.¹¹ The chassis with the circuit parts and controls, including the highly insulated grid switch, is fixed externally on the cabinet, which is firmly secured to the room-wall. The grid lead from the electrode assembly is a rigid high-insulated shielded cable of the coaxial

type, and care is taken to avoid having vibrations transmitted to this cable.

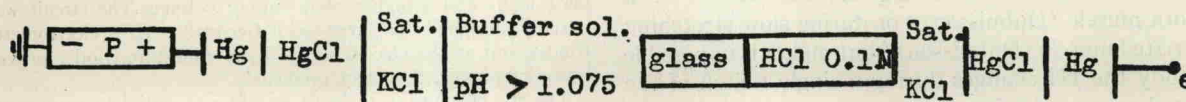
The electrometer stage is followed by a battery operated dc preamplifier. Its gain is $\times 300$, and it is similar to that described by Copeland.¹² It has four valves: two type E37A, operating as a long tailed-pair whose outputs are converted to a single-sided signal by a 6SC7 valve and fed at earth level to the oscilloscope amplifier by a cathode follower (6J5). The oscilloscope dc amplifier is the high gain channel ($\times 900$) of the Cossor model 1049 double-beam oscilloscope with camera attachment.

The gain of the electrometer stage being $\times 0.7$, the over-all full voltage gain of the whole instrument is $\times 190\,000$. The tube sensitivity is $160\ \mu V/cm$ ($\approx 0.003\ pH/cm$). The oscilloscope attenuator is used for different gain settings. The signal acceptance at full gain without distortion is 50 mv. The over-all drift, after a 1 hr warmup, at full amplification, is about $300\ \mu V/hr$ ($\approx 0.005\ pH$ unit). The drift is continuous, without sudden jumps. The noise level is about $50\ \mu V$ peak to peak (band width reduced to dc—4.6 kc/sec, input grid earthed or connected to the electrode assembly). Stepwise reduction of the band width is provided for, down to dc—50 c/sec, the peak to peak noise decreasing to less than $20\ \mu V$.

B. Glass Electrodes, Calomel Electrodes, and Cell Assembly

The glass electrodes are of the Mac Innes and Dole type,¹³ and made by sealing a thin membrane of 015 Corning glass onto an ordinary glass tube (inner ϕ 6.5 mm, outer ϕ 8 mm). The extremity of the glass tubes is ground flat and perpendicular to the stem axis. The tubes are 10 or 12 cm long, and coated with paraffin wax up to 2 or 3 cm from the membrane. Electrodes with skew, rippled, or sucked-in membranes are discarded.

The inside of the electrode is filled with 0.1 N HCl, and the electrode is connected in the following cell assembly:



(P is the compensating potentiometer; e is connected to the input grid of the electrometer.)

The calomel electrodes are of the conventional type and care is taken to connect them in opposition during 48 hrs after filling with saturated KCl, to eliminate any potential difference. The salt bridges are filled with agar-agar jelly saturated with KCl.

The glass electrodes are checked by measuring their potential in an acetate buffer pH 4.66. The electrodes giving stable potentials between 205 and 215 mv are retained.

The membrane thickness of the electrodes ranges from 5 to $10\ \mu$. These values have been found by comparing the edge thickness of about 100 glass flakes taken from the bulbs blown to make electrodes, to a calibrated wire gauge under a microscope with micrometer scale.

C. Equipment to Produce a Step pH Change Under the Electrodes

To measure the distortion introduced by the glass electrode in the recording of fast pH changes, the shape of the pH step variation has to be known. As the response of other electrodes, sensitive to H^+ ions or other ions, is unknown,* there is no direct

way to solve this problem. An indirect way is to produce a pH change so fast compared to the delay due to the electrode, that it can safely be assumed to approximate a rectangular step.

Fast pH changes can be produced mechanically, either by mixing solutions of different pH under the electrode, or by quickly displacing one solution by another. The shape of the pH variation will depend on the mechanical arrangement and may be affected by diffusion, adsorption, viscosity, and surface wetting. The faster the pH variation, the less its shape will depend on the first two of these factors, the last three appearing as speed limiting factors for a given solution.

Mixing was first tried, but soon proved to give irregular and too-slow pH changes, however vigorous the stirring.

The quick displacement of one solution by another of different pH gave much better results from the start. After much trial, the apparatus shown in Fig. 2 was built.

The inverted glass bottle A , the rubber tube B , the Y-shaped glass tube y , are filled with solution 1. The liquid does not flow as long as valve a is closed. A droplet of solution 2 ($pH_1 - pH_2 = 0.3$) is suspended under the electrode E . When valve a is opened, solution 1 bursts out of the flattened tip of tube y and washes

depends very much on the degree of polish of the metallic surface. With the specimens tried, the response has been found much slower than that of a glass electrode. Only a few experiments have been made, and no conclusions can be safely drawn.

¹² K. Copeland, *J. of Physiol. (London)* **117**, 15 P (1952).

¹³ D. A. Mac Innes and M. Dole, *J. Am. Chem. Soc.* **52**, 29 (1930).

¹⁰ A. V. Hill, *J. Sci. Instr.* **25**, 225 (1948).

¹¹ B. Frankenhaeuser and D. K. C. Mac Donald, *J. Sci. Instr.* **26**, 145 (1949).

* Some experiments have been tried with a platinum electrode and quinhydrone, and with a Cu/CuSO₄ electrode of the same shape as the glass-electrode. The response of these electrodes