SHORT COMMUNICATIONS

AUTOMATIC CONTINUOUS VARIATION OF SOLUTE CONCENTRATION IN A PERFUSATE

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Many physiological experiments involve switching between two or more solutions. For example, when drugs are applied to tissues the concentration of the drug is usually changed as a step function, by switching from a control saline to a saline containing the drug. There are many instances where it would be advantageous to change the concentration according to some other function such as a ramp. This paper describes how this has been achieved by a mixing valve under electronic control.

The mixing valve (incorporating a Lee Products, solenoid valve No LFAX050303AC) was arranged to control the mixing of two solutions and thus vary the composition of the effluent stream. The composition of the solution leaving the output is directly proportional to the time that the valve allows each of the inputs to be connected to the output (Cook & Hokanson, 1976).

The electronic circuit for control of the valve is shown in Fig. 1. Driving current for the valve is provided by TR1 under control of a pulse width modulator, IC1 Fig. 1(A). Permanently connected to the non-inverting input of IC1 is a triangular wave generator IC2, and IC3 whose mathematical function is $V_{\text{max}} \pm (2V_{\text{max}}/\pi)w_1t$, (where $w_1 = 2\pi f_1$), $f_1$ is the frequency in Hertz, and $t$ the time in seconds. With this function alone, and zero volts (earth), connected to the inverting input of the modulator IC1, a square wave of mark space ratio 1:1 is fed to the valve. Thus the concentration of a solute in the effluent equals one half of the difference between the two input concentrations. By connecting a signal to the inverting input, the mark-space ratio of the square wave can be made to vary, and continuous variations are produced in the concentration of the effluent.

To continuously vary solute concentration, two functions were applied: a sine wave generated by IC4 (Fig. 1 B), and a ramp, produced by the diagram shown in Fig. 1(C). The sine-wave generator generates the function $V \sin w_2t$, where $w_2$ is $2\pi f_2$, $f_2$ is the frequency in Hertz, and $t$ the time in seconds. $V_{\text{max}}$, the peak amplitude reached by the waveform, is arranged to be equal to $V_{\text{max}}$ of the triangular wave. As sin $w_2t$ approaches 1 the mark-space ratio of the modulator output square wave becomes large, as sin $w_2t$ approaches -1 the ratio becomes small.

In the ramp generator (Fig. 1 C) a voltage-controlled oscillator, IC6, runs at a
Fig. 1. Resistors are 1/4 W high-stability carbon-film variable, pre-set resistors are cermet, and capacitors are polyester, except where otherwise stated. (A) Integrated circuits (IC) 2 and 3 with associated components form the triangular wave generators, IC1 is the pulse-width modulator, which drives the solenoid transistor BC109. (B) IC4 with the components shown provide a low-frequency sine-wave generator with IC5 acting as the output stage. The potential divider with a 10 K cermet variable resistor gives manual control. (C) Block diagram of the triangular wave generator; circuit details are well covered in manufacturer’s literature. The power supply is ±15 V with ±0.6% load regulation between 0 and 100 mA.

frequency determined by the setting of the 10 K potentiometer. This frequency is divided by 10 in IC7; IC8 is a ripple counter which gives an output in binary equal to the number of oscillator pulses counted. The binary output of IC8 is converted to analogue voltage by IC9, a digital-to-analogue converter, and subsequently amplified by IC10. Should the ramp require inversion this is done by amplifier IC11. Output limits of the ramp generator exceed $V_{\text{max}}$ of the triangular wave generator by 10%, which causes the concentration of the valve effluent to remain at the lower or upper concentration for a period of time, at the beginning and end of the ramp, that depends upon the slope selected. The slope and the frequency selected by the 10 K potentiometer are directly proportional.
Variation of solute concentration in a perfusate

Fig. 2. (A) The sinusoidal variation of absorbance at three modulation frequencies: 0.01, 0.03 and 0.06 Hz. (B) Radioactivity (expressed as a percentage of maximum count rate) in the output from the device under the control of a linear ramp. Arrowed circles are points obtained by linear regression. (C) The effect of applying ramp changes in potassium concentration varying between zero and 100 mM on the output of a potassium ion-sensitive micro-electrode (upper trace). A short period of recovery time is necessary and was allowed on the third test. This became an automatic feature and is in evidence in (D). (D) The effect of applying ramp changes in 5-HT concentration to a salivary gland varying between zero and 1 x 10^-8 M. Concentration was held constant at each end of the ramp to allow recovery time for the biological tissue. This was done by simply increasing the height of the ramp above V_max of the triangular wave and serves to show the versatility of the system.

Test performance

The performance of the valve was monitored by measuring solute concentration in the effluent, using spectrophotometry, radio-isotopes and ion-selective electrodes. Test of sinusoidal variation of the solute concentration by absorption measurement, was carried out by passing the effluent through a flow cell (Pye Unicam UW 109644) in a Pye Unicam (SP 800 B) spectrophotometer. Input to the valve was, to one side, distilled water and, to the other, a dye (Registriertintne Red B180/551, recording ink) of a sufficient concentration to give almost 100% absorbance (λ = 500 nm). With a flow-rate of 10 ml/min absorbance of the effluent closely followed the modulation waveform, C_max sin ωt, where C max is 100% concentration (Fig. 2 A). The distortion present in the 120 s period sinusoid is due to distortion in the modulating waveform produced by IC4 at this low frequency of 0.01 Hz. Note the reduced amplitude of the two remaining sinusoids. This can be accounted for by the limited
Testing for a linear change in concentration using the ramp generator was carried out with a radioactive tracer ([8-3H]guanosine-3'-5'-cyclic phosphate) in one of the reservoirs, and distilled water in the other. The tracer was introduced as a linear ramp by modulating with \( f(t) = Vt \), causing the radioactive content of the effluent to increase as \( f(t) = Ct \). Flow-rate was adjusted to give 1 drop every 2 s; samples of five drops each were taken at 30 s intervals and a total of 56 samples was taken during a period of 28 min. The samples, collected in counting vials, were prepared for scintillation counting by adding 3·8 ml of Brays fluid, and were subsequently counted in a Packard 3255. With a background count of 23 cpm and a maximum count of 2075 cpm the result is plotted in Fig. 2(B) after normalizing, to give individual counts as a percentage of the maximum counting rate.

An ion-sensitive potassium electrode, prepared as described by Berridge & Schlue (1978), was also used to monitor a linear increase in potassium concentration in the effluent using the ramp, with input concentrations of zero and 100%. Chart recordings of three runs where potassium was increased from zero to 100 mm are shown in Fig. 2(C). The potassium electrode converts the linear ramp into a log function, as predicted by the Nernst equation.

**Experimental result**

The salivary gland of *Calliphora erythrocephala* responds to 5-HT with a characteristic change in transepithelial potential. At certain 5-HT concentrations the potential oscillates with characteristic frequency, which is high at low doses (1 × 10^{-9} to 5 × 10^{-8} M) but increases as concentration rises to finally damp out at 1 × 10^{-8} M (Berridge & Prince, 1972).

When a salivary gland was perfused with a 5-HT ramp, which passed through this sensitive region, the oscillations in transepithelial potential displayed the characteristic range of frequencies (Fig. 2D) previously obtained by applying a series of step functions.

**Conclusion**

This device gives much greater flexibility to many physiological experiments, by automatically giving an almost infinite variability to a concentration. Sensitivity of a biological tissue to a specific concentration may be found, easily and swiftly, thus eliminating the need for many step changes. Calibration of ion-sensitive electrodes, and comparisons between them, becomes straightforward, and may be done in minutes.

More advanced work may possibly be done by feeding biological tissues with forcing functions, eventually leading to the solving of coefficients and constants in differential equations which describe the biological phenomena such as oscillatory activity (Berridge & Rapp, 1979; Rapp, 1979).
REFERENCES


